Sport and Exercise Medicine

Theory and Practice

Raghav Trivedi

Sport and Exercise Medicine: Theory and Practice

Sport and Exercise Medicine: Theory and Practice

Raghav Trivedi

VIDYA BOOKS

Published by Vidya Books,
305, Ajit Bhawan,
21 Ansari Road,
Daryaganj, Delhi 110002

Raghav Trivedi
ISBN: 978-93-5429-367-2

Contents

Chapter 1 An age-adapted plyometric exercise program improves dynamic strength, jump performance and functional capacity in older men either similarly or more than traditional resistance training 1

Chapter 2 Motion sickness symptoms during jumping exercise on a short-arm centrifuge 23

Chapter 3 High plasma soluble levels of the immune checkpoint HLA-G molecule among bodybuilders 33

Chapter 4 Soccer heading and concussion are not associated with reduced brain volume or cortical thickness 44

Chapter 5 Salivary endocrine response following a maximal incremental cycling protocol with local vibration 52

Chapter 6 Caffeine increases motor output entropy and performance in 4 km cycling time trial 65

Chapter 7 A 2-year longitudinal follow-up of performance characteristics in Chinese male elite youth athletes from swimming and racket sports 78

Chapter 8 The effect of an airflow restriction mask (ARM) on metabolic, ventilatory, and electromyographic responses to continuous cycling exercise 96

Chapter 9 Preschool environment and preschool teacher's physical activity and their association with children's activity levels at preschool 108

Chapter 10 Motor control integrated into muscle strengthening exercises has more effects on scapular muscle activities and joint range of motion before initiation of radiotherapy in oral cancer survivors with neck dissection: A randomized controlled trial 124

Chapter 11 Evidence for the effects of prehabilitation before ACL-reconstruction on return to sportrelated and self-reported knee function: A systematic review 141

Chapter 12 Are heart rate methods based on ergometer cycling and level treadmill walking interchangeable? 162

Chapter 13 The effects of post activation potentiation warm-up and pre-shot routine programs on driving performance in amateur golfers 180

Chapter 14 Subconcussive head impact exposure between drill intensities in U.S. high school football 190

Chapter 15 Effects of high intensity interval exercise on cerebrovascular function: A systematic review 204

Chapter 16 Reproducibility of isokinetic knee testing using the novel isokinetic SMM iMoment dynamometer 221

1

An age-adapted plyometric exercise program improves dynamic strength, jump performance and functional capacity in older men either similarly or more than traditional resistance training

Evelien Van Roie[1]*, **Simon Walker**[2], **Stijn Van Driessche**[1], **Tijs Delabastita**[3], **Benedicte Vanwanseele**[3], **Christophe Delecluse**[1]

1 Physical Activity, Sports and Health Research Group, Department of Movement Sciences, KU Leuven, Leuven, Belgium, **2** Neuromuscular Research Center, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland, **3** Human Movement Biomechanics Research Group, Department of Movement Sciences, KU Leuven, Leuven, Belgium

* evelien.vanroie@kuleuven.be

Abstract

Power declines at a greater rate during ageing and is more relevant for functional deterioration than either loss of maximum strength or muscle mass. Human movement typically consists of stretch-shortening cycle action. Therefore, plyometric exercises, using an eccentric phase quickly followed by a concentric phase to optimize power production, should resemble daily function more than traditional resistance training, which primarily builds force production capacity in general. However, it is unclear whether older adults can sustain such high-impact training. This study compared the effects of plyometric exercise (PLYO) on power, force production, jump and functional performance to traditional resistance training (RT) and walking (WALK) in older men. Importantly, feasibility was investigated. Forty men (69.5 ± 3.9 years) were randomized to 12-weeks of PLYO ($N = 14$), RT ($N = 12$) or WALK ($N = 14$). Leg press one-repetition maximum (1-RM), leg-extensor isometric maximum voluntary contraction (MVC) and rate of force development (RFD), jump and functional performance were evaluated pre- and post-intervention. One subject in RT (low back pain) and three in PLYO (2 muscle strains, 1 knee pain) dropped out. Adherence to (91.2 ± 4.4%) and acceptability of ($\geq$ 7/10) PLYO was high. 1-RM improved more in RT (25.0 ± 10.0%) and PLYO (23.0 ± 13.6%) than in WALK (2.9 ± 13.7%) ($p < 0.001$). PLYO improved more on jump height, jump power, contraction time of jumps and stair climbing performance compared to WALK and/or RT ($p < 0.05$). MVC improved in RT only ($p = 0.028$) and RFD did not improve ($p > 0.05$). To conclude, PLYO is beneficial over RT for improving power, jump and stair climbing performance without compromising gains in strength. This form of training seems feasible, but contains an inherent higher risk for injuries, which should be taken into account when designing programs for older adults.

Editor: Massimo Sacchetti, Universita degli Studi di Roma 'Foro Italico', ITALY

Competing interests: The authors have declared that no competing interests exist.

Introduction

During the ageing process, muscle mass, muscle force and power production decline progressively [1–3]. This decline is more pronounced in power and rapid force production than in maximal force or muscle mass [3, 4]. In addition, the ability to generate a high amount of power enables individuals to perform better on everyday activities, such as rising from a chair and climbing stairs [5]. In reactive motor tasks, such as balance recovery following sudden perturbations, muscle force needs to be generated in short time frames [6]. Consequently, reduced lower-limb power and slowing of force production have been proposed as important predictors of age-related deterioration in functional performance and should be targeted in exercise programs for older adults.

Resistance exercise has been widely recognized as an effective strategy to improve muscle mass, muscle strength and functional performance in older adults. The majority of interventions prescribed slow-speed resistance exercise with 1–4 sets of about 8–15 repetitions at moderate to high loads for at least 2x/week, in line with previous international recommendations [7]. Although the benefits are well documented, limited improvements in power and rate of force production have been reported when exercises are typically performed at slow and controlled speeds [8]. As mentioned above and supported by recent insights and exercise guidelines, there are important arguments to justify the inclusion of explosive type of resistance exercise in older adults [9, 10]. Machine-based resistance exercise performed with an explosive concentric phase followed by a controlled, slower eccentric phase is feasible, even in institutionalized elderly [11]. This type of explosive resistance exercise has shown greater effects on functional performance than traditional slow-speed resistance exercise [12, 13].

However, human locomotion rarely involves pure concentric movements, but consists of rapidly coupled eccentric-concentric multi-joint muscle actions, known as stretch-shortening cycle (SSC) activities. Fast eccentric-concentric transition in a SSC movement facilitates subsequent power generation through storage and reutilization of elastic energy [14]. Therefore, multi-joint SSC movements represent a mechanism behind optimal power production. This is an important argument for the use of plyometric training, which specifically targets multi-joint SSC, in older adults, especially considering that the utilization of elastic energy becomes gradually impaired due to neural and structural changes in aged muscles [15, 16].

Plyometric training elicits numerous positive changes in neural and musculoskeletal systems, muscle function and performance of healthy individuals (for a review, see [17]). However, this training modality has primarily been used in athletes and/or young adults, while limited research exists on plyometric training for older adults [18]. In addition, knowledge on the potential adverse events of such high-impact training is lacking, as papers often fail to comment on feasibility and injuries [19].

Therefore, we designed a 12-week multi-joint plyometric exercise program (PLYO) for older men. This program was age-adapted to maximize feasibility by slowly progressing from slow speed exercises over submaximal to maximal jumps, using only low-intensity drills, allowing using wall bars for support and introducing short breaks between every jump. Its effects on muscle strength, muscle power, jump performance and functional capacity were compared to a traditional resistance training program (RT) and a walking program (WALK). The walking group was considered a control group, as the exercise was not likely to improve muscle strength or power, but did consist of low-load eccentric-concentric lower-limb movements that might already affect functional performance. As a secondary outcome, the feasibility of the plyometric exercise program was investigated. We hypothesized that PLYO would improve more on muscle power, jump performance and functional performance than RT and WALK and that the program would be feasible in healthy community-dwelling older men.

Methods

Trial design

This randomized trial was designed as a parallel-group study, with three different exercise interventions. The intervention duration was 12 weeks. Outcome measurements were obtained at baseline (pre-intervention, from February to March 2018) and within one week after the last exercise session (post-intervention, from May to June 2018). The study was approved by the Human Ethics Committee Research UZ/KU Leuven in accordance with the declaration of Helsinki. All subjects provided written informed consent.

Subjects

Community-dwelling older men aged 65 to 80 years were recruited through advertisements in local newspapers. Exclusion criteria were unstable cardiovascular disease, neurological disorders, cognitive malfunctioning, severe knee or hip problems, previous rupture of the Achilles tendon and systematic engagement in (resistance) exercise in the 12 months prior to participation. In total, 42 men (age: 69.4 ± 3.8 years, body mass: 82.7 ± 10.5 kg, body height: 174.8 ± 6.4 cm, BMI: 27.1 ± 3.2 kg/m^2) were found eligible and agreed to participate in the study (Fig 1).

Randomization

Subjects were randomly assigned to one of three intervention conditions by block randomization (block size of 3): traditional resistance training (RT, n = 14), plyometric exercise (PLYO, n = 14) or walking (WALK, n = 14). Allocation ratio was 1:1:1. In RT, two subjects were not able to perform baseline measurements because of recurrent headache or muscle injury so they were excluded from the study (Fig 1).

Exercise protocols

Resistance training protocol. Subjects exercised three times weekly on non-consecutive days over a period of 12 weeks (total of 36 sessions). Exercise sessions were performed in small groups of maximum four subjects and were supervised by at least one expert. Session duration was about 35 minutes. After a standard 15-minute warm-up on a cycle ergometer (Technogym, Bike Excite) at self-selected resistance and 70–80 revolutions per minute, three exercises for the lower-limb muscles were performed: the bilateral leg press and straight-legged calf raises (both on the plate-loaded linear leg press, Life Fitness Signature Series) and leg extension (Life Fitness Optima Series). These exercises were chosen because they train the muscles responsible for triple extension in the lower-limb (i.e. knee extensors, hip extensors and plantar flexors), a multi-joint movement that is crucial in daily life activities such as walking and stair climbing. Training variables and progression are shown in Table 1. Subjects were instructed to perform the last set to concentric failure. When they were able to perform more repetitions than the prescribed training zone, the load was increased in the next exercise session. The rest period between sets and exercises was one minute and at least two minutes, respectively. At the end of the exercise session, basic static stretching exercises were performed for the trained muscle groups.

Plyometric exercise protocol. Similar to RT, training sessions (35-minute duration) in PLYO were performed three times weekly on non-consecutive days for 12 weeks in small groups of maximum four subjects and supervised by at least one expert. The warm-up consisted of 10-minutes of cycling, followed by plyometric warm-up exercises: 4 x 10m high knee skips, 4 x 10m sideways skips and 8 consecutive hops with short contact times. The core program similarly consisted of three exercises, using the same muscle groups (triple extension) as

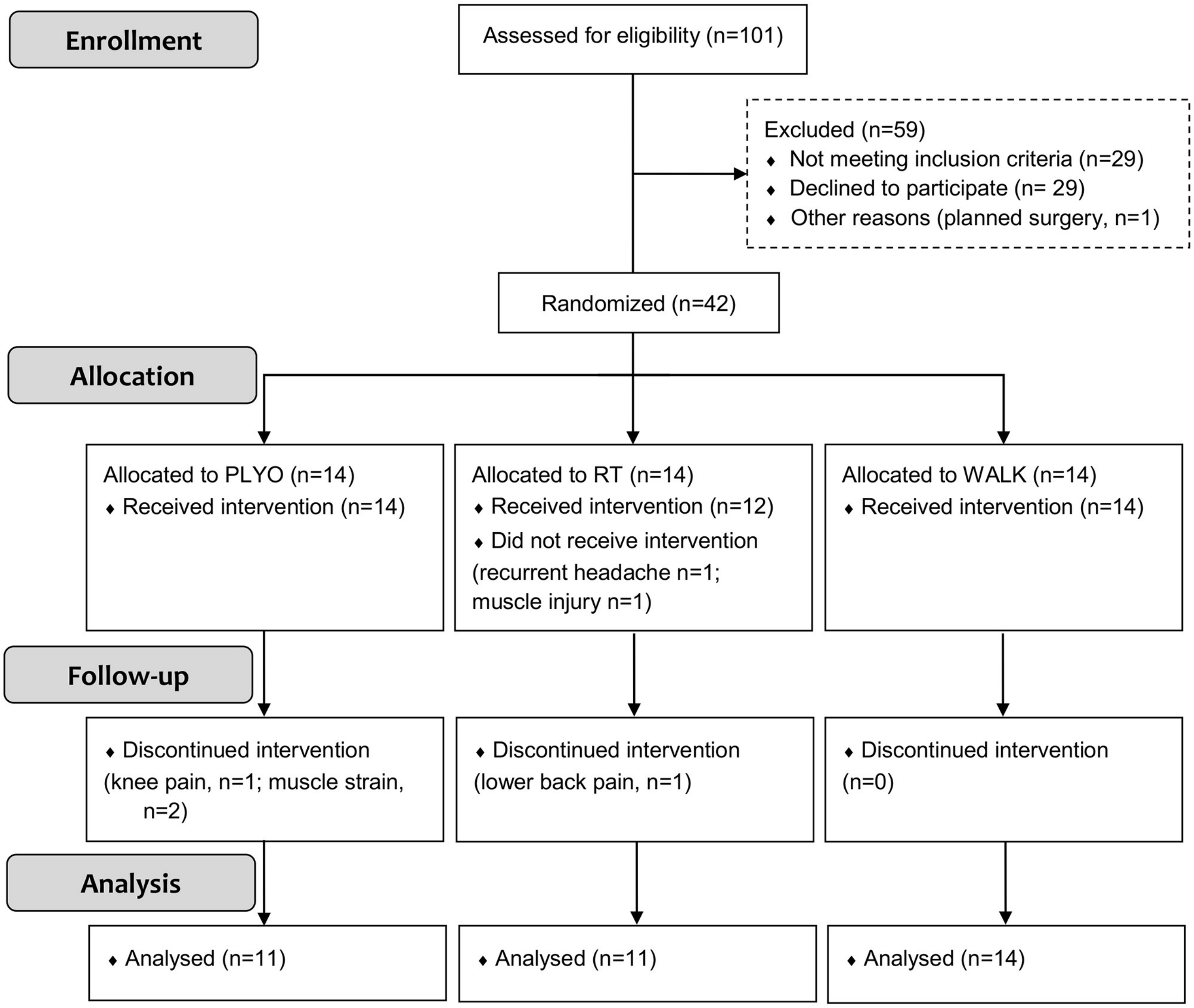

Fig 1. Flowchart of the study. PLYO = plyometric training; RT = resistance training; WALK = walking.

in RT: two unilateral exercises, i.e. forward step-up (jump) and lateral step-up (jump), and one bilateral exercise, i.e. countermovement jump. Exercise intensity progressively increased every three weeks (see Table 1). The rest period between sets and exercises was one minute and at least two minutes, respectively, in all training phases. From week 1–3, exercises were performed at regular speed without jumping, i.e. either stepping up on a box (20-30-40 cm) in forward or lateral direction or performing a squat (body mass with or without weight vest) with bouncing movement at the lowest point. Weight vests (5–10 kg) were only used for the squat exercise prior to progressing to jumping. From week 4–6, all exercises progressed to submaximal jumping with a short eccentric phase before all jumps (i.e. SSC action) (Fig 2) and weight vests were no longer used as the squat exercise progressed to a countermovement jump exercise. Subjects were instructed to have a short 5s-break between the repetitions to ensure proper performance and to avoid fatigue. From week 7–9, subjects were instructed to jump as explosively and as high as possible in every jump, with 5s-breaks between the repetitions. From week 10–12, both step-up jumps were performed consecutively (no breaks between

Table 1. Training variables for the resistance training (RT) and plyometric (PLYO) training program.

	Sets	Repetitions	External load	Break between repetitions	Inter-set rest	Performance
RT (leg press, straight-legged calf raise, leg extension)						
Week 1–4	2	12–15	12-15RM	/	1 min.	3s ecc– 3 sec conc
Week 5–8	3	10–12	10-12RM	/	1 min.	3s ecc– 3 sec conc
Week 9–12	4	8–10	8-10RM	/	1 min.	3s ecc– 3 sec conc
PLYO (forward step-up, lateral step-up, countermovement jump)						
Week 1–3	2	15–20	BM*	/	1 min.	Regular speed, no jump
Week 4–6	3	8–12	BM	5s	1 min.	Fast ecc–submax. jump
Week 7–9	4	6–8	BM	5s	1 min.	Fast ecc–max. jump
Week 10–12	4	6–8	BM	/	1 min.	Fast ecc–max. jump

* Progression of intensity during week 1–3 in PLYO was based on subjects' feelings of muscle fatigue: forward and lateral step-up: increase in step height (to maximum 40 cm); squat: adding weight vest of 5 to 10 kg.

BM = body mass; RM = repetition maximum (last set performed to concentric failure in RT); ecc = eccentric.

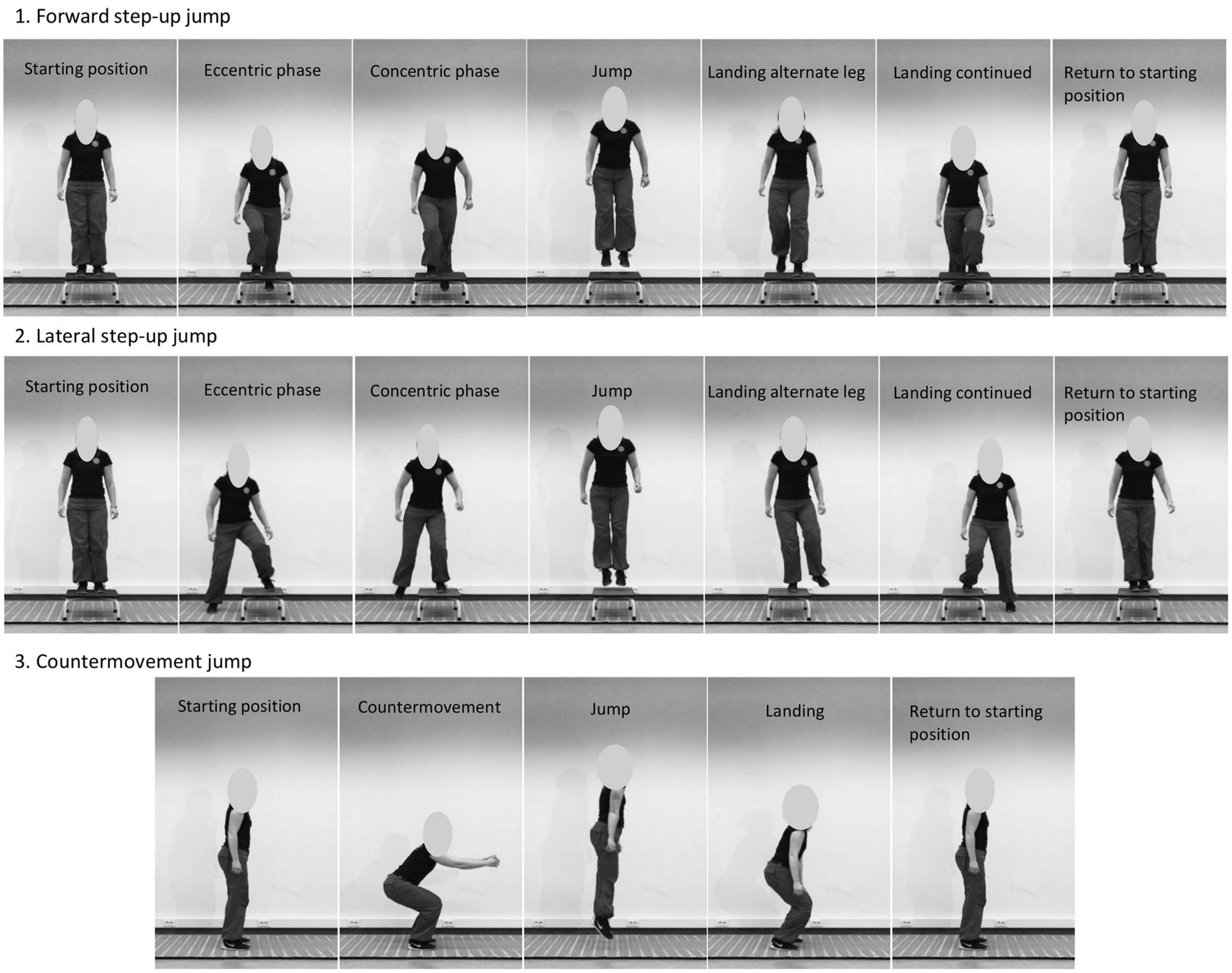

Fig 2. Visual representation of the three plyometric exercises from week 4 to 6.

Table 2. Walking program (prescribed number of steps per day).

level	N	day 1	day 2	day 3	day 4	day 5	day 6	day 7	sessions per week	volume per week
1		1000**	1000**	1000**	1000**	1000**	1000**	1000**	7	7000
2		1000*	1000*	1000*	1000*	1000*	1000*	1000*	7	7000
3		1000	1000	1000	1000	1000	1000	1000	7	7000
4		1500	1000	1500	1000	1500	1000	1500	7	9000
5		1500	1500	1500	1500	1500	1500	1500	7	10500
6		2000	1500	2000	1500	2000	1500	2000	7	12500
7	4	2000	2000	2000	2000	2000	2000	2000	7	14000
8		2500	2000	2500		2500	2000	2500	6	14000
9	1	3000	2000	3000		3000	2000	3000	6	16000
10	2	3000	3000	3000		3000	3000	3000	6	18000
11	2	4000		3000		4000		3000	4	14000
12	5	4000		4000		4000		4000	4	16000
13		4000		4000		4000		4000	4	16000
14		6000		4000		6000		4000	4	20000
15		6000		4000		6000		4000	4	20000
16		8000		4000		6000		4000	4	22000
17		8000		4000		8000		4000	4	24000
18		8000		8000			8000		3	24000
19		8000		6000			10000		3	24000
20		10000		5000			10000		3	25000
21		10000		7000			10000		3	27000
22		10000		10000			10000		3	30000
23		10000		10000			10000		3	30000

*1 resting break allowed,

**2 resting breaks allowed.

N represents the number of subjects with the respective level as starting level of the program. Every week, subjects increased one level until the 12-week intervention was finished.

repetitions, short contact times) and as high as possible. The countermovement jumps were preceded by a two pre-hops, the second one with countermovement to load both ankle and hip/knee joints. In all exercise phases, subjects were allowed to use wall bars for support if needed and were instructed to stop the exercise when feeling unable to perform maximally. At the end of the exercise session, basic static stretching exercises were performed for the trained muscle groups.

Walking protocol

The walking program was adapted from a 10-week progressive individualized walking program, described in detail in previous work [20], by adding two additional weekly schedules to complete a 12-week program (see Table 2). Briefly, subjects were assigned to a starting level of the walking program based on the results of a 6-minute walk test at baseline. Each walking schedule prescribed walks of a certain number of steps on 3–7 days a week, depending on the starting level. Training volume was progressively increased weekly to a maximum of walks of 10000 steps performed three times weekly. Subjects received their personalized walking schedule and a pedometer. They were instructed to walk at a moderate and comfortable pace that increased breathing and heart rate without restricting the ability to talk. Subjects were allowed to perform their walks at home, but were encouraged to engage in group walks that were provided three times weekly at the training facility. All subjects were asked to document their walk sessions, including the amount of steps performed, in a diary. Diaries were reviewed by the research team when subjects joined the on-site group walks (on average once per week) or at least once every two weeks through contact via e-mail or telephone.

Outcome measurements

Feasibility. Feasibility of the exercise protocols were assessed by the following criteria: recruitment rate, exercise session adherence, number of drop-outs, acceptability and adverse events [21]. Recruitment rate was calculated as the number of study subjects divided by the total number of individuals that showed an initial interest in study participation. Adherence rate was calculated as the number of training sessions performed divided by the recommended training frequency (3x/week for 12 weeks, 36 sessions for PLYO and RT; 3-6x/week for 12 weeks, 41–56 sessions for WALK). Given that subjects in WALK could potentially perform more sessions than recommended, which was not the case for PLYO and RT, we additionally corrected the adherence rate in WALK. More specifically, the corrected adherence rate in WALK was calculated by excluding any exercise sessions above the weekly prescribed number of exercise sessions. For example, if a subject performed 5 exercise sessions instead of 4 in a certain week, adherence was corrected to 100% instead of 125% for that week. The number of drop-outs was recorded, including the time of and the reason for drop-out. All subjects were asked to report any adverse events during the intervention.

Acceptability was evaluated through a short questionnaire completed 2-weekly in RT and PLYO only. The questionnaire consisted of 5 questions answered on a 11-point Likert scale (ranging from 0 = 'not at all. . .' to 10 = 'very. . .'): (1) How much did you enjoy the exercises while doing them? (2) How proud are you that you were able to complete these exercises? (3) How confident are you that you will be able to complete these exercises in the next training session? (4) How motivated are you to complete these exercises in the next training session? (5) How feasible do you think that these exercises are for people of your age? The first four questions were employed previously in similar populations [22, 23], while question 5 was added. In week 12, a sixth question was added to the questionnaire: (6) How likely is it that you will engage in similar exercise programs after the end of the intervention? The questionnaire in week 12 was also provided to the subjects of WALK. Internal consistency of the 5-item questionnaire was good, with a Cronbach's α coefficient of 0.79. Therefore, we calculated the mean of the 5 items into one global scale representing acceptability of the intervention program.

Leg press one-repetition maximum. Leg press one-repetition maximum (1-RM) was assessed on the plate loaded linear leg press device (Life Fitness Signature Series). In accordance with previous research [22], the assessment started with a standardized warm-up of 8 repetitions at 50% of the estimated 1-RM, followed by 5 repetitions at 70% of the estimated 1-RM. After this warm-up, single lifts with progressively heavier loads were performed until failure. To standardize, these lifts were performed as concentric lifts only, starting in a knee and hip joint angle of 90° and 65° respectively (full extension = 180°). Rest periods between warm-up sets and between single attempts were 1 to 5 minutes. The heaviest successful lift (in kg) was determined as 1-RM.

Force production and jump performance. A sledge apparatus was used to assess leg-extensor force production and jump performance [24]. The inclination of the sledge was 20° to horizontal and the seat was inclined backwards (130°). A force platform was built in perpendicular to the jumping direction (Fig 3) and a velocity sensor was attached to the seat of the sledge. The force platform consisted of four S style load cells (YZC-516, capacity of 300kg

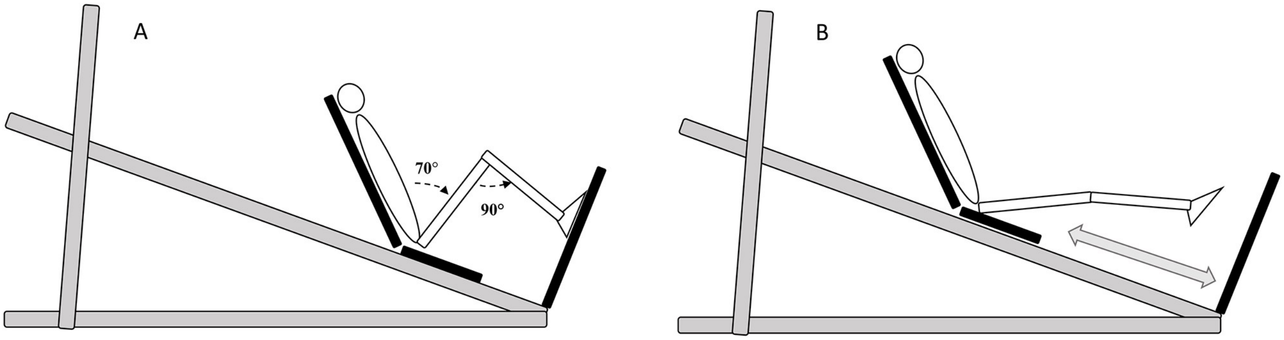

Fig 3. Sledge apparatus with a force platform built in perpendicular to the jumping direction and a speed sensor attached to the seat of the sledge. Position A represents the lowest position in the isometric tests and in the squat jumps, position B represents the flight phase during jumps.

each) that were attached to a custom-built platform. Prior to attachment to the platform, each of the load cells was calibrated with a weight of 155kg. In addition, the platform was calibrated by measuring the unloaded condition prior to each test. The velocity sensor consists of a permanent magnet DC motor riding along with the seat. A wheel running on a rail next to the seat of the sledge drives the motor shaft. That way the motor acts as a generator, producing a current linear with the rotational velocity. Calibration was done by measuring the current while running the wheel at different velocities. Rotational velocity was measured with an electronic pulse counter (HP53131A) counting the pulses of a temporarily added pulse generator on the shaft. The current was measured with a high precision multimeter (HP34405A). Linear regression analysis showed a R^2 of 0.9998 between current and velocity. The shoulders and hips were stabilized with a 4-point seatbelt. Subjects wore standard flat non-cushioning shoes to minimize the cushioning effect during explosive movements.

The test protocol consisted of explosive isometric voluntary contractions, squat jumps (SJ), countermovement jumps (CMJ), and drop jumps (DJ). Prior to all tests, 2 to 4 practice trials were allowed. All tests were performed three times. All data were relayed to a pc via an AD converter (Micro 1401, Cambridge Electronic 180 Design, UK) and recorded using Signal 4.03 software (Cambridge Electronic Design, UK). Data (time, force and velocity) were sampled at 1000Hz and force data were filtered using a fourth-order low-pass Butterworth filter with a 70Hz cut-off frequency. The best trial (maximal force, maximal rate of force development or highest jump) was used for further analyses.

Explosive isometric multi-joint leg-extensor contractions were performed unilaterally with the right leg while the sledge was locked in position. The knee joint angle was set at 90˚ and the hip angle at 70˚. The point of force application was aligned with the head of the fifth metatarsal. Subjects were instructed to push as fast and as hard as possible and maintain their maximum force for approximately 3s. The start of the force-time curve was defined as the first point where the force exceeded body weight by 3% and where the increase in force after 50ms was at least 50N (to ensure the start of a slope). Maximal force (N) was defined as the highest mean 500 ms epoch (rolling average) over the force-time curve. The rate of force development (N/s) was defined as the linear slope of the force-time curve and was measured from the onset of movement until 100 ms (RFD0-100).

In the bilateral SJ, subjects jumped with the sledge as high as possible from a squatting position (90˚ knee flexion and 70˚ hip angle, same position as isometric tests) without countermovement by performing a fast upward movement. If the test leader visually detected a countermovement, the jump was repeated. The start of the jump was defined as the first point where the force exceeded body weight by 3% and the increase in velocity exceeded 0.02 m/s after 300 ms. Any jump preceded by a countermovement, defined as a drop in velocity of at least -0.02 m/s in the 400 ms preceding the start of the jump, was deleted from the analyses.

In the bilateral CMJ, subjects jumped as high as possible by starting from an extended knee joint position and performing a fast downward movement to about 90° knee flexion and 80° hip flexion, immediately followed by a fast upward movement. The start of the jump was defined as the first point where the force dropped below body weight by 3% and the decrease in velocity dropped below -0.02 m/s after 300 ms.

Bilateral drop jumps were performed as three maximal consecutive jumps. Feet were placed lower on the force plate to allow for continuous jumps on the forefoot (hip joint angle of 130° in extended position). Subjects were asked to reverse the downward velocity as soon as possible after landing on the force platform into an upward one. The second and third jump were used as maximal drop jump performances. The start of the second or third jump was defined as the point of landing (time point before force $\geq$ 10N) after the first or second flight phase.

For the jump tests, instantaneous power was calculated as the product of force and velocity. Instantaneous position of the chair was derived from the velocity signal. The following parameters were calculated: (1) jump height (m), i.e. the difference between the maximal value of the position-time curve and the position at take-off (point before force $\leq$ 10N); (2) contraction time (s), i.e. the time from the start of the jump until take-off; (3) eccentric time (s) (for CMJ and DJ), i.e. the time from the start of the jump until velocity > 0; (4) concentric time (s) (for CMJ and DJ), i.e. the time from the end of the eccentric phase until take-off; (5) reactive strength index (RSI, mm/s, for DJ), i.e. jump height (in mm) divided by contraction time; (6) concentric peak power (watt) (Ppeak), i.e. the highest value of the power-time curve in the concentric phase; and (7) concentric rate of power development (RPD, watt/s), i.e. the linear slope of the power-time curve from start until peak in the concentric phase.

All settings of the sledge and subjects' positioning were identical at baseline and post intervention. Comparison of familiarization and baseline measurements showed that coefficients of variation (CV, in %) ranged from 5.7% to 18.9% for all parameters measured with the sledge apparatus. Additional reliability values are reported in S1 and S2 Tables. Acceptable reliability was determined as an intraclass correlation coefficient (ICC) > 0.60 [25] and a CV $< 15\%$ [26].

Functional performance. Functional performance was assessed by a test battery, consisting of a 6-minute walk test (6MWT), a 10m fast walk, a 5-repetition sit-to-stand (5xSTS) test and a 6-step stair ascent (SA) test.

In the 6MWT, subjects were asked to walk a 20m course (back and forth) at a fast but comfortable pace, and the total distance covered (m) was noted. In the 10m fast walk test, subjects were asked to walk as fast as possible. Time (s) was registered through timing gates (Racetime2 Light Radio, Microgate, IT). During the 5xSTS and SA test, data were collected by means of 3D accelerometry positioned at the lower back (DynaPort MoveTest, McRoberts, The Hague, NL). Sampling rate was 100Hz and data were analyzed using commercially available software (DynaPort MoveTest, McRoberts, The Hague, NL). In the 5xSTS, subjects were instructed to perform five sit-to-stand cycles as fast as possible with the arms crossed over the chest. Total STS duration (from start until the fifth standing position) in seconds was calculated from the accelerometer data [27]. Mean power (watt) was calculated for each single sit-to-stand transition and the highest mean power output was used in the analyses. In the SA test, subjects ascended a flight of 6 stairs as fast as possible without using the handrail. Total SA duration (s) and mean power (watt) during the rise phase (defined as vertical velocity > 0.1m/s) of each single step were calculated. The highest mean power output was used in the analyses. For more detailed information on the calculation of power by means of 3D accelerometry, see previous work [28].

The 6MWT was performed once and all other functional tests twice. The best result was used in the analyses.

Statistical analyses

One-way analysis of variance with Bonferroni post hoc testing was used to test for baseline differences and for differences in exercise adherence between groups. Non-parametric statistics were used for the questionnaire variables (ordinal scales). Between-group differences on the acceptability questionnaire were assessed with Mann-Whitney U (two groups) or Kruskal-Wallis tests (three groups) at all points in time. Time-effects were assessed with Friedman tests. Fisher's exact test was used to check for differences in the number of drop-outs between groups.

To assess between-group differences in changes over time for the performance variables, linear mixed-model analysis with an unstructured covariance structure was used, with time as repeated factor and group as fixed factor. Post hoc analyses were conducted for within-group changes and to determine which groups differed in changes. Because of the risk for type II errors, these post hoc analyses were performed when the time effect or the time by group interaction effect showed at least a trend ($p < 0.1$) towards significance. In order to test the normality assumption for multilevel regression models, we checked for all models whether the residuals were normally distributed by means of Shapiro-Wilk tests. If a dependent variable was non-normally distributed, a log or square-root transformation was conducted. Only when these transformations did not result in normality, non-parametric tests were used as alternative. In that case, time effects from baseline to post intervention were analyzed with Friedman tests, and within-group changes from baseline to post were analyzed with Wilcoxon-signed rank tests. Percent changes from baseline to post were calculated and then used in Kruskal–Wallis tests to determine differences in changes between groups, with Mann-Whitney U tests as post hoc tests. The following parameters were not normally distributed: SA duration and power (non-parametric), STS duration (log transformation), SJ jump height and contraction time (non-parametric), CMJ concentric time (log transformation), DJ RPD (log transformation).

Cohen's *d* effect sizes for between-group differences in percent changes from baseline to post-intervention were calculated. This was done for the variables that showed a significant difference in change between groups. Thresholds 0.20, 0.50 and 0.80 were used to interpret small, medium and large effect sizes [29].

All statistical tests were executed with SPSS software version 25 (SPSS Inc., Chicago, IL). Level of significance was set at $p < 0.05$.

Results

Table 3 shows the baseline characteristics of the subjects in each group.

Feasibility

In total, 101 older men were assessed for eligibility. Twenty-nine men declined to participate and thirty-two were excluded (for reasons, see flowchart in Fig 1). Recruitment rate was 39.6%.

Of the 40 subjects that started the study, three in PLYO (one in week 5, two in week 6) and one in RT (in week 11) dropped out. The number of drop-outs was not different between groups (p = 0.190). Reasons for drop-out were knee pain (PLYO, n = 1), muscle strain in the m. gastrocnemius during the forward or sideways step-up exercise (PLYO, n = 2) and lower back pain (RT, n = 1). Other minor adverse effects included knee pain (n = 4 PLYO, n = 1 RT),

Table 3. Means ± SD for baseline characteristics of the subjects.

Characteristic	RT (N = 12)	PLYO (N = 14)	WALK (N = 14)	p-value
Age (years)	68.2 ± 2.7	69.6 ± 3.3	70.5 ± 5.1	0.334
Body height (cm)	170.1 ± 5.4	175.6 ± 5.8	177.6 ± 6.5*	0.008
Body mass (kg)	81.1 ± 9.9	86.1 ± 11.8	80.2 ± 10.2	0.305
BMI (kg/m^2)	28.0 ± 2.8	27.9 ± 3.5	25.4 ± 2.8	0.060

RT = resistance training; PLYO = plyometric training; WALK = walking.

p-values: results of one-way analysis of variance.

*Significant difference with RT ($p < 0.05$).

mild muscle soreness (n = 5 PLYO, n = 4 RT), pain in glutes (n = 1 RT, n = 1 WALK), pain in feet (n = 1 WALK). Three subjects in PLYO, six in RT and twelve in WALK did not report any side effects over the 12-week period.

Exercise session adherence was higher in WALK (106.7 ± 18.6%) than in PLYO (79.8 ± 23.3%, p = 0.001) and RT (91.9 ± 8.4%, p = 0.137). However, when the adherence rate in WALK was corrected by excluding any exercise sessions above the weekly prescribed number, adherence in WALK dropped to 93.1 ± 7.8 and was no longer different from PLYO or WALK (p = 0.052). In addition, when subjects who dropped out were deleted from the analysis, adherence increased to 91.2 ± 4.4% in PLYO and 93.9 ± 4.8% in RT. The number of on-site group walks attended was 12.4 ± 9.6 (ranging from 1 to 29), which corresponds to once per week on average. Group size during these walks was 4 ± 2 subjects.

Acceptability of the exercise program was very high in both groups, with no difference between groups and no change over time (all $p > 0.05$). All subjects indicated that they were likely to engage in similar exercise programs in the future, apart from two subjects in RT, who gave a neutral answer (5/10) (Table 4).

Performance outcomes

Analyses on baseline differences revealed that PLYO showed higher STS power than RT, PLYO produced more concentric power in SJ and CMJ than WALK, and RT had shorter concentric times during CMJ than WALK. Given that PLYO improved most on power production

Table 4. Means ± SD for questionnaire variables on acceptability of the exercise program.

Variables	RT	PLYO	WALK	p-value
Acceptability of the program				
Week 2	8.2 ± 1.1	8.5 ± 0.7		0.379
Week 4	8.1 ± 1.3	8.2 ± 1.0		0.935
Week 6	8.2 ± 1.3	8.3 ± 1.0		0.853
Week 8	8.2 ± 1.4	8.6 ± 0.6		0.510
Week 10	8.3 ± 1.1	8.8 ± 0.6		0.289
Week 12	8.4 ± 0.8	8.5 ± 0.6	8.6 ± 1.0	0.729
Likelihood of participation in similar exercise programs in the future				
Week 12	8.0 ± 1.8	9.1 ± 1.0	8.8 ± 1.7	0.305

RT = resistance training; PLYO = plyometric training; WALK = walking.

p-values: results of Mann-Whitney U tests (two groups) or Kruskal-Wallis tests (three groups).

Table 5. Estimated means and SE at baseline (pre-) and posttest and % change (±SD) for leg press one repetition maximum, leg-extensor maximal isometric force and rate of force development in the three intervention groups.

		RT			PLYO			WALK			Statistics	
		Mean	**SE**	**%**	**Mean**	**SE**	**%**	**Mean**	**SE**	**%**	**Time**	**Time x group**
1-RM (kg)	Pre	194.2	14.6		175.7	13.6		162.1	13.6			
	Post	244.3	15.1	25.0 ± 10.0*†	211.8	14.2	23.0 ± 13.6*†	161.7	14.1	2.9 ± 13.7	**F (1, 31.1) = 72.0; $p < 0.001$**	**F (2, 31.1) = 20.5; $p < 0.001$**
MVC (N)	Pre	815.7	32.8		787.9	30.4		725.3	30.4			
	Post	862.1	31.7	6.8 ± 10.7*	807.3	30.2	2.9 ± 7.1	727.7	29.3	-0.2 ± 9.7	F (1, 32.9) = 4.0; p = 0.053	F (2, 32.9) = 1.3; p = 0.285
RFD0-100 (N/s)	Pre	2982.2	282.0		2638.7	261.1		2632.8	261.1			
	Post	2717.0	254.2	-6.1 ± 25.0	2887.0	245.7	4.3 ± 15.7	2507.0	234.6	-4.0 ± 24.5	F (1, 33.3) = 1.1; p = 0.301	F (2, 33.3) = 1.0; p = 0.374

Statistics of Linear Mixed Models analyses.

*Significant change from pre to post ($p < 0.05$);

†Significant difference with WALK ($p < 0.05$).

PLYO = plyometric training, RT = resistance training, WALK = walking, MVC = maximal voluntary contraction, RFD = rate of force development.

(see later) and that the comparison of RT and WALK was not the focus of this research, no corrections for these baseline differences were conducted.

Jump data from one subject in PLYO were deleted due to not being able to perform the tests in the correct manner and all performance data from one in WALK due to the influence of illness during testing.

1-RM, force production and jump performance. Leg press 1-RM improved similarly in RT and PLYO and significantly more than in WALK ($p < 0.001$, *d* = 1.84 (RT vs WALK) and *d* = 1.47 (PLYO vs WALK)) (Table 5). The explosive isometric leg-extensor test did not show any time-by-group interaction effects. However, RT was the only group that increased maximal force (p = 0.028). There was no within-group change for RFD (Table 5). PLYO improved the most on jump performance. For an overview of the results, see S3–S5 Tables. With regard to SJ, PLYO was the only group that improved jump height (p = 0.017) (Fig 4A), reduced contraction time (p = 0.059) and exerted more power (both Ppeak and RPD) post-intervention ($p < 0.05$) (Fig 4B–4D). This improvement in contraction time and RPD was significantly different from WALK (p = 0.026, *d* = 0.98 and p = 0.014, *d* = 1.06 respectively). Similar results were found for CMJ. In PLYO, overall contraction time reduced because of a reduction in eccentric time, jump height increased and more concentric power was produced ($p < 0.05$). Overall contraction time and eccentric time improved significantly more in PLYO than in both RT and WALK (all $p < 0.05$, *d* ranged from 1.05 to 1.44), while jump height improved significantly more in PLYO than in RT (p = 0.030, *d* = 1.16) (Fig 4A–4D). With regard to DJ, PLYO was the only group that significantly increased jump height (Fig 4A) and RSI, and these increases were greater than in WALK (p = 0.017, *d* = 0.57 and p = 0.006, *d* = 0.98 respectively). In addition, PLYO was able to produce more power (Ppeak) in the concentric phase post-intervention. This gain in Ppeak was significantly different from both RT (p = 0.014, *d* = 1.14) and WALK (p = 0.001, *d* = 1.13) (Fig 4D).

Functional performance. Both 10m fast walk and 6MWD improved similarly in all groups, while STS duration did not change. No time-by-group interaction effect was found for STS power (p = 0.405), although only RT (p = 0.018) and PLYO (p = 0.011) showed a within-group gain. Interestingly, stair-climbing performance, represented by stair ascent duration

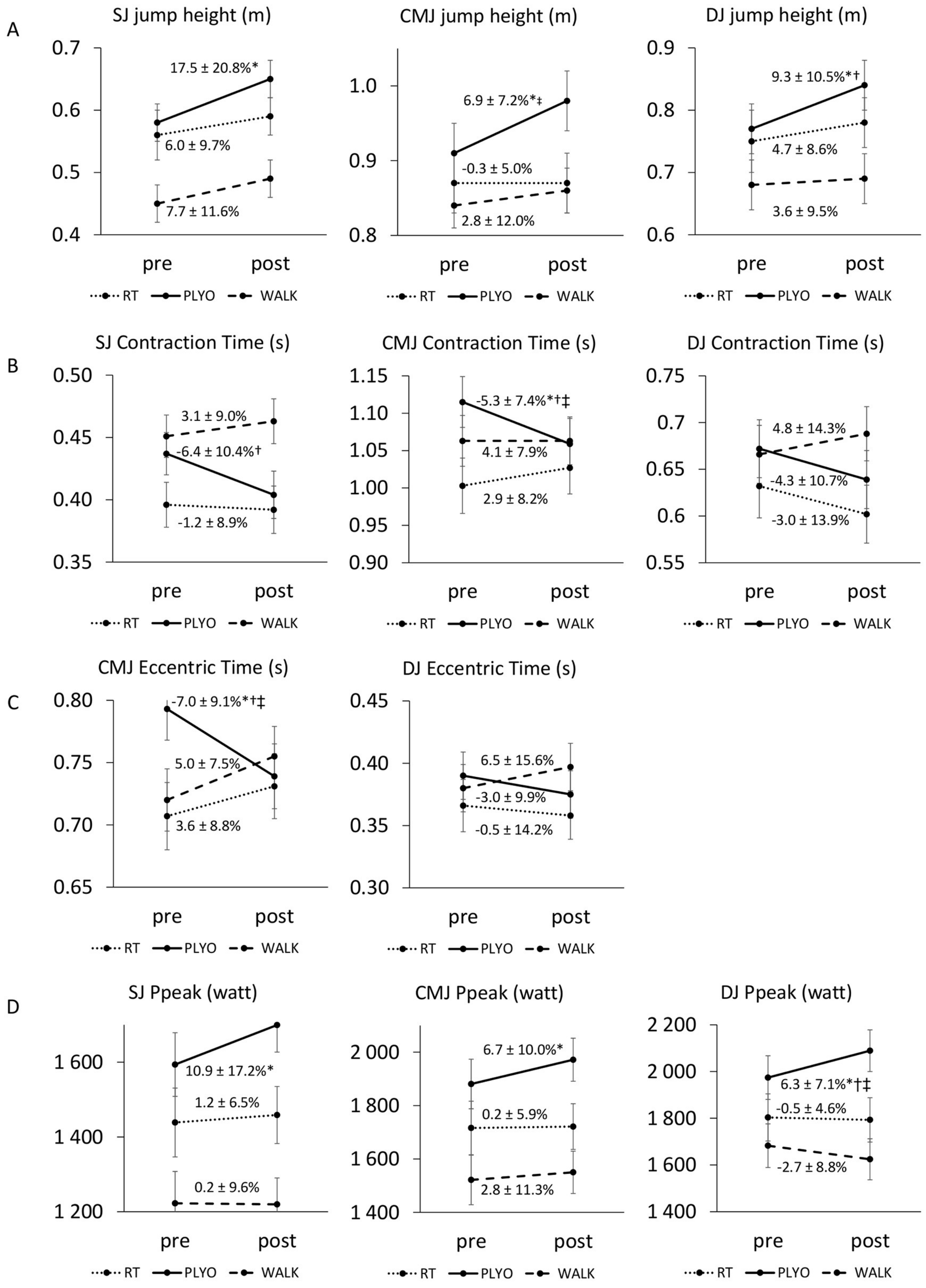

Fig 4. Estimated means and SE for jump height (A), contraction time (B), eccentric time (C), concentric peak power (Ppeak) (D) at baseline and post 12-weeks of plyometric training (PLYO), resistance training (RT) and walking (WALK) in squat jump (SJ), countermovement jump (CMJ) and drop jump (DJ). *Significant change from pre to post ($p < 0.05$); †Significant difference with WALK ($p < 0.05$); ‡ Significant difference with RT ($p < 0.05$).

Table 6. Estimated means and SE at baseline (pre-) and posttest and % change (±SD) for functional performance in the three intervention groups.

		RT			**PLYO**			**WALK**			**Statistics**	
		Mean	**SE**	**%**	**Mean**	**SE**	**%**	**Mean**	**SE**	**%**	**Time**	**Time x group**
10m fast walk (s)	Pre	4.50	0.23		4.41	0.21		4.42	0.21			
	Post	4.23	0.21	-5.3 ± 9.2*	4.12	0.20	-5.5 ± 8.6*	4.12	0.19	-6.5 ± 7.2*	**F (1, 34.3) = 23.2; p < 0.001**	F (2, 34.3) = 0.02; p = 0.983
6MWD (m)	Pre	579.6	21.9		600.6	20.2		604.2	20.2			
	Post	618.9	23.9	6.7 ± 6.5*	635.0	22.4	6.1 ± 4.9*	631.2	22.1	4.4 ± 3.8*	**F (1, 32.0) = 44.2; p < 0.001**	F (2, 32.0) = 0.5; p = 0.596
SA duration (s)	Pre	1.64	0.13		1.95	0.12		1.96	0.12			
	Post	1.67	0.10	2.1 ± 9.5	1.71	0.09	-10.1 ± 12.1*‡	1.85	0.09	-4.8 ± 7.6*	**χ^2 (1) = 4.2; p = 0.040** (np)	**χ^2 (2) = 6.0; p = 0.049** (np)
SA power (watt)	Pre	846.4	66.7		854.9	61.8		753.4	61.8			
	Post	825.7	64.2	-1.5 ± 9.8	961.9	60.7	18.0 ± 23.8*‡	774.1	59.3	2.0 ± 15.0	χ^2 (1) = 0.1; p = 0.739 (np)	χ^2 (2) = 4.8; p = 0.089 (np)
STS duration (s)	Pre	8.44	0.42		8.06	0.41		9.25	0.38			
	Post	8.90	0.42	5.4 ± 10.8	8.42	0.40	4.8 ± 7.5	9.38	0.40	1.6 ± 12.6	**F (1, 31.9) = 4.6; p = 0.040**	F (1, 31.9) = 0.5; p = 0.601
STS power (watt)	Pre	291.7	18.7		370.2	17.3		316.7	17.3			
	Post	329.6	18.3	14.6 ± 19.1*	410.6	17.7	12.9 ± 9.2*	331.9	16.9	5.8 ± 16.3	**F (1, 32.2) = 13.3; p = 0.001**	F (1, 32.2) = 0.9; p = 0.405

Statistics of Linear Mixed Models analyses; stair ascent duration and power were not normally distributed and non-parametric (np) tests were performed and reported for these variables; STS duration was not normally distributed and log transformed for the analyses. For easier interpretation, non-transformed data means are reported for all variables.

[*] Significant change from pre to post (p < 0.05)

[‡] Significant difference with RT (p < 0.05)

PLYO = plyometric training, RT = resistance training, WALK = walking, 6MWD = 6-minute walk distance, SA = stair ascent, STS = sit-to-stand.

and power, improved significantly more in PLYO than in RT (p < 0.05, *d* = 1.12 and *d* = 1.07 respectively) (Table 6).

Discussion

This study developed and implemented a 12-week age-adapted and progressive plyometric exercise program for community-dwelling older men and compared its effects to traditional resistance training and walking. Primary outcomes were muscle strength, muscle power, jump performance and functional capacity. In addition, the feasibility of the plyometric training program was investigated. The results show that (1) PLYO is more effective than RT and WALK for improving muscle power (Ppeak and RPD) and jump performance (jump height and contraction time), (2) PLYO is equally effective compared to RT for improving muscle strength (1-RM), (3) all interventions equally improve walking performance, but PLYO seems favorable for stair climbing performance, and (4) older men seem to accept the PLYO program to a similar extent as either the RT or WALK program, although risk for injuries might be greater in PLYO.

In line with previous findings [24, 30, 31] and the principle of training specificity, plyometric exercise resulted in more optimal jump performance than traditional resistance training or walking. In all jumps, PLYO improved jump height post-intervention (6.9–17.5%) and this coincided with an increase in concentric power production (both Ppeak and RPD) and a decrease in (eccentric) contraction time. In other words, PLYO was able to jump higher and more powerfully, while needing less time to jump. We should acknowledge that ICC appeared poor for the duration of the eccentric phase in the countermovement jump (0.48), even though CV% was good (9.7%). However, we should keep in mind that our reliability values are based on a comparison between familiarization and baseline measurements. In subjects that are new to jump tests, we can expect an improvement in jump strategy, i.e. a faster eccentric time and/or faster transition from eccentric to concentric movement, from familiarization to baseline. The ICC quantifies the between-subject variance in relation to the total variance, which also contains the variance within subjects [25]. As the variance within subjects will increase in case of a learning effect from familiarization to baseline, a lower ICC value will be obtained, especially when between-subject differences in means are small. This result should therefore be interpreted with caution and replication in future studies is warranted.

Although we can only speculate on the underlying mechanisms behind improved jumping performance, Hoffren-Mikkola et al. [30] demonstrated that improvements in jumping performance after plyometric exercise were achieved with shorter operating lengths of the m. gastrocnemius and, therefore, increased fascicle stiffness and improved tendon utilization [30]. In addition, Piirainen et al. [24] reported no increase in muscle activity of the triceps surae during explosive isometric tests after plyometric exercise, suggesting that mechanisms other than improved voluntary drive, such as increased utilization of elastic energy and/or stretch reflex activity, may be responsible for enhanced jump performance [24]. With regard to muscle architectural changes, 6 weeks of plyometric training has been shown to result in increased muscle thickness, fascicle length and pennation angle, which likely contributed to the observed changes in power [32].

In the current study, we did not observe a significant increase in leg-extensor RFD after either plyometric or resistance exercise. Such null findings should be set in context with respect to the body position specificity of the test and training exercises: a significant increase in RFD may be more likely if the test is more body position-specific or if training is performed with the intent to rapidly contract muscles [33]. Although RT included dynamic leg press training and a multi-joint isometric leg press set-up was used to assess RFD, training was performed bilaterally while testing unilaterally. In contrast, part of the training in PLYO was performed unilaterally, but in different body positions regarding knee-joint angle. We should however note that the percent change does seem to point out an exercise-induced improvement in PLYO, but this improvement was not significant because of a large variability in training responses and small sample size. Coefficient of variation (18.9%) was high, but that is quite typical in early phase RFD, even in young adults [34]. A longer time interval of 0–200 ms was considered (CV of 9.9%), but as it did not lead to any different conclusions, we decided not to include this information in the results section for brevity. In line with the results on RFD, no time-by-group interaction effect was found for isometric MVC, although RT was the only group that improved.

To our knowledge, limited reports exist on changes in 1-RM after plyometric exercise in older adults. Bolam et al. (2016) reported no increase in leg press 1-RM after 9 months of plyometric exercises performed at high (40–80 jumps) or moderate (20–40 jumps) dose 4x/weekly in middle-aged and older men. However, these findings might have been related to the limited adherence rates (i.e. 53–65%) [35]. On the contrary, Correa et al. [36] reported similar gains in knee-extension 1-RM after 6 weeks of plyometric compared to traditional resistance exercise. Both groups followed the same 6-week program of generalized strength training prior to division in two exercise groups. Gains in 1-RM from week 6 to 12 were similar (+20–21%) between groups [36]. This is in line with our findings, showing that short-term plyometric exercise is able to induce similar gains in leg press 1-RM as traditional resistance exercise. This gain in 1-RM might be linked to muscle architectural changes (i.e. increased muscle thickness and pennation angle) and improved muscle recruitment, as found previously after 6 weeks of plyometric training in older men [32]. Although the long-term effects should be investigated, these results show that PLYO is beneficial over RT for improving muscle power and jump performance without compromising gains in strength, at least when older individuals begin a high-intensity (resistance or plyometric) training program.

Improved (rapid) power production in PLYO was hypothesized to result in a greater gain in functional capacity compared to RT and WALK [12, 13, 36]. However, this hypothesis was only partly confirmed. Walking performance (10m fast walk and 6MWD) equally improved in RT, PLYO and WALK, and to a similar extent (4.4–6.7%) as previously reported after resistance exercise [22] or walking [20]. 5xSTS duration did not change significantly in either of the groups. This test was probably not challenging or training-specific (i.e. no SSC) enough in our sample of older men, as all subjects were classified as not mobility-limited (6MWD > 400m). Although 5xSTS duration did not change, the highest mean power output in a single sit-to-stand transition improved in both RT and PLYO. This result was not influenced by gains in body mass, as there was no correlation between gains in power and in body mass nor a significant gain in body mass post-intervention. Interestingly, PLYO showed a greater improvement in stair climbing performance than RT. This result was not surprising, given that PLYO improved power production in the leg-extensor (i.e. knee- and hip-extensor) muscles, which play a dominant role in developing the power needed to progress from one step to the next during stair ascent [37, 38]. In addition, the forward step-up (jump) exercises in PLYO are mechanically very similar to stair climbing, enhancing the potential of inducing training-specific adaptations. As stair climbing is one of the most demanding functional tasks in older adults, improvements are crucial in maintaining independence.

It should be noted that some of our key findings of between-group differences (i.e. results on stair ascent and squat jump) are based on non-parametric analyses. However, in most cases, non-parametric tests are considered to have lesser statistical power than parametric analyses, meaning that the latter are more likely to detect an effect when it actually exists. Parametric analyses of our non-normally distributed parameters resulted in the same conclusions, which indicates towards the robustness of our results.

A secondary aim of this study was to assess the feasibility of plyometric exercise in healthy older men. Recruitment rate was 39.6%, which is acceptable and in line with recruitment rates of (resistance) exercise trials in similar populations [22, 23, 39]. Exercise adherence was very high (>80%) in all individuals, except in the four dropouts, independent of training group. Subjects in WALK appeared to have higher adherence rates than the other groups, with some individuals even exceeding the recommended amount of training sessions. This might have been caused by the self-report in that group, with subjects noting down every daily life walk instead of solely the walks as part of the training program. When adherence rates in WALK were corrected by excluding any sessions above the prescribed training frequency, adherence was similar in all groups.

Acceptability of the exercise program was very high in both PLYO and RT, demonstrating that plyometric exercise is at least perceived by healthy older men as being feasible (and to a similar extent as resistance exercise). All subjects in PLYO indicated that they were likely to participate in similar exercise programs in the future. However, as shown previously, this intention to participate in future programs appears to have limited predictive value for actual long-term exercise behavior [40]. Also, inevitable in research with volunteers, a self-selection bias might have occurred, by only including highly motivated subjects in the intervention. In addition, subjects might have tended to give socially desirable answers to the questionnaires. Acceptability should therefore be investigated in larger study samples to confirm these findings.

Although our subjects seemed to enjoy the plyometric exercise program and considered it to be feasible, we cannot ignore the adverse events. Two in particular need further attention: knee pain and muscle strain of the m. gastrocnemius. Five out of 14 subjects in PLYO reported some kind of knee pain during the exercise program. In one subject, this was related to a non-treated knee injury in the past and not specifically caused by the training program. In three subjects, knee pain was only reported once in the beginning weeks of the exercise program and disappeared later on. This might have been related to the height of the box (up to 30–40 cm) during step-up exercises, which may have caused an unfavorable knee joint angle (more flexed), or poor technique (e.g. inversion at the knee, corrected when inspected by supervisors). Height of the box was reduced once subjects progressed to jumping (20–30 cm) and knee pain was no longer reported. In only one subject, knee pain was sufficiently severe to cause a drop-out. It is not clear whether this pain was caused by the stepping exercises as such or by the impact of the landing phase during jumping. A more severe adverse event, causing two subjects to drop out, was a strain in the m. gastrocnemius. In both cases, the injury occurred in the second phase of the plyometric program (week 5 or 6), in which slight, submaximal jumps were performed to familiarize with the exercises. The injuries did not seem to be caused by insufficient warm-up, nor by excessive fatigue, as they occurred in set 3 or set 6 (out of the total exercise volume of 9 pre-programmed sets). Importantly, the strain occurred during the concentric phase of either the forward or the sideways step-up jump. These are both unilateral exercises in which the calf muscles are part of the prime movers. Considering that both subjects could be classified as obese (BMI of 30.5 and 33.45 kg/m^2 respectively), the calf muscles had to comply with high absolute loading forces. Excessive body weight in combination with a likely decrease in gastrocnemius fascicle length [15] and Achilles tendon stiffness [15, 41] with ageing inevitably increases the risk of muscle injuries during such exercises.

With this in mind, we should reflect on the proper design and exercises to be included in a plyometric exercise program for older adults. To be able to compare the effects of a plyometric-only protocol to traditional resistance exercise, we did not include a preparation phase of resistance training before introducing jumps. It can be argued that a proper periodization design is warranted, starting with traditional resistance exercise aimed to induce hypertrophy and maximal strength gains, before progressing to explosive-type of exercises. However, we did progress slowly from slow-speed exercises without jumping over submaximal jumps to maximal jumps. The three plyometric exercises are all considered to be low-intensity drills [42]. Countermovement jumps have been used multiple times in exercise programs for healthy older adults without any adverse events [43, 44]. Step-up exercises were chosen considering their beneficial effects on muscle strength and functional performance [23] and their resemblance to stair climbing, i.e. one of the most demanding functional tasks for older adults. In addition, the lateral step-up jumps were already used in older women without any adverse events [36]. Subjects in our study were recommended to use wall bars if necessary (both to reduce intensity and the balance component), but only one subject chose to do so. Training volume per session, in plyometrics typically expressed as total number of foot contacts, was limited to recommended guidelines for beginners (i.e. 80 to 100) [42] and even decreased with increasing training intensity to keep injury risk to the minimum. Adequate recovery, i.e. between repetitions (5 s, consecutive jumps only in last training phase), between sets (1 min.), between exercises (at least 2 min.) and between training sessions (48 – 72h), was provided [42]. Although we did not measure markers of muscle damage, subjects in PLYO did not report excessive muscle soreness after training. However, given that plyometric exercise interventions in older adults remain scarce in literature, more research is urgently needed to set the appropriate training dose (volume, intensity, frequency, duration) [18] and exercises for optimizing gains in power and functional capacity and for minimizing injury risk. In addition, we should acknowledge that it is challenging to quantify exercise intensity (i.e. both mechanical load and neuromuscular demand) [45] and fatigue during plyometric exercises, although it would definitely be of added value to optimize training dose.

Based on the findings of the current study, people might question whether it really is worth doing more stressful plyometric jump training as opposed to regular walking. Aside from training-specific gains in strength, power, jump and stair climbing performance, more 'neutral' functional performance tests (i.e. walking and STS) do not seem to improve as robustly. However, the following aspects need to be considered. Firstly, it should be noted that the relationship between muscle power and functional performance is curvilinear [5]. Hence, for low levels of muscle power, the improvement in power leads to a substantial improvement in functional performance. However, above a certain level of baseline muscle power, further increases in power do not lead to further increases in the parameters usually assessed to register functional performance (e.g. STS ability). Our subjects were well-functioning older men with relatively high baseline levels of muscle power, which might explain the null findings with regard to STS ability. Notwithstanding, any improvement in muscle power in well-functioning older adults should be recognized as important, even if it does not result in further improvements in functional performance. Higher levels of muscle power can at least postpone the drop below the disability threshold. Secondly, STS ability is subject to a ceiling effect in well-functioning adults, as noted previously [28]. Instead of questioning the beneficial effect of plyometric exercise on functional performance, it may be necessary to question whether traditional functional performance tests are sensitive enough to capture changes in well-functioning older adults. Jumping is a more sensitive measure of power performance than chair rising [46]. Even though jumping is not an activity that older adults do on a regular basis, the inability to jump is related to poorer self-reported health, more comorbidities, worse cognitive functioning, more limitations in daily life activities and higher fall incidence [47]. Thirdly, intensity is not something to be feared, as discussed by Hunter et al. [48] and commented by Gentil et al. [49]. Ageing is associated with both a decline in type II fiber size and in the ability to activate these fibers [50, 51], resulting in decreased strength and power production. To train these type II fibers, high efforts are needed, either through using relatively high loads (as in RT), performing exercises at high velocity (as in PLYO) or training to momentary failure [49]. According to Henneman's size principle, walking at comfortable pace is not sufficient to target the type II fibers and is therefore incapable of countering this age-related decline.

Considering the cost-benefit of our plyometric exercise program, we cannot claim that plyometric training is better than concentric-only machine-based power training, as previously performed by several research groups [13, 52]. While concentric-only machine-based power training or alternative plyometric training with machines designed to limit the impact of the landing phase (e.g. [24, 32]) might be considered a safer modality for older adults, adverse events in the current study did not seem to be the result of high impact during landing. Because of differences in the design of the traditional resistance exercise protocol as reference, in the study population and in measurement outcomes, it is also difficult to compare our results to previous findings in machine-based power training [13, 52]. What we can say is that both plyometric and machine-based power training will improve power and functional performance in older adults previously unaccustomed to systematic training, although the mechanisms behind these improvements are very likely training-mode specific. While neuromuscular improvements due to machine-based power training are mainly attributable to improved voluntary neural drive, plyometric training seems to result in a better utilization of the advantaged provided by the SCC [24]. Both aspects are vulnerable to age-related deterioration [15, 16, 51] and deserve attention in exercise programs for older adults.

To conclude, plyometric exercises are beneficial over traditional resistance training for improving muscle power, jump and stair climbing performance without compromising gains in muscle strength. This form of training seems feasible in older men, although proper supervision is warranted and caution is advised when applying unilateral exercise drills because of a potential increase in the risk for calf muscle injuries. Box heights of 20–30 cm are feasible for step-up jumps in older men, but higher heights might result in more reports of knee pain because of unfavorable knee-joint angles. Given the beneficial performance-related effects of plyometric exercise in older adults, future research should focus on optimizing the training dose, exercise drills and periodization schemes.

Supporting information

S1 Table. Reliability values for jump parameters by comparing familiarization and baseline measurements.
(DOC)

S2 Table. Reliability values for the explosive isometric leg-extensor test by comparing familiarization and baseline measurements.
(DOC)

S3 Table. Estimated means and SE at baseline (pre-) and post-intervention and % change (±SD) for squat jump in the three intervention groups.
(DOC)

S4 Table. Estimated means and SE at baseline (pre-) and posttest and % change (±SD) for countermovement jump (CMJ) in the three intervention groups.
(DOC)

S5 Table. Estimated means and SE at baseline (pre-) and posttest and % change (±SD) for drop jump in the three intervention groups.
(DOC)

Author Contributions

Conceptualization: Evelien Van Roie, Simon Walker, Benedicte Vanwanseele, Christophe Delecluse.

Data curation: Evelien Van Roie, Stijn Van Driessche, Tijs Delabastita.

Formal analysis: Evelien Van Roie.

Funding acquisition: Evelien Van Roie, Christophe Delecluse.

Investigation: Evelien Van Roie, Tijs Delabastita.

Methodology: Evelien Van Roie, Simon Walker, Christophe Delecluse.

Project administration: Evelien Van Roie.

Resources: Christophe Delecluse.

Supervision: Simon Walker, Benedicte Vanwanseele, Christophe Delecluse.

Writing – original draft: Evelien Van Roie.

Writing – review & editing: Simon Walker, Stijn Van Driessche, Tijs Delabastita, Benedicte Vanwanseele, Christophe Delecluse.

References

1. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R. Aging of skeletal muscle: a 12-yr longitudinal study. J Appl Physiol. 2000; 88(4):1321–6. https://doi.org/10.1152/jappl.2000.88.4.1321 PMID: 10749826
2. Charlier R, Knaeps S, Mertens E, Van Roie E, Delecluse C, Lefevre J, et al. Age-related decline in muscle mass and muscle function in Flemish Caucasians: a ten-year follow-up. Age. 2016; 38(2):36. https://doi.org/10.1007/s11357-016-9900-7 PMID: 26961694
3. Van Roie E, Van Driessche S, Inglis AJ, Thomis M, Delecluse C. Rate of power development of the knee extensors across the adult life span: A cross-sectional study in 1387 Flemish Caucasians. Exp Gerontol. 2018; 110:260–6. https://doi.org/10.1016/j.exger.2018.06.021 PMID: 29953950
4. Izquierdo M, Aguado X, Gonzalez R, Lopez JL, Hakkinen K. Maximal and explosive force production capacity and balance performance in men of different ages. Eur J Appl Physiol Occup Physiol. 1999; 79(3):260–7. https://doi.org/10.1007/s004210050504 PMID: 10048631
5. Bean JF, Kiely DK, Herman S, Leveille SG, Mizer K, Frontera WR, et al. The relationship between leg power and physical performance in mobility-limited older people. J Am Geriatr Soc. 2002; 50(3):461–7. https://doi.org/10.1046/j.1532-5415.2002.50111.x PMID: 11943041
6. Pijnappels M, van der Burg PJ, Reeves ND, van Dieen JH. Identification of elderly fallers by muscle strength measures. Eur J Appl Physiol. 2008; 102(5):585–92. https://doi.org/10.1007/s00421-007-0613-6 PMID: 18071745
7. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc. 2011; 43(7):1334–59. https://doi.org/10.1249/MSS.0b013e318213fefb PMID: 21694556
8. Walker S, Peltonen H, Hakkinen K. Medium-intensity, high-volume "hypertrophic" resistance training did not induce improvements in rapid force production in healthy older men. Age (Dordr). 2015; 37(3):9786. https://doi.org/10.1007/s11357-015-9786-9 PMID: 25911469
9. Cadore EL, Pinto RS, Reischak-Oliveira A, Izquierdo M. Explosive type of contractions should not be avoided during resistance training in elderly. Exp Gerontol. 2018; 102:81–3. https://doi.org/10.1016/j.exger.2017.12.003 PMID: 29247789
10. Fragala MS, Cadore EL, Dorgo S, Izquierdo M, Kraemer WJ, Peterson MD, et al. Resistance Training for Older Adults: Position Statement From the National Strength and Conditioning Association. J Strength Cond Res. 2019; 33(8):2019–52. https://doi.org/10.1519/JSC.0000000000003230 PMID: 31343601
11. Cadore EL, Casas-Herrero A, Zambom-Ferraresi F, Idoate F, Millor N, Gomez M, et al. Multicomponent exercises including muscle power training enhance muscle mass, power output, and functional outcomes in institutionalized frail nonagenarians. Age (Dordr). 2014; 36(2):773–85.
12. Tschopp M, Sattelmayer MK, Hilfiker R. Is power training or conventional resistance training better for function in elderly persons? A meta-analysis. Age Ageing. 2011; 40(5):549–56. https://doi.org/10.1093/ageing/afr005 PMID: 21383023
13. Ramirez-Campillo R, Castillo A, de la Fuente CI, Campos-Jara C, Andrade DC, Alvarez C, et al. High-speed resistance training is more effective than low-speed resistance training to increase functional capacity and muscle performance in older women. Exp Gerontol. 2014; 58:51–7. https://doi.org/10.1016/j.exger.2014.07.001 PMID: 25014621
14. Komi PV, Bosco C. Utilization of stored elastic energy in leg extensor muscles by men and women. Med Sci Sports. 1978; 10(4):261–5. PMID: 750844
15. Stenroth L, Peltonen J, Cronin NJ, Sipila S, Finni T. Age-related differences in Achilles tendon properties and triceps surae muscle architecture in vivo. J Appl Physiol (1985). 2012; 113(10):1537–44.
16. Aagaard P, Suetta C, Caserotti P, Magnusson SP, Kjaer M. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. Scand J Med Sci Sports. 2010; 20(1):49–64. https://doi.org/10.1111/j.1600-0838.2009.01084.x PMID: 20487503
17. Markovic G, Mikulic P. Neuro-musculoskeletal and performance adaptations to lower-extremity plyometric training. Sports Med. 2010; 40(10):859–95. https://doi.org/10.2165/11318370-000000000-00000 PMID: 20836583
18. Ramirez-Campillo R, Alvarez C, Garcia-Hermoso A, Ramirez-Velez R, Gentil P, Asadi A, et al. Methodological Characteristics and Future Directions for Plyometric Jump Training Research: A Scoping Review. Sports Med. 2018; 22(10):018–0870.

19. Vetrovsky T, Steffl M, Stastny P, Tufano JJ. The Efficacy and Safety of Lower-Limb Plyometric Training in Older Adults: A Systematic Review. Sports Med. 2019; 49(1):113–31. https://doi.org/10.1007/s40279-018-1018-x PMID: 30387072

20. Pelssers J, Delecluse C, Opdenacker J, Kennis E, Van Roie E, Boen F. "Every step counts!": effects of a structured walking intervention in a community-based senior organization. J Aging Phys Act. 2013; 21(2):167–85. https://doi.org/10.1123/japa.21.2.167 PMID: 22898451

21. Van Roie E, Martien S, Hurkmans E, Pelssers J, Seghers J, Boen F, et al. Ergometer-cycling with strict versus minimal contact supervision among the oldest adults: A cluster-randomised controlled trial. Arch Gerontol Geriatr. 2017; 70:112–22. https://doi.org/10.1016/j.archger.2017.01.010 PMID: 28131050

22. Van Roie E, Delecluse C, Coudyzer W, Boonen S, Bautmans I. Strength training at high versus low external resistance in older adults: Effects on muscle volume, muscle strength, and force-velocity characteristics. Exp Gerontol. 2013; 48(11):1351–61. https://doi.org/10.1016/j.exger.2013.08.010 PMID: 23999311

23. Baggen RJ, Van Roie E, Verschueren SM, Van Driessche S, Coudyzer W, van Dieen JH, et al. Bench stepping with incremental heights improves muscle volume, strength and functional performance in older women. Exp Gerontol. 2019; 120:6–14. https://doi.org/10.1016/j.exger.2019.02.013 PMID: 30797825

24. Piirainen JM, Cronin NJ, Avela J, Linnamo V. Effects of plyometric and pneumatic explosive strength training on neuromuscular function and dynamic balance control in 60-70year old males. J Electromyogr Kinesiol. 2014; 24(2):246–52 https://doi.org/10.1016/j.jelekin.2014.01.010 PMID: 24581701

25. Bartko JJ. The intraclass correlation coefficient as a measure of reliability. Psychol Rep. 1966; 19(1):3–11. https://doi.org/10.2466/pr0.1966.19.1.3 PMID: 5942109

26. Garcia-Ramos A, Haff GG, Padial P, Feriche B. Reliability of power and velocity variables collected during the traditional and ballistic bench press exercise. Sports Biomech. 2018; 17(1):117–30. https://doi.org/10.1080/14763141.2016.1260767 PMID: 28632056

27. van Lummel RC, Walgaard S, Maier AB, Ainsworth E, Beek PJ, van Dieen JH. The Instrumented Sit-to-Stand Test (iSTS) Has Greater Clinical Relevance than the Manually Recorded Sit-to-Stand Test in Older Adults. PLoS One. 2016; 11(7):e0157968. https://doi.org/10.1371/journal.pone.0157968 PMID: 27391082

28. Van Roie E, Van Driessche S, Huijben B, Baggen R, van Lummel RC, Delecluse C. A body-fixed-sensor-based analysis of stair ascent and sit-to-stand to detect age-related differences in leg-extensor power. PLoS One. 2019; 14(1):e0210653. https://doi.org/10.1371/journal.pone.0210653 PMID: 30653542

29. Cohen J. A power primer. Psychol Bull. 1992; 112(1):155–9. https://doi.org/10.1037//0033-2909.112.1.155 PMID: 19565683

30. Hoffren-Mikkola M, Ishikawa M, Rantalainen T, Avela J, Komi PV. Neuromuscular mechanics and hopping training in elderly. Eur J Appl Physiol. 2015; 115(5):863–77. https://doi.org/10.1007/s00421-014-3065-9 PMID: 25479729

31. Zubac D, Paravlic A, Koren K, Felicita U, Simunic B. Plyometric exercise improves jumping performance and skeletal muscle contractile properties in seniors. J Musculoskelet Neuronal Interact. 2019; 19(1):38–49. PMID: 30839302

32. Franchi MV, Monti E, Carter A, Quinlan JI, Herrod PJJ, Reeves ND, et al. Bouncing Back! Counteracting Muscle Aging With Plyometric Muscle Loading. Front Physiol. 2019; 10:178. https://doi.org/10.3389/fphys.2019.00178 PMID: 30890953

33. Blazevich A. Are training velocity and movement pattern important determinants of muscular rate of force development enhancement? Eur J Appl Physiol. 2012; 112(10):3689–91. https://doi.org/10.1007/s00421-012-2352-6 PMID: 22350361

34. Buckthorpe MW, Hannah R, Pain TG, Folland JP. Reliability of neuromuscular measurements during explosive isometric contractions, with special reference to electromyography normalization techniques. Muscle Nerve. 2012; 46(4):566–76. https://doi.org/10.1002/mus.23322 PMID: 22987699

35. Bolam KA, Skinner TL, Jenkins DG, Galvao DA, Taaffe DR. The Osteogenic Effect of Impact-Loading and Resistance Exercise on Bone Mineral Density in Middle-Aged and Older Men: A Pilot Study. Gerontol. 2015; 62(1):22–32. https://doi.org/10.1159/000435837 PMID: 26226987

36. Correa CS, LaRoche DP, Cadore EL, Reischak-Oliveira A, Bottaro M, Kruel LF, et al. 3 Different types of strength training in older women. Int J Sports Med. 2012; 33(12):962–9. https://doi.org/10.1055/s-0032-1312648 PMID: 22782384

37. McFadyen BJ, Winter DA. An integrated biomechanical analysis of normal stair ascent and descent. J Biomech. 1988; 21(9):733–44. https://doi.org/10.1016/0021-9290(88)90282-5 PMID: 3182877

38. Larsen AH, Sorensen H, Puggaard L, Aagaard P. Biomechanical determinants of maximal stair climbing capacity in healthy elderly women. Scand J Med Sci Sports. 2009; 19(5):678–86. https://doi.org/10.1111/j.1600-0838.2008.00845.x PMID: 18694432

39. Ihalainen JK, Inglis A, Makinen T, Newton RU, Kainulainen H, Kyrolainen H, et al. Strength Training Improves Metabolic Health Markers in Older Individual Regardless of Training Frequency. Front Physiol. 2019; 10:32. https://doi.org/10.3389/fphys.2019.00032 PMID: 30774600

40. Van Roie E, Bautmans I, Coudyzer W, Boen F, Delecluse C. Low- and high-resistance exercise: long-term adherence and motivation among older adults. Gerontol. 2015; 61(6):551–60. https://doi.org/10.1159/000381473 PMID: 25997778

41. Delabastita T, Bogaerts S, Vanwanseele B. Age-Related Changes in Achilles Tendon Stiffness and Impact on Functional Activities: A Systematic Review and Meta-Analysis. J Aging Phys Act. 2018:1–12. https://doi.org/10.1123/japa.2017-0359 PMID: 29722592

42. Haff GG, Triplett NT, editor. Essentials of strength training and conditioning. Fourth ed. Champaign, IL: Human Kinetics; 2016.

43. Pereira A, Izquierdo M, Silva AJ, Costa AM, Bastos E, Gonzalez-Badillo JJ, et al. Effects of high-speed power training on functional capacity and muscle performance in older women. Exp Gerontol. 2012; 47(3):250–5. https://doi.org/10.1016/j.exger.2011.12.010 PMID: 22234287

44. Bassey EJ, Rothwell MC, Littlewood JJ, Pye DW. Pre- and postmenopausal women have different bone mineral density responses to the same high-impact exercise. J Bone Miner Res. 1998; 13(12):1805–13. https://doi.org/10.1359/jbmr.1998.13.12.1805 PMID: 9844097

45. Jarvis MM, Graham-Smith P, Comfort P. A Methodological Approach to Quantifying Plyometric Intensity. J Strength Cond Res. 2016; 30(9):2522–32. https://doi.org/10.1519/JSC.0000000000000518 PMID: 24787677

46. Zemkova E, Jelen M, Schickhofer P, Hamar D. Jumping From a Chair is a More Sensitive Measure of Power Performance In Older Adults Than Chair Rising. Exp Aging Res. 2016; 42(5):418–30. https://doi.org/10.1080/0361073X.2016.1224665 PMID: 27749205

47. Strotmeyer ES, Winger ME, Cauley JA, Boudreau RM, Cusick D, Collins RF, et al. Normative Values of Muscle Power using Force Plate Jump Tests in Men Aged 77–101 Years: The Osteoporotic Fractures in Men (MrOS) Study. J Nutr Health Aging. 2018; 22(10):1167–75. https://doi.org/10.1007/s12603-018-1081-x PMID: 30498822

48. Hunter GR, Plaisance EP, Carter SJ, Fisher G. Why intensity is not a bad word: Optimizing health status at any age. Clin Nutr. 2018; 37(1):56–60. https://doi.org/10.1016/j.clnu.2017.02.004 PMID: 28214041

49. Gentil P, Steele J, Fisher J. Why intensity is not a bad word—Benefits and practical aspects of high effort resistance training to the older. Clin Nutr. 2017; 36(5):1454–5. https://doi.org/10.1016/j.clnu.2017.05.024 PMID: 28592355

50. Lexell J. Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci. 1995; 50:11–6. https://doi.org/10.1093/gerona/50a.special_issue.11 PMID: 7493202

51. Lexell J. Evidence for nervous system degeneration with advancing age. J Nutr. 1997; 127(5 Suppl):1011S–3S. https://doi.org/10.1093/jn/127.5.1011S PMID: 9164286

52. Bottaro M, Machado SN, Nogueira W, Scales R, Veloso J. Effect of high versus low-velocity resistance training on muscular fitness and functional performance in older men. Eur J Appl Physiol. 2007; 99(3):257–64. https://doi.org/10.1007/s00421-006-0343-1 PMID: 17146693

2

Motion sickness symptoms during jumping exercise on a short-arm centrifuge

Timo Frett[1]*, **David Andrew Green**[2,3,4], **Michael Arz**[1], **Alexandra Noppe**[1], **Guido Petrat**[1], **Andreas Kramer**[5], **Jakob Kuemmel**[5], **Uwe Tegtbur**[6], **Jens Jordan**[1,7]

1 Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany, **2** Space Medicine Team (HRE-OM), European Astronaut Centre, European Space Agency, Cologne, Germany, **3** KBRwyle GmbH, Cologne, Germany, **4** King's College London, London, United Kingdom, **5** Institute for Sport Sciences, University Konstanz, Konstanz, Germany, **6** Institutes of Sports Medicine, Hannover Medical School, Hannover, Germany, **7** Chair of Aerospace Medicine, University of Cologne, Cologne, Germany

* Timo.frett@dlr.de

Editor: Sakamuri V. Reddy, Charles P. Darby Children's Research Institute, 173 Ashley Avenue, Charleston, SC 29425, USA, UNITED STATES

Abstract

Artificial gravity elicited through short-arm human centrifugation combined with physical exercise, such as jumping, is promising in maintaining health and performance during space travel. However, motion sickness symptoms could limit the tolerability of the approach. Therefore, we determined the feasibility and tolerability, particularly occurrence of motion sickness symptoms, during reactive jumping exercises on a short-arm centrifuge. In 15 healthy men, we assessed motion sickness induced by jumping exercises during short-arm centrifugation at constant +1*Gz* or randomized variable +0.5, +0.75, +1, +1.25 and +1.5 *Gz* along the body axis referenced to center of mass. Jumping in the upright position served as control intervention. Test sessions were conducted on separate days in a randomized and cross-over fashion. All participants tolerated jumping exercises against terrestrial gravity and on the short-arm centrifuge during 1 *Gz* or variable *Gz* at the center of mass without disabling motion sickness symptoms. While head movements markedly differed, motion sickness scores were only modestly increased with jumping on the short-arm centrifuge compared with vertical jumps. Our study demonstrates that repetitive jumping exercises are feasible and tolerable during short-arm centrifugation. Since jumping exercises maintain muscle and bone mass, our study enables further development of exercise countermeasures in artificial gravity.

Introduction

Lack of terrestrial gravity during space travel produces multiple physiological adaptations challenging astronaut performance and health. The issue is particularly relevant for future deep space missions. Countermeasures relying on strength and endurance exercises help maintaining skeletal muscle [1] and cardiopulmonary fitness [2]. Current exercise countermeasures on the International Space Station are individually tailored for each astronaut. In general, an integrated resistance and aerobic training schedule is prescribed [3–5]. Crewmembers typically exercise six days per week, which consumes significant crew time and resources [6,7]. Yet,

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing interests: KBRwyle GmbH provided the salary for D.G but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

with current countermeasures, lower limb bone mass and muscle volume was still reduced after 16–28 weeks in space [8]. Moreover, countermeasures for potentially serious changes in ocular and brain structures likely resulting from chronic cephalad fluid shifts, the so called space associated neuro-ocular syndrome [9, 10], have not been established. Other approaches such as passive axial loading suits or lower body negative pressure systems [11–13] affect only parts of the complex physiological adaption process during long-term space missions.

Artificial gravity elicited through axial acceleration on short-arm human centrifuges, which distributes fluids to the lower part of the body, has been developed as potential countermeasure. Centrifugation may also help maintaining coordination and vestibular function, which are crucial when arriving on other celestial bodies. Yet, centrifugation when simply added to current countermeasures may not be practical given the tight schedule of astronauts. Combined centrifugation and physical exercise may be more efficient. Because reactive jumps appear to maintain skeletal muscle as well as bone mass in bed rest [5], jumping exercises during centrifugation are particularly promising. However, exercise-induced head movements within a rotating environment can produce severe motion sickness symptoms or illusory sensations through cross-coupled angular accelerations of semi-circular canals [6]. The issue is complicated by the steep g gradient away from the rotation axis during short-arm centrifugation [7]. Leg press exercises were tolerated during centrifugation, however, subjects were restrained to avoid head movements [8]. Therefore, the aim of our study was to determine the feasibility and tolerability of reactive jumping exercises during short-arm human centrifugation.

Methods

Study participants

We included 15 healthy men (26.4 ± 5.8 yrs; 180.9 ± 4.0 cm; 77.2 ± 5.8 kg) who were naïve to jumping exercises during centrifugation. Prior to the study, participants completed a brief medical questionnaire detailing their drug and medical history and passed a standardized centrifuge medical screening that includes clinical-chemical analyses of blood and urine, stress electrocardiogram, and orthostatic testing. Participants were excluded if they were in pain, or had any significant current or history of musculoskeletal, cardiovascular or neurological disorder or injury that could affect the ability to perform exercise. All participants were recreationally active (engaging in a minimum of two sport sessions per week) in order to facilitate exercise performance and minimize risk of injury during centrifugation. All participants gave written informed consent to participate in the study. The study was approved by the North Rhine ethical committee (Ref: 2017122).

Protocol

Participants attended to the laboratory at: envihab (DLR, Cologne, Germany) on four testing sessions separated by at least three resting days to allow for muscle recovery. In a fifth session participants ran on a treadmill at the German Sports University in Cologne. Participants were not permitted to take anti-emetic medication (i.e. scopolamine) and were offered light food (bananas, cereal bars) and non-sparkling water during each protocol to ensure hydration and glycaemia. Our experiment on motion sickness was part of a broader physiological investigation of jumping exercises during centrifugation that will be published elsewhere. Briefly, we compared effects of jumping exercises in the supine position on a short-arm centrifuge during spinning at different gravity level with jumping in upright position in terrestrial gravity (see Table 1).

Table 1. Exercise conditions for each participant.

Condition	Description
Terrestrial Gravity	15 x 15 vertical jumps in terrestrial gravity
Continuous AG	15 x 15 jumps at constant +1 Gz* at CoM
Variable AG	SAHC: 3 x 15 jumps at +0.5 Gz* 3 x 15 jumps at +0.75 Gz* 3 x 15 jumps at +1 Gz* 3 x 15 jumps at +1.25 Gz* 3 x 15 jumps at +1.5 Gz* in randomized order

*The value refers to *Gz* at the center of mass

Prior to recording, participants were familiarized with equipment and testing procedures including a brief centrifugation run. In two testing sessions, subjects performed jumping exercises in artificial gravity (AG) on the DLR-short-arm centrifuge at constant +1 *Gz* along the subject´s body axis (Continuous AG) and with +0.5, +0.75, +1, +1.25 and +1.5 *Gz* along the subject´s body axis in randomized order (Variable AG). Jumping in the upright position against terrestrial gravity served as control intervention (Terrestrial gravity). The study was conducted in a randomized controlled cross-over fashion.

Participants performed jumping exercises in the supine position on the short-arm centrifuge using a horizontal sledge (Figs 1 and 2) against a fixed footplate. The jumping sledge was attached to the short-arm centrifuge via low friction bearings that by riding along rails permitted linear movements along the centrifuge arm (Fig 3A). In addition, the sledge allowed for pitch at participants´ center of mass to facilitate natural jumping movements (Fig 1).

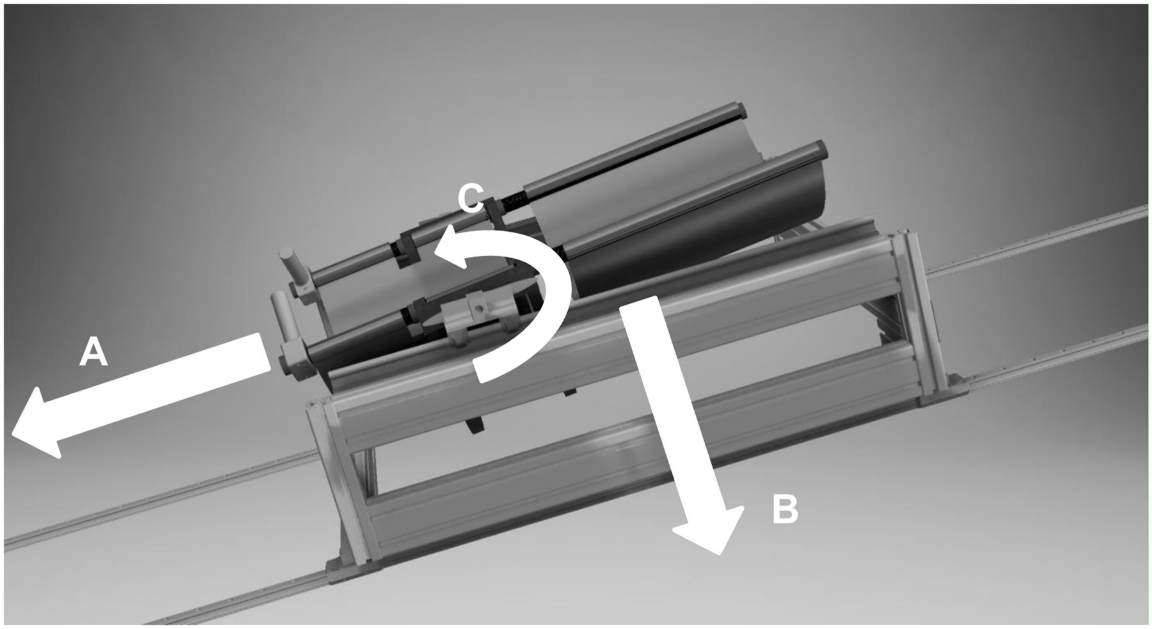

Fig 1. Schematic of the jumping sledge used on the short-arm human centrifuge. Participants were secured in supine position with a safety belt controlling their movement using two hand grips while jumping against a footplate mounted to the centrifuge. Due to the sledge design, movements along the centrifuge radius (A) against earth´s gravity (B) and in pitch axis around the center of mass (C) are possible.

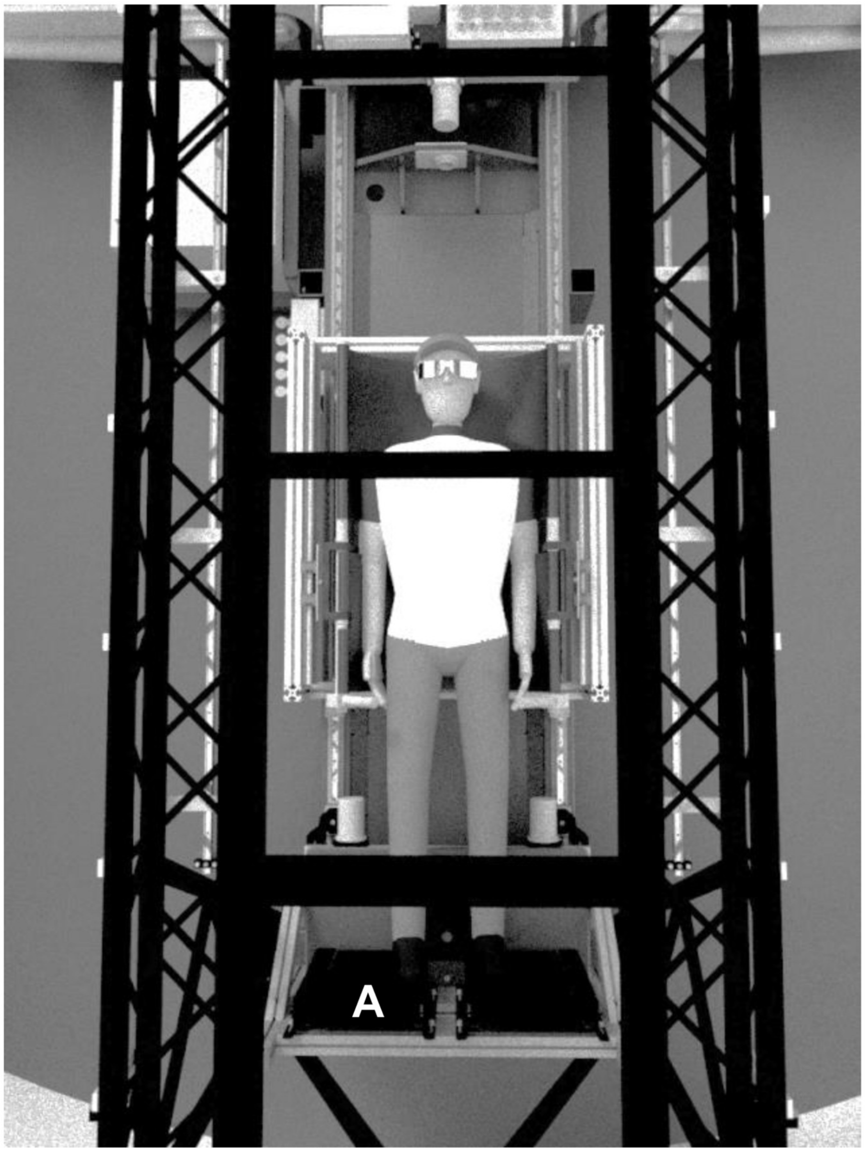

Fig 2. Presentation of participants position on the short-arm human centrifuge in bird's-eye perspective. During centrifugation participants performed jumping exercises against a footplate (A).

Participants were fastened on the sledge by safety belts around hip. The head was not restrained. Each centrifugation session lasted approximately 30 min. In protocol 2, each *G*–level lasted for around 6 min. Onset and offset acceleration of the centrifuge were 0.1 *G*/sec. We terminated centrifugation when participants demonstrated pre-syncopal signs or symptoms.

Data acquisition and analysis

During centrifugation, five lead electrocardiogram, brachial cuff blood pressure, finger pulse oximetry (Philipps IntelliVue®), and a live video feed were continuously monitored subjects by an experienced physician.

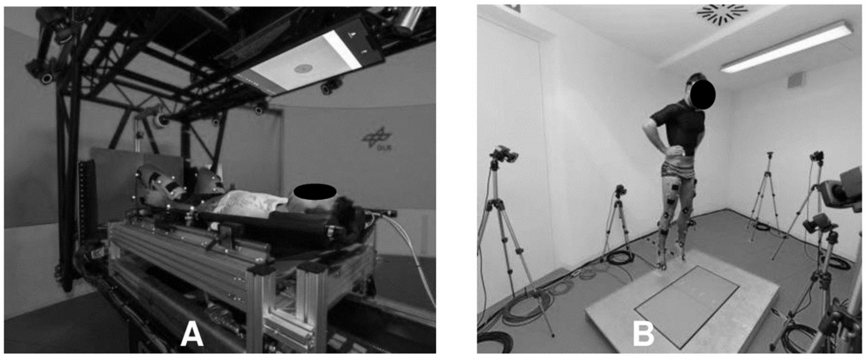

Fig 3. Participant´s jumping position during (A) continuous or variable centrifugation on the short-arm human centrifuge and (B) vertically against terrestrial gravity.

We assessed tri-axial (pitch, yaw, and roll) head movement velocities throughout using a wearable inertial sensor (Shimmer3, Shimmer, Dublin, Ireland) secured with an elasticated band on the forehead. We determined motion sickness susceptibility prior to the study using the Motion Sickness Susceptibility Questionnaire (MSSQ) short-form [10] that yields MSA (based on childhood experience (before age 12) and MSB for that over the last 10 years (max score = 54).

Directly before, and immediately following each condition, participants completed Subjective Motion Sickness Rating, Motion Sickness Assessment Questionnaire (MSAQ), Positive and Negative Affect Schedule (PANAS), and Epworth Sleepiness Scale (ESS) questionnaires. Subjective Motion Sickness Rating's range from 0 "I am feeling fine" to 20 "I am about to vomit" [14]. The MSAQ was used to measure (1 to 9 max) various dimensions (e.g. gastrointestinal, sopite) of motion sickness [15]. PANAS was used to measure the effect of symptoms induced by jumping upon mood. Participants rated each item on a Likert scale from 1 "not at all" to 5 "very much". The ESS (which via rating from 0 (non-) to 3 "high chance of dozing" in 8 contexts) since "drowsiness" is a cardinal symptom of motion sickness [16–18]

In addition, participants were asked regularly during centrifugation whether they were experiencing any motion sickness symptoms, and to report any unexpected symptoms such as tunnel vision or tumbling sensations.

During centrifugation five lead ECG (Philipps IntelliVue®), cuff blood pressure and SpO_2 as well as a live video feed were used to continuously monitor subjects by an experienced physician. Any run where participants demonstrated pre-syncopal symptoms was terminated immediately.

Statistical analysis

Mean head movement (Pitch, Yaw, Roll) velocities were compared between jumping sessions 1–15 for each condition using analysis of variance with repeated measurement. All questionnaire pre and post data was compared between conditions per participant. Pre-data represented scoring from every questionnaire before starting of the individual condition and post-data for every questionnaire after completion of each condition. Non-parametric tests (Friedman´s Chi-Square) were performed to evaluate whether there was an effect of condition. If significant differences across conditions were observed, post-hoc tests with pairwise comparisons using Dunn-Bonferroni were performed to determine which condition was significant different.

All statistical tests were conducted using SPSS version 21 (IBM Corp., USA) with $\alpha < 0.05$ indicating significance.

Results

All participants tolerated well jumping exercises against terrestrial gravity and on the short-arm centrifuge during both, the continuous and the variable centrifugation protocol. Only one subject experienced presyncopal symptoms requiring termination of the Variable AG protocol but completed all other protocols without similar symptoms. No disabling motion sickness symptoms occurred that required termination of testing. Serious adverse events did not occur.

Mean head movement velocities in pitch axes did not differ between centrifugation protocols (Fig 4) but compared to terrestrial condition (p = 0.000, dfs = 14). In the eccentric phase of the jumps, mean positive peak pitch angular velocity (Fig 5) was significantly greater during continuous (t (14) = 5.06, $p < 0.001$) and variable centrifugation (t (14) = 6.27, $p < 0.001$) compared to the terrestrial control condition. During concentric movements against the centrifuge´s gravity vector, mean negative pitch angular velocity was also significantly greater in continuous (t (14) = -8.503, $p < 0.001$) and variable centrifugation protocols (t (14) = -3.055, p = 0.009) compared with the control intervention. We observed no significant changes in head movements across time, F = 0.827, p = 0.643, partial η2 = 0.045, n = 15 (Greenhouse-Geisser).

No participant reported motion sickness before the training sessions commenced. Motion Sickness Susceptibility (MSSQ) scores were 10.84 ± 4.52 with sub-scores for MSA (5.68 ± 2.70) and MSB (5.37 ± 2.93).

After the interventions, Subjective Motion Sickness Ratings were low with 1.33±0.48 following Terrestrial gravity intervention, 2.53 ± 1.45 following Continuous AG, and 2.15 ± 1.14 following Variable AG. Post-hoc analysis (Dunn-Bonferroni) across conditions showed that

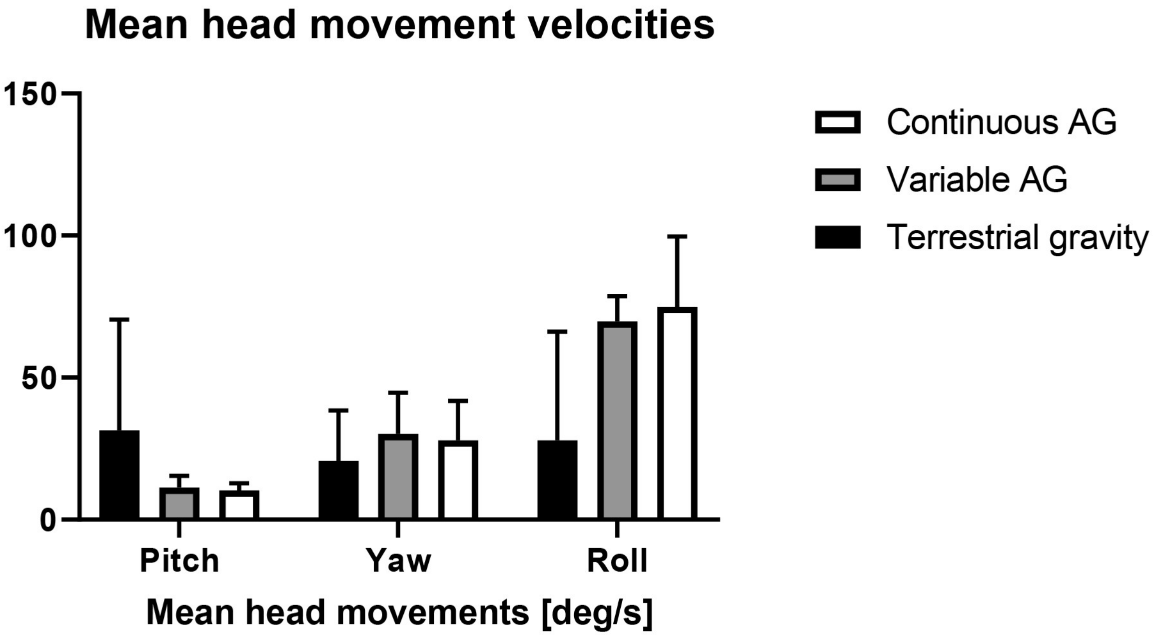

Fig 4. Mean (± SD) head movement velocities in roll, yaw and pitch for each condition.

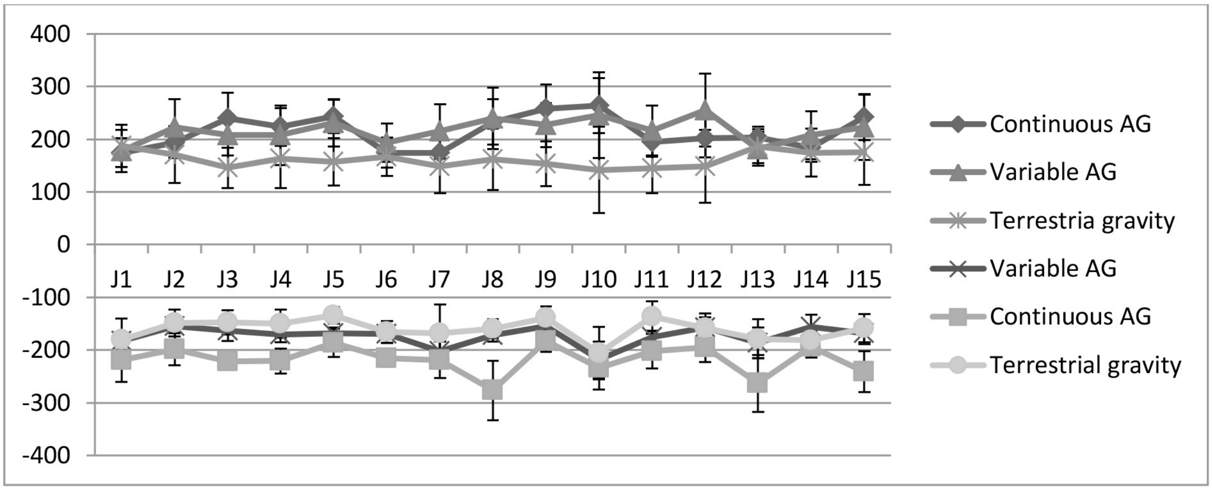

Fig 5. Mean (± SD) peak pitch angular velocities during each jumping session and in each condition. Subsequent jumps are labeled as J1 to J 15.

Subjective Motion Sickness ratings were significantly higher during continuous centrifugation compared to terrestrial control condition (z = 2.527, p = 0.034).

Post condition mean Motion Sickness Assessment Questionnaire scores were relatively low (Fig 6) and did not differ between conditions (Friedman´s Chi-Square χ^2 (2) = 0.792, p = 0.673).

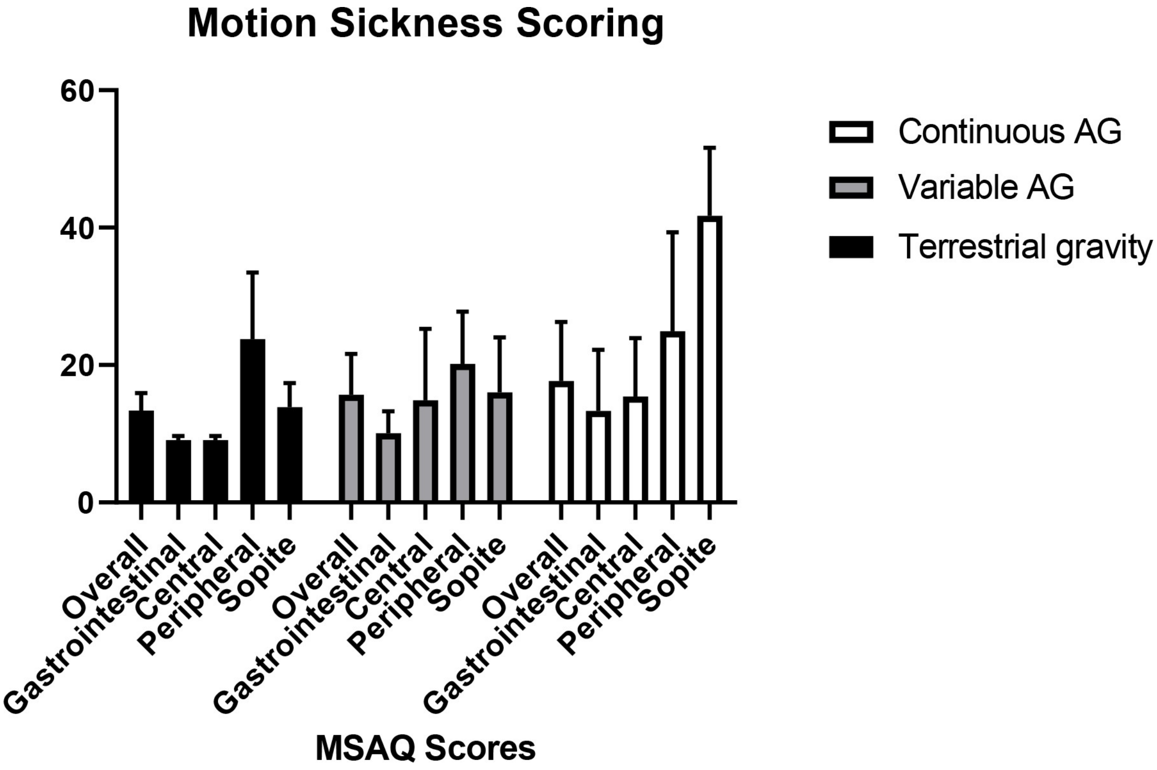

Fig 6. Motion sickness scoring from MSAQ questionnaire for each condition.

Post condition PANAS scores tended to be more positive in all conditions with PA: 26.25 ± 9.63, NA: 13.23 ± 4.81 following Continuous AG, PA: 29.76 ± 10.00, NA: 10.61 ± 1.66 following Variable AG and PA: 23.26 ± 8.72, NA: 15.73 ± 5.31 following terrestrial control condition,. Between the terrestrial control condition and both centrifuge conditions no significant effect (PANAS P χ^2 (2) = 5.636, p = 0.060, χ^2 (2) = 4.769, p = 0.092) occurred.

Post condition Epworth Sleepiness Scale scores did not differ significantly between conditions ratings were numerically slightly higher with centrifugation (Continuous AG: 8.28 ± 5.31, Variable AG: 8.07 ± 5.89, terrestrial control condition: 6.33 ± 3.67).

Discussion

Our study demonstrates that repetitive voluntary jumping exercises are both feasible and tolerable during short-arm centrifugation at levels ranging from +0.5 to +1.5 *Gz* at the center of mass along the body axis. Indeed, the intervention was well tolerated by recreationally fit individuals who were naïve to jumping exercises during centrifugation as long as they were briefly familiarized. Study participants could move their heads freely within certain safety limits on the centrifuge and perform jumping exercises without experience increased motion sickness levels. Thus, contrary to the common perception that whole-body movements, including head motion during short-arm centrifugation result in motion sickness and related symptoms *per se*, we demonstrated that vigorous repetitive jumping is possible without induction of negative motion sickness symptoms.

Head movements within a rotating environment produce cross-coupled angular accelerations in the semicircular canals. The mechanism can trigger adverse vestibular stimulation with symptoms ranging from mild discomfort (e.g. sweaty palms) to severe nausea, vomiting or even loss of consciousness [6]. Yet, not only was repetitive jumping possible but no participant needed to drop out due to motion sickness symptoms. In fact, Motion Sickness Scores and Motion Sickness symptoms were low in all conditions. The finding is remarkable given the high values for head yaw, pitch, and roll velocities being generated in all conditions that are excess of those previously defined as being associated with comfort zones [9]. Moreover, the comparison between both centrifuge conditions reveals the interesting fact that alternating gravity levels seems to have only minor effects on the increase of motion sickness scoring or other related symptoms.

Our study extends the recent findings of Piotrowski et al [8] who demonstrated that leg press exercises on a sledge during centrifugation albeit with head movement restraint, could be tolerated. Thus, contrary to that previously thought rapid, forceful and complex voluntary repetitive movement such as jumping can be implemented during short-arm centrifugation. The cardiovascular burden imposed by short-arm centrifugation may promote presyncopal symptoms that can progress to frank syncope. The fact, that only one presyncopal event occurred during the Variable AG condition is reassuring. It is likely that jumping or squat exercise during centrifugation can help to maintain orthostatic tolerance even in a steep +*Gz* gravity gradient.

PANAS Negative Affect (NA) Scores tended to be slightly lower during centrifugation. These findings, albeit non-significant may be explained by participants perceiving centrifugation as exciting–particularly for unexperienced participants.

The fact that only men were included is a limitation that was part of the study design in which our experiment was included. In our study, both average MSA and MSB MSSQ scores were relatively low compared to normative populations [10,11]. Thus, whether similar results would be observed in more or highly sensitive individuals is unknown. While some subjects in our study scored relatively high in terms of motion sickness sensitivity (MSB $>$ 11), none featured motion sickness requiring test termination. While the issue warrants further study, astronaut populations undergo tight medical screening and are not likely to have high motion sickness susceptibility. We cannot exclude that repeated exposure as part of a countermeasure protocol mitigates motion sickness symptoms completely. Since our study only included men, our findings cannot be simply extrapolated to women. Indeed, previous studies reported impaired vasoconstriction leading to impaired orthostatic tolerance in women after bed rest [12].

Despite these issues, we suggest that jumping exercises on a short-arm centrifuge are not generally restricted by disabling motion sickness symptoms. We speculate that being 'in control' may have increased the tolerability against cross-coupled effects during head movements while exercising on the short-arm centrifuge. This could be explained with increased controllability of the unknown setting on a centrifuge [13]

Since jumping exercise have been proven efficient in maintaining bone and muscle mass, our study enables further development of exercise countermeasures in Artificial Gravity.

Supporting information

S1 File.
(RAR)

Acknowledgments

The authors would like to thank the DLR centrifuge team and DLR study team for the conduction of the experiment. Further gratitude belongs to the principle investigator teams from University of Konstanz and the German Sport University for their cooperation.

Author Contributions

Conceptualization: Timo Frett.

Data curation: Michael Arz.

Formal analysis: Timo Frett, David Andrew Green.

Investigation: Michael Arz, Alexandra Noppe, Guido Petrat, Jakob Kuemmel.

Project administration: Jens Jordan.

Software: Michael Arz.

Supervision: Uwe Tegtbur.

Writing – original draft: Timo Frett.

Writing – review & editing: Timo Frett, David Andrew Green, Alexandra Noppe, Andreas Kramer, Jens Jordan.

References

1. Clément G. & Bukley A. *Artificial gravity.* Vol. 20 (Springer Science & Business Media, 2007).
2. Arnauld E. Nicogossian, R. S. W., Carolyn L. Huntoon,Charles R. Doarn,James D. Polk,Victor S. Schneider. Space Physiology and Medicine: From Evidence to Practice. (2016).
3. Lee A. G., Mader T. H., Gibson C. R., Brunstetter T. J. & Tarver W. J. Space flight-associated neuro-ocular syndrome (SANS). *Eye* (2018) 32, 1164–1167, https://doi.org/10.1038/s41433-018-0070-y PMID: 29527011

4. Roberts D. R. et al. Effects of Spaceflight on Astronaut Brain Structure as Indicated on MRI. *New England Journal of Medicine* (2017) 377, 1746–1753, https://doi.org/10.1056/NEJMoa1705129 PMID: 29091569
5. Kramer A., Gollhofer A., Armbrecht G., Felsenberg D. & Gruber M. How to prevent the detrimental effects of two months of bed-rest on muscle, bone and cardiovascular system: an RCT. *Scientific Reports* (2017) 7, 13177, https://doi.org/10.1038/s41598-017-13659-8 PMID: 29030644
6. Bertolini G. & Straumann D. Moving in a Moving World: A Review on Vestibular Motion Sickness. *Frontiers in Neurology* (2016) 7, 14, https://doi.org/10.3389/fneur.2016.00014 PMID: 26913019
7. Kaderka J, Y. L., Paloski WH. in Acta Astronautica 1090–1102 (2010).
8. Timothy Piotrowski J. R., Jochen Zane. A Comparison of Squatting Exercise on a Centrifuge and with Earth Gravity. *Front. Physiol.*, (2018) https://doi.org/10.3389/fphys.2018.01759 PMID: 30568604
9. Hall T. W. Artificial gravity and the architecture of orbital habitats. *Journal of the British Interplanetary Society* (1999) 52, 290–300.
10. Golding J. F. Predicting individual differences in motion sickness susceptibility by questionnaire. *Personality and Individual Differences* (2006) 41, 237–248, https://doi.org/10.1016/j.paid.2006.01.012.
11. Golding J. F. Motion sickness susceptibility questionnaire revised and its relationship to other forms of sickness. *Brain Res Bull* 47, 507–516 (1998). https://doi.org/10.1016/s0361-9230(98)00091-4 PMID: 10052582
12. Custaud M.-A. et al. Orthostatic tolerance and spontaneous baroreflex sensitivity in men versus women after 7 days of head-down bed rest. *Autonomic Neuroscience* (2002) 100, 66–76, https://doi.org/10.1016/S1566-0702(02)00132-7 PMID: 12422962
13. Rolnick A. & Lubow R. E. Why is the driver rarely motion sick? The role of controllability in motion sickness. *Ergonomics* (1991) 34, 867–879, https://doi.org/10.1080/00140139108964831 PMID: 1915252
14. Young L. R., Sienko K. H., Lyne L. E., Hecht H. & Natapoff A. Adaptation of the vestibulo-ocular reflex, subjective tilt, and motion sickness to head movements during short-radius centrifugation. *Journal of vestibular research*: *equilibrium & orientation* (2003) 13, 65–77.
15. Gianaros P. J., Muth E. R., Mordkoff J. T., Levine M. E. & Stern R. M. A Questionnaire for the Assessment of the Multiple Dimensions of Motion Sickness. *Aviation*, *space*, *and environmental medicine* (2001) 72, 115–119. PMID: 11211039
16. Graybiel A. & Knepton J. Sopite syndrome: a sometimes sole manifestation of motion sickness. *Aviat Space Environ Med* (1976) 47, 873–882. PMID: 949309
17. Lawson B. D. & Mead A. M. The sopite syndrome revisited: drowsiness and mood changes during real or apparent motion. *Acta Astronaut* (1998) 43, 181–192. https://doi.org/10.1016/s0094-5765(98)00153-2 PMID: 11541923
18. Van Ombergen A., Lawson B. D. & Wuyts F. L. Motion sickness and sopite syndrome associated with parabolic flights: a case report. *International journal of audiology* (2016) 55, 189–194, https://doi.org/10.3109/14992027.2015.1111526 PMID: 26624575

3

High plasma soluble levels of the immune checkpoint HLA-G molecule among bodybuilders

Talita M. Fernandes[1]*, Enrico F. Puggina[1], Celso T. Mendes-Junior[2], Milena C. de Paula[3], Paulin Sonon[4], Eduardo A. Donadi[5], Ana Paula M. Fernandes[3]

1 School of Physical Education and Sport of Ribeirão Preto, University of São Paulo at Ribeirão Preto, Ribeirão Preto, SP, Brazil, **2** Ribeirão Preto Faculty of Philosophy, Sciences and Letters, University of São Paulo at Ribeirão Preto, Ribeirão Preto, SP, Brazil, **3** College of Nursing, General and Specialized Nursing Department, University of São Paulo at Ribeirão Preto, Ribeirão Preto, SP, Brazil, **4** FIOCRUZ Oswaldo Cruz Foundation–Instituto Aggeu Magalhães, Federal University of Pernambuco, Recife, Brazil, **5** Medical School, Department of Medicine, University of São Paulo at Ribeirão Preto, Ribeirão Preto, SP, Brazil

* talitafernandes@usp.br

Abstract

Introduction

Studies report that intense physical activity influences the down-regulation of immune function in athletes as well as the interaction between adipose tissue and the immune system.

Aim

This study aimed to compare the plasma soluble levels of the immune checkpoint HLA-G (sHLA-G) molecule with the fat mass and muscle mass index among 77 bodybuilders and 64 controls.

Results

The comparisons of the percentage of body fat (%BF) revealed that the groups of male and female bodybuilders showed a statistically significant reduction in the percentage of body fat when compared to their control group, ($P < 0.0001$, for both comparisons). Regarding sHLA-G levels, the comparisons showed that the group of male bodybuilders had significantly higher sHLA-G levels compared to the group of female bodybuilders ($P = 0.0011$).

Conclusion

Our results showed that in bodybuilders with less body fat, the systemic levels of soluble HLA-G, an immunological molecule with recognized immunosuppressive function, are significantly higher and suggest that this immune mechanism may corroborate the immunosuppressive state in athletes undergoing intense and prolonged physical training.

Editor: Mauro Lombardo, San Raffaele Roma Open University, ITALY

Competing interests: The authors have declared that no competing interests exist.

Introduction

In contrast to moderate or intermittent physical activity, prolonged and intensive exertion causes numerous changes in immunity that possibly reflects on immunological suppression. In fact, evidence indicates that intense physical activity may downregulate the immune function and increase the risk of certain types of infection [1, 2]. Indeed, bodybuilding athletes are submitted to high intensity resistance training to develop muscular hypertrophy (size), to reduce the amount of subcutaneous fat, and to slow down the rate of recovery from fatigue after exercise [3–5].

Exercise-induced immune depression has a multifactorial origin, depending on mechanisms related to neuro/immune/endocrine systems. Evidence shows that prolonged periods of intense training may alter the profile of immune cells, including lymphopenia [6, 7] mucosal immunoglobulin levels [3], impaired phagocytosis [8, 9], and natural killer cell cytotoxicity (NKCA) [10].

Human leukocyte antigens (HLA) are involved in several important functions of the immune system, starting from antigen presentation to lymphocytes, performed by the classical class I histocompatibility molecules (HLA-A/B/C), and extending until the control of the immune response, as performed by the non-classical class I molecules (HLA-E/F/G). Among these molecules, HLA-G is the most studied one, and its major function is the down-regulation of the activity of the innate and adaptive immune system cells, by means of the interaction with ILT-2/4 inhibitory leukocyte receptors. Accordingly, HLA-G may inhibit the proliferation of T and B lymphocytes [11, 12], the activity of antigen-presenting cells (APC) [13] and the cytotoxicity of TCD8 and Natural Killer (NK) cells [11]. Because of these properties, HLA-G has been recognized as an immune check point molecule. In certain infections and in some types of cancer, the overexpression of HLA-G can create a tolerogenic environment; inhibiting several steps of the immune response, propitiating the spreading of infectious and malignant cells [14]. In contrast, in transplanted organs and autoimmune disorders the expression of HLA-G may produce beneficial effects.

Considering that: i) numerous studies report that intense physical activity may down-regulate the immune function in athletes [5, 8–10], ii) the reported interactions between adipose tissue and the immune system [15] may be affected by the low amount of subcutaneous fat in bodybuilders [3, 4], and iii) HLA-G down-regulates the function of cells of the innate and adaptive immune system [11–14], this study aimed to evaluate the soluble HLA-G levels among bodybuilders, stratified according to fat mass and muscle mass index.

Materials and methods

Sample

The sample consisted of 141 healthy individuals categorized into 77 bodybuilders and 64 controls, who presented no infections, immunological or metabolic disorders at the time of inclusion in the study. The bodybuilder group was composed of 50 (65%) male bodybuilders (MB) with a mean age of 33.6 years and 27 (35%) female bodybuilders (FB) with a mean age of 33.2 years. The control group was composed of 24 (37.5%) female controls (FC) with a mean age of 28.0 years and 40 (62.5%) male controls (MC) with a mean age of 23.5 years. Due to lack of data for all individuals, analyses related to height, total body weight, lean, fat and body mass indexes were performed only with 14 females and 31 males in the control and 19 females and 33 males in the bodybuilders. The group of high performance bodybuilders was selected from the participants of the 48th Brazilian Championship of Bodybuilding and Fitness–IFBB 2018, held in the city of Ribeirão Preto, SP, Brazil. The athletes were assessed before or after the standard weighing process to championship. All bodybuilders reported following the American College of Sports Medicine (ACSM) recommendations for training with respect to hypertrophy. Bodybuilders preparing for competition followed self- or coach- prescribed diets, with the sole aim of supplying specific amounts of protein, fat and carbohydrate. The control group was selected from bodybuilders with at least 1 year of practicing the sport. This investigation was approved by the Ethics in Research Committee of the University of São Paulo at Ribeirão Preto School of Physical Education and Sport of Ribeirão Preto (EEFERP-USP, process#2,808,296). The athletes agreed to take part in this investigation by signing an informed consent form.

Anthropometry

Measurements were taken according to the International Society for the Advancement of Kineanthropometry guidelines (ISAK) [16], and from each subject the following variables were taken: i) anthropometric assessment for the determination of body composition (% of fat, lean mass, fat mass and BMI—Body Mass Index), ii) total weight, total stature, nine skinfolds (tricipital, subscapular, bicipital, chest, medium axillary, suprailiac, abdominal, front thigh, and medium calf), two muscle girths (flexed biceps, calf standing), and three bone breadths (elbow, ankle and knee).

The following evaluations were taken: i) weight was measured using the *Welmy*® (Santa Barbara D´Oeste, BRA) scale, with accuracy of 100 g; ii) height with a vertical metric scale with a 1 mm accuracy; iii) skinfold thickness was assess by a adipometer *Cescorf*® with a 0.2 mm accuracy; and iv) muscle girths were measured with a measuring tape *Sanny Medical* (Guangdong, China) with a 1 mm accuracy scale. After obtaining these values, we used the following equation to estimate body density of male athletes: DC (g/cm^2) = 1,112–0.00043499 (∑7doc) + 0.00000055 $(\sum 7doc)^2$- 0.00028826 (age); and, for women: DC (g/cm^2) = 1.0970 - [0.00046971 (ST) + 0.00000056 $(ST)^2$]—[0.00012828 (age)], developed by Jackson and Pollock, 1978 [17], followed by converting the result into a percentage of fat by applying the Siri Equation (1961): % G = [(4.95/D) - 4.50] x 100 [18].

Soluble HLA-G quantification

Soluble HLA-G (sHLA-G) was quantified using MEM-G/9 antibody, which recognizes the most abundant soluble isoforms (shed sHLA-G1 and secreted HLA-G5), and an anti-human β2-microglobulin antibody, respectively, as capture and detection antibodies [19]. Microtitration plates were coated with 10 μg/mL MEM-G/9 mouse-anti-human HLA-G mAb (Exbio, Praha, Czech Republic) and incubated overnight at 4˚C. Plates were saturated with 300 μL ready to use diluent buffer (DAKO, Carpinteria, CA, USA) for 2 h. Plasma samples were diluted (½) in diluent buffer, tested in duplicate and incubated for 2 h. After incubation and washing, monoclonal anti-human beta2-microglobulin antibody (DAKO, Glostrup, Denmark), which recognizes the immobilized antibody sHLA-G complex, was added to the wells and incubated for 1 h. The plates were then incubated for 1 h with 100 μL (1:200) envision buffer + system HRP (DAKO, Carpinteria, CA, USA) to obtain anti-β2-microglobulin-horseradish peroxidase complex to improve the efficiency of the reaction. All incubation steps were performed at room temperature and followed by four washes using washing buffer (H_2O, PBS 1X, 0.1% Tween 20). The plates were incubated for 30 min with substrate (Tetramethylbenzidine super sensitive-TMB, Sigma Aldrich, Saint Louis, MO, USA) and absorbance was measured at 450 nm after adding HCL (1 N). Total sHLA-G levels were determined from a five-point standard calibration curve using dilutions (12.5–200 ng/mL) of HLA-G5 purified from M8-HLA-G5 cell line culture supernatants. Results are presented in ng/mL.

Statistical analysis

Categorical variables were described using proportions and 95% confidence interval, and the continuous variables were described by the mean, median, amplitude and correlation. Continuous variables were compared by means of Student T test, Wilcoxon-Mann-Whitney rank-sum test and analysis of variance (ANOVA). We checked for normality of distribution using Kolmogorov-Smirnov normality test. Since deviations from normality were found, non-parametric tests were used, Kruskal-Wallis and Mann-Whitney tests. In all analyzes performed, two-tailed versions of the tests were used and a significance level of 5% (alpha = 0.05) was adopted. Such analyzes were performed using the program GraphPad InStat version 3.06, GraphPad Software (www.graphpad.com). For intergroup analyzes, Spearman's correlation was used, an analysis used to discover the relationship between the two variables that do not have a normal joint distribution.

Results

Anthropometric assessment

Total body mass (body weight) and height were evaluated, and the body weight for the MB group ranged from 67–108 kg (84.97 ± 10.45), for the FB group it ranged from 45.5–77 kg (58.45 ± 9.56), for MC it ranged from 59–98 kg (79.17 ± 11.64) and FC of 48–74 kg (642.42 ± 8.80). The height of individuals in the MB group ranged from 160–186 cm (175.63 ± 6.29), for the FB group from 153–176 cm (164.68 ± 6.94), for MC it ranged from 164–192 cm (176.10 ± 6.80) and FC of 153–170 cm (162.18 ± 6.151) (Table 1).

Comparisons of the Body Mass Index (BMI) between the different groups showed that the group of Male Bodybuilders has a significantly higher BMI compared to the Male Control (P = 0.0213) and Female Bodybuilders group (P <0.0001) (Table 1).

Percentage of Body Fat (%BF)

Comparisons of the percentage of body fat (%BF) between the groups revealed that the groups of male and female bodybuilders showed a statistically significant reduction in the percentage of body fat when compared to their control group, male (P <0.0001) and female (P <0.0001), respectively. The comparison between the groups of male and female bodybuilders showed that the group of male bodybuilders showed a significantly greater reduction in the percentage of body fat compared to the group of female bodybuilders (P <0.0001) (Table 1).

Lean Mass Index (LMI)

To enhance fitness in bodybuilders, LMI must have high levels associated with low levels of fat mass index. The LMI was calculated using the formula LMI = TBM-FMI, where: LMI = Lean Mass Index, TBM = Total Body Mass, FMI = Fat Mass Index. Table 1 shows the medians and comparisons between the different groups studied.

Comparisons of the LMI between the groups showed that the groups of male and female bodybuilders have significantly higher LMI compared to the male (P<0.0001) and female (P = 0.0062) control groups, respectively. The control groups and male bodybuilders had significantly higher LMI compared to the control (P<0.0001) and female bodybuilder (P<0.0001) groups, respectively.

Fat Mass Index (FMI)

For the calculation of fat mass, the formula FMI = TBM x %BF/100 was used, where: FMI = Fat Mass Index, TBM = Total Body Mass, %BF = Body Fat Percentage. Table 1 shows the medians and comparisons between the different groups studied.

Table 1. Anthropometric variables and comparison of the groups of individuals studied.

							Statistical Comparisons (Mann-Whitney Test)				
Variables	**FC (*n* = 24)**	**MC (*n* = 40)**	**FB (*n* = 27)**	**MB (n = 50)**	**TC (*n* = 64)**	**TB (*n* = 77)**	**FC vs MC**	**FC vs FB**	**MC vs MB**	**FB vs MB**	**TC vs TB**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**					
	Median	**Median**	**Median**	**Median**	**Median**	**Median**					
	Min—max	**Min—max**	**Min—max**	**Min—max**	**Min—max**	**Min—max**					
Age (years)	29.042 (9.134)	24.325 (6.391)	33.222 (5.807)	33.420 (8.732)	26.094 (7.813)	33.351 (7.791)	**0.0321**	**0.0416**	< **0.0001**	0.5861	<**0.0001**
	27.500	22.500	33.000	31.500	24.000	32.000					
	16–48	18–44	24–45	20–57	16–48	20–57					
Height (cm)*	162.18 (6.151)	176.10 (6.808)	164.68 (6.945)	175.63 (6.291)	171.77 (9.234)	171.63 (8.378)	< **0.0001**	0.3579	> 0.9999	< **0.0001**	0.9222
	165	175	164	176	172	173					
	153–170	164–192	153–176	160–186	153–192	153–186					
Total body mass (kg)*	62.429 (8.806)	79.173 (11.641)	58.453 (9.561)	84.971 (10.454)	73.963 (13.295)	75.282 (16.343)	**0.0002**	0.2258	0.0729	< **0.0001**	0.5850
	66.700	77.600	56.000	83.000	74.000	77.500					
	48–74	59–98	45.5–77	67–108	48–98	45.5–108					
BMI (Kg/m^2)*	23.685 (2.773)	25.448 (2.854)	23.014 (3.196)	27.526 (2.959)	24.900 (2.917)	25.305–4.033	0.0931	**0.0213**	**0.0156**	< **0.0001**	0.7106
	24.250	25.230	22.310	26.570	24.540	25.715					
	19.540–29.900	20.720–31.770	18.000–26.040	22.340–35.270	19.540–31.770	18.000–35.270					
%BF	28.741 (8.766)	16.715 (6.438)	10.593 (2.798)	6.460 (5.183)	21.225 (9.389)	7.909 (4.893)	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**
	28.335	16.620	10.560	5.055	20.320	6.800					
	3.030–42.610	5.560–31.790	4.220–17.810	0.4500–29.950	3.030–42.610	0.4500–29.950					
LMI (kg)*	44.471 (5.015)	65.170 (7.824)	52.461 (8.572)	78.555 (10.493)	58.731 (11.962)	69.021 (2.219)	< **0.0001**	**0.0062**	< **0.0001**	< **0.0001**	**0.0008**
	44.185	66.070	49.540	76.630	59.660	70.385					
	36.270–54.650	49.300–82.150	41.170–70.680	56.740–102.87	36.270–82.150	41.170–102.87					
FMI (kg)*	17.957 (5.876)	14.002 (7.459)	5.992 (2.029)	6.416 (5.534)	15.233 (7.181)	6.261 (4.551)	**0.0485**	< **0.0001**	< **0.0001**	0.1514	< **0.0001**
	18.800	13.660	5.890	4.910	13.970	5.045					
	9.320–28.410	3.940–31.150	2.700–10.710	2.220–27.010	3.940–31.150	2.220–27.010					
sHLA-G (ng/mL)	86.549 (87.606)	166.42 (56.300)	76.518 (61.300)	129.17 (135.29)	136.47 (287.76)	110.70 (117.16)	0.0856	0.7989	0.2574	**0.0011**	0.3767
	60.000	90.000	67.270	109.29	78.335	99.290					
	0.000–355.45	4.580–2266.4	8.890–330.00	0.000–932.14	0.000–2266.4	0.000–932.14					

Statistical significant values are highlighted in boldface.

*Sample sizes for these variables are reduced. For FC, *n* = 14; for MC, *n* = 31; for FB, *n* = 19; for MB, *n* = 33; for TC, *n* = 45; and for TB, *n* = 52.

BMI = body mass index (median); FC = female control; MC = male control; FB = female bodybuilder; MB = male bodybuilder; vs = versus.

Comparisons of the FMI between the groups showed that male and female bodybuilders have significantly lower FMI compared to the male (P <0.0001) and female (P <0.0001) control groups, respectively. Among the controls, the male control group had significantly lower FMI compared to the female control group (P = 0.0485).

Plasma levels of soluble HLA-G

Plasma levels of soluble HLA-G were analyzed and for the FM group their levels ranged from 0–932.14 ng/mL (109.29 ± 135.29), for the FF group from 8.89–330 ng/mL (67.27 ± 31.3), for CM of 4.58–604.58 ng/mL (88.75 ± 105.37) and CF of 0–355.45 ng/mL (60 ± 87.6) (Fig 1).

Comparisons of plasma levels of soluble HLA-G (sHLA-G) showed that the group of male bodybuilders had significantly higher levels of sHLA-G compared to the group of female bodybuilders (P = 0.0011) (Table 1).

Considering that the age difference between the groups MB (33.6 years) and MC (23.5 years) was of almost 10 years, we also correlated results according to age and gender in the four studied groups. According to age, the following correlations were performed: i) (MB: rho = 0.0233; P = 0.8724; ii) FB: rho = -0.0724; P = 0.7196; iii) MC: rho = -0.0330; P = 0.8400; and FC: rho = 0.2936; P = 0.1638). According to gender, the following correlations were evaluated: i) (males: rho = 0.1032; P = 0.3330; females: rho = 0.1708; P = 0.2307) or in the entire sample (rho = 0.0872; P = 0.3037). These analyses revealed that age and gender did not influence intragroup results.

When compared to the different groups, male bodybuilders (MB) had significantly higher values of sHLA-G and significantly lower values of Fat Mass Index (FMI) and Percentage of Body Fat (%BF), we explored intra-group comparisons (Table 1).

Intragroup correlations (Table 2) showed that MB individuals with lower %BF had significantly higher levels of sHLA-G (P = 0.0073). Similarly, individuals with lower FMI had significantly higher levels of sHLA-G (P = 0.0087).

Discussion

The immune system presents several mechanisms for regulating the inflammatory response. Several immunological mediators have anti-inflammatory and immunoinhibitory functions,

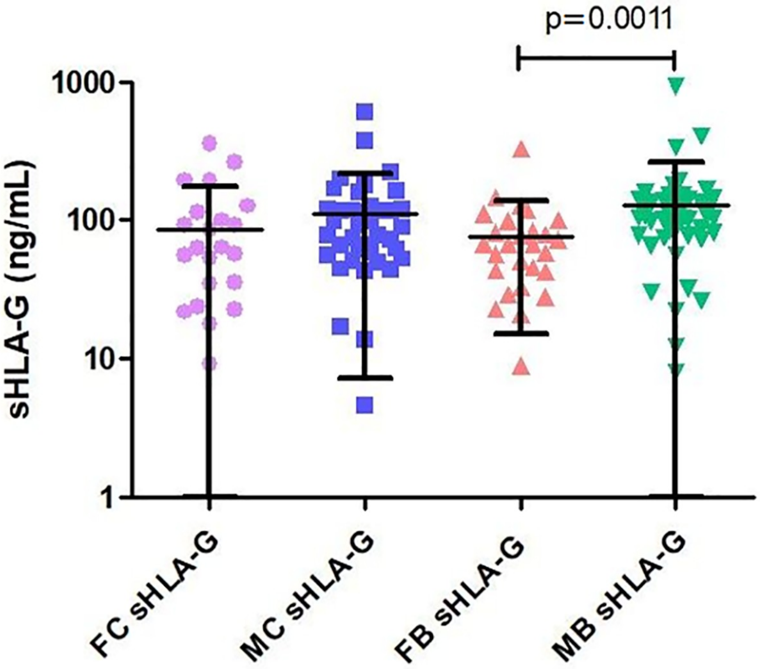

Fig 1. Plasma levels of soluble HLA-G (sHLA-G) from individuals in the FC (female control), MC (male control), FB (female bodybuilder), MB (male bodybuilder) groups. sHLA-G levels among MB were significantly higher when compared to FB. The horizontal bars in bold represent the medians.

Table 2. Spearman correlations between plasma levels of soluble HLA-G with Body Fat Percentage, Lean Mass Index and Fat Mass Index among bodybuilders.

sHLA-G (ng/mL)	%BF	LMI (kg)	FMI (kg)
Female (n = 27)[a]	p = 0.1689	p = 0.8194	p = 0.1827
Male (n = 50)[b]	p = **0.0073**	p = 0.8224	p = **0.0087**

Significant values are highlighted in boldface.

[a] For correlation with LMI and FMI, a reduced sample size of 19 women was used.

[b] For correlation with LMI and FMI, a reduced sample size of 33 men was used.

among these, HLA-G presents a well-recognized immunomodulatory activity [17–20]. This is the first study to assess plasmas HLA-G levels in bodybuilders, highlighting male to female and intragroup differences. In short, sHLA-G levels were augmented in: i) male bodybuilders (MB) compared to female bodybuilders (FB) (P = 0.0011), ii) the correlation between MB evidenced an inverse relationship between body fat and sHLA-G levels.

Studies have shown that adipose tissue secretes various inflammation-related cytokines, such as leptin and IL—6, which can induce chronic low-grade inflammation in obese individuals, with a concomitant stimulation of sHLA-G production, which has anti-inflammatory activities [21, 22]. This mechanism is possibly due to compensate the chronic pro-inflammatory state of obesity. Other studies indicate that the presence of higher levels of sHLA-G in patients with chronic diseases could, provisionally, represent a way to compensate the inflammatory condition [23].

Studies that measured the chronic effects of moderate or intermittent physical exercise point to local and systemic reduction of pro-inflammatory mediators, with attenuation in the production and secretion of acute phase proteins [24], and greater production and secretion of cytokines with anti-inflammatory function (especially IL-6 in skeletal muscle tissue and blood) [25]. The literature also suggests that the beneficial effects of physical training on the modulation of inflammation depend on the quality and quantity of stimuli, which are directly related to the rest time between stimuli to avoid the emergence of the stress condition [26]. In this context, it is also reported that physical exercise when intensively performed contributed to increase the incidence of upper respiratory tract infection, while exercise of moderate intensity would have a protective effect against the risk of infections [27, 28].

Considering the relationship between susceptibility to infections and inflammatory\immunologicalmediators associated with the practice of physical exercise, we sought to understand what possible mechanisms may modulate the immune response, primarily focusing on HLA-G. According to Costa Rosa and Vaisberge, 2002 [26], cellular and humoral components are mobilized from the immune system in response to mechanical (hypoxia, hyperthermia and muscle damage), metabolic (glutamine) and hormonal (adrenaline, cortisol) changes imposed by exercise [27].

In normal muscle, HLA-G is undetectable. However, in the context of inflammation, muscle fibers express HLA-G in a distribution similar to classical HLA class I molecules, as well as under stimulation of inflammatory cytokines, such as IFN-gamma [29]. In inflammatory myopathy, CD8 T cells destroy non-necrotic muscle fibers, showing a response mediated by HLA class I restricted cytotoxic T cells against surface antigens expressed in muscle fibers. Marzuillo et al. [30] reported that muscle fibers, in an inflamed environment, co-express HLA-G, raising the intriguing possibility that HLA-G protects muscle fibers from injury mediated by NK cells [31, 32]. Locally accumulated inflammatory cells produce a multitude of pro-inflammatory cytokines, including TNF-alpha and IFN-gamma. As these cytokines are known to induce HLA expression in many cell types, it seems likely that HLA-G expression, seen in inflammatory myopathies, at least in part, results from stimulation by locally produced cytokines after injury [33].

Insufficient physical exercise is related to the development of several diseases and metabolic changes, such as decreased insulin sensitivity, changes in lipid metabolism, increased visceral adiposity, decreased lean body mass and loss of muscle strength, resulting in low-grade chronic inflammation [34]. On the other hand, muscle damage, resulting from physical exercise, may initially result in a condition of acute inflammation with an increase in the production of myocins and activation of immune system cells to the site of muscle damage in order to initiate the tissue remodeling and repair process [35]. Noteworthy, it has been shown that systematic physical training can lead to a local and systemic anti-inflammatory state that enables tissue regeneration and adaptation and, at the same time, protects the organism against the development of chronic inflammatory pathologies [36]. In other words, an increase in the pro-inflammatory organic condition, caused by the stimulation of acute exercise, would be counterbalanced by the chronic anti-inflammatory environment, caused by systematic exercise, which would restrict the magnitude and duration of the inflammation. Thus, the beneficial effects of physical training on the modulation of inflammation depend on the quality and quantity of stimuli, which are directly related to the rest time between stimuli [37]. Thus, transversal and longitudinal data suggest that people who perform regular exercises of moderate intensity maintain a protective state [28, 38]. However, intense and prolonged exercise has been associated with greater morbidity and mortality [4, 39]. These findings gave rise to the "J"-shaped model, related the relationship between the exercise dose and the risk of infection. This model reports that moderate exercise may lower the risk of upper respiratory infection, whereas excessive exercise may increase the risk. Indeed, greater risk for upper respiratory infection is observed in over-trained compared with well-trained athletes [40].

Finally, our results present important evidence related to the immunological profile in bodybuilders, associating the decreased body fat to an increased plasma sHLA-G levels, indicating that, at least in part, HLA-G may contribute to the immunosuppressive state of athletes undergoing intense and prolonged physical training. Indeed, bodybuilding performed with extreme training to optimize lean mass and reduce the rate of body fat [31] may be associated with immunosuppression process [41], which has been attributed to exhaustive and prolonged exercises [32, 42, 43]. This cross-sectional study presents data obtained only at the time of championship, and long-term follow-up studies evaluating the role of diet, supplements and the role of the innate and adaptive immune response are needed to clarify the influence of intense physical exercise on the immune function.

Author Contributions

Conceptualization: Talita M. Fernandes, Enrico F. Puggina, Eduardo A. Donadi, Ana Paula M. Fernandes.

Data curation: Talita M. Fernandes, Celso T. Mendes-Junior, Milena C. de Paula, Paulin Sonon, Eduardo A. Donadi, Ana Paula M. Fernandes.

Formal analysis: Talita M. Fernandes, Celso T. Mendes-Junior, Milena C. de Paula, Ana Paula M. Fernandes.

Funding acquisition: Talita M. Fernandes, Ana Paula M. Fernandes.

Investigation: Talita M. Fernandes, Celso T. Mendes-Junior, Ana Paula M. Fernandes.

Methodology: Talita M. Fernandes, Eduardo A. Donadi, Ana Paula M. Fernandes.

Project administration: Talita M. Fernandes, Eduardo A. Donadi, Ana Paula M. Fernandes.

Resources: Talita M. Fernandes, Ana Paula M. Fernandes.

Software: Talita M. Fernandes, Ana Paula M. Fernandes.

Supervision: Talita M. Fernandes, Eduardo A. Donadi, Ana Paula M. Fernandes.

Validation: Talita M. Fernandes, Eduardo A. Donadi, Ana Paula M. Fernandes.

Visualization: Talita M. Fernandes, Eduardo A. Donadi, Ana Paula M. Fernandes.

Writing – original draft: Talita M. Fernandes, Ana Paula M. Fernandes.

Writing – review & editing: Talita M. Fernandes, Milena C. de Paula, Eduardo A. Donadi, Ana Paula M. Fernandes.

References

1. Stewart LK, Flynn MG, Campbell WW, et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. *Med Sci Sports Exerc.* 39:1714–1719, 2007. https://doi.org/10.1249/mss.0b013e31811ece1c PMID: 17909397
2. Uchida MC, Nosaka K, Ugrinowitsch C, et al. Effect of bench press exercise intensity on muscle soreness and inflammatory mediators. *J Sports Sci.* 27: 499–507, 2009. https://doi.org/10.1080/02640410802632144 PMID: 19253084
3. Nieman DC, Nehlsen-Cannarella SL. The effects of acute and chronic exercise of immunoglobulins. *Sports Med.* 11:183–201, 1991. https://doi.org/10.2165/00007256-199111030-00003 PMID: 2047622
4. Ekblom B, Ekblom O, Malm C. Infectious episodes before and after a marathon race. *Scandinavian Journal of Medicine and Science in Sports* 16(4):287–293, 2006. https://doi.org/10.1111/j.1600-0838.2005.00490.x PMID: 16895535
5. Huygens W, Claessens AL, Thomis M, et al. Body composition estimations by BIA versus anthropometric equations in body builders and other power athletes. *J Sports Med Phys Fitness* 42: 45–55, 2002. PMID: 11832874
6. Jürimäe J, Purge P, Jürimäe T. Adiponectin and stress hormone responses to maximal sculling after volume-extended training season in elite rowers. *Metabolism* 55:13–19, 2006. https://doi.org/10.1016/j.metabol.2005.06.020 PMID: 16324914
7. Kleiner SM, Bazzarre TL, Ainsworth BE. Nutritional status of nationally ranked elite bodybuilders. *Int J Sport Nutr.* 4:54–69, 1994. https://doi.org/10.1123/ijsn.4.1.54 PMID: 8167655
8. Kleiner SM, Bazzarre TL, Litchford MD. Metabolic profiles, diet, and health practices of championship male and female bodybuilders. *J Am Diet Assoc* 90:962–967, 1990. PMID: 2365938
9. Kokkinos PF, Hurley BF, Vaccaro P, et al. Effects of low- and high-repetition resistive training on lipoprotein-lipid profiles. *Med Sci Sports Exerc* 20:50–54,1988.
10. Kraemer WJ, Ratamess NA. Hormonal responses and adaptations to resistance exercise and training. *Sports Med* 35:339–361, 2005. https://doi.org/10.2165/00007256-200535040-00004 PMID: 15831061
11. Rouas-Freiss N, Goncalves RM, Menier C, Dausset J, Carosella ED. Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. *Proc Natl Acad Sci U S A* 94(21):11520–11525, 1997. https://doi.org/10.1073/pnas.94.21.11520 PMID: 9326642
12. Naji A, Menier C, Morandi F, et al. Binding of HLA-G to ITIM-bearing Ig-like transcript 2 receptor suppresses B cell responses. *J Immunol* 192(4): 1536–1546, 2014. https://doi.org/10.4049/jimmunol.1300438 PMID: 24453251
13. Horuzsko A, Lenfant F, Munn DH, Mellor AL. Maturation of antigen-presenting cells is compromised in HLA-G transgenic mice. *Int Immunol* 13(3): 385–394, 2001. https://doi.org/10.1093/intimm/13.3.385 PMID: 11222508
14. Fagerberg P. Negative Consequences of Low Energy Availability in Natural Male Bodybuilding: A Review. *Int J Sport NutrExercMetab* 28(4): 385–402, 2018.
15. Maurizi G, Della Guardia L, Maurizi A, Poloni A. Adipocytes properties and crosstalk with immune system in obesity-related inflammation. *J Cell Physiol* 233(1): 88–97, 2018. https://doi.org/10.1002/jcp.25855 PMID: 28181253
16. ISAK. (2001). International Standards for Anthropometric Assessment. Under dale, S.A., ISAK.

17. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. *Br J Nutr* 40: 497–504, 1978. https://doi.org/10.1079/bjn19780152 PMID: 718832

18. GUEDES D.P. Composição corporal: princípios, técnicas e aplicações. 2ª edição. Londrina: APEF. 1994.

19. Rebmann V, Lemaoult J, Rouas-Freiss N, Carosella ED, Grosse-Wilde H. Report of the wet workshop for quantification of soluble HLA-G in Essen. *Hum. Immunol* 66: 853–863, 2005. https://doi.org/10.1016/j.humimm.2005.05.003 PMID: 16216668

20. González A, Rebmann V, LeMaoult J, et al. The immunosuppressive molecule HLA-G and its clinical implications. *Critical Reviews in Clinical Laboratory Sciences*, 49: 63–84, 2012. https://doi.org/10.3109/10408363.2012.677947 PMID: 22537084

21. Gonen-Gross T, Achdout H, Gazit R, et al. Complexes of HLA-G protein on the cell surface are important for leukocyte Ig-like receptor-1 function. *The Journal of Immunology* 171(3): 1343–1351, 2003. https://doi.org/10.4049/jimmunol.171.3.1343 PMID: 12874224

22. Zidi I, Yahia HB, Bortolotti D, et al. Association between sHLA-G and HLA-G 14-bp deletion/insertion polymorphism in Crohn's disease. *International Immunology* 27(6): 289–296, 2015. https://doi.org/10.1093/intimm/dxv002 PMID: 25577194

23. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *Journal of the American College of Cardiology* 45(10): 1563– 1569, 2005. https://doi.org/10.1016/j.jacc.2004.12.077 PMID: 15893167

24. Kim JI, Ho IC, Grusby MJ, Glimcher LH. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. *Immunity*, 10(6): 745–751, 1999. https://doi.org/10.1016/s1074-7613(00)80073-4 PMID: 10403649

25. Smith DA, Nicholls S, Morgan BA, Brown AJ, Quinn J. A conserved stress-activated protein kinase regulates a core stress response in the human pathogen Candida albicans. *Molecular biology of the cell* 15(9): 4179–4190, 2004. https://doi.org/10.1091/mbc.e04-03-0181 PMID: 15229284

26. Rosa C, Bicudo LFP, Vaisberg MW. Influências do exercício na resposta imune. *Revista brasileira de Medicina do Esporte* 8(4): 167–172, 2002.

27. Calder PC, Dimitriadis G, Newsholme P. Glucose metabolism in lymphoid and inflammatory cells and tissues. Current Opinion in Clinical Nutrition & Metabolic *Care* 10(4): 531–540, 2007.

28. Nieman GW, Weertman JR, Siegel RW. Micro-hardness of nanocrystalline palladium and copper produced by inert-gas condensation. Scripta metallurgica 23(12): 2013–2018, 1989.

29. Yang Y, Chu W, Geraghty DE, Hunt JS. Expression of HLA-G in human mononuclear phagocytes and selective induction by IFN-gamma. *The Journal of Immunology* 156(11):4224–4231, 1996. PMID: 8666791

30. Marzuillo P, Bellini G, Punzo F, et al. Association between 14 bp insertion/deletion HLA-G functional polymorphism and insulin resistance in a cohort of Italian children with obesity. *Pediatric diabetes* 19 (8): 1357–1361, 2018. https://doi.org/10.1111/pedi.12768 PMID: 30203576

31. Braud VM, Allan DS, McMichael AJ. Functions of nonclassical MHC and non-MHC-encoded class I molecules. *Current opinion in immunology* 11(1): 100–108, 1999. https://doi.org/10.1016/s0952-7915(99)80018-1 PMID: 10047540

32. Le Bouteiller P, Blaschitz A. The functionality of HLA-G is emerging. *Immunological reviews* 167(1): 233–244, 1999.

33. Petersen EW, Carey AL, Sacchetti M, et al. Acute IL-6 treatment increases fatty acid turnover in elderly humans *in vivo* and in tissue culture *in vitro*: evidence that IL-6 acts independently of lipolytic hormones. *Am. J. Physiol. Endocrinol. Metab* 288: 155–162, 2005.

34. Pedersen BK. The diseasome of physical inactivity–and the role of myokines in muscle–fat cross talk. *The Journal of physiology* 587(23): 5559–5568, 2009.

35. Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 298(5): 1173–1187, 2010.

36. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nature Reviews Endocrinology* 8(8): 457–465, 2012. https://doi.org/10.1038/nrendo.2012.49 PMID: 22473333

37. Silva FOCD, Macedo D.V. Exercício físico, processo inflamatório e adaptação: uma visão geral. *Revista Brasileira de Cineantropometria & Desempenho Humano* 13(4): 320–328, 2011.

38. Wong PC, Chia M, Tsou IY, et al. Effects of a 12-week exercise training programme on aerobic fitness, body composition, blood lipids and C-reactive protein in adolescents with obesity. *Ann Acad Med Singapore* 37(4): 286–93, 2008. PMID: 18461212

39. Heath GW, Macera CA, Nieman DC. Exercise and upper respiratory tract infections. *Sports Medicine* 14(6): 353–365, 1992. https://doi.org/10.2165/00007256-199214060-00003 PMID: 1470789

40. Nielsen HG. Exercise and Immunity. In: Hamlin M, Draper N and Kathiravel Yeditors. Current Issues in Sports and Exercise Medicine; 2013. pp.121–140. 2013.

41. Gleeson M. Immune function in sport and exercise. *Journal of applied physiology* 103(2): 693–699, 2007. https://doi.org/10.1152/japplphysiol.00008.2007 PMID: 17303714

42. Pedersen BK, Ostrowski K, Rohde T, Bruunsgaard H. The cytokine response to strenuous exercise. *Canadian journal of physiology and pharmacology* 76(5): 505–511, 1998. https://doi.org/10.1139/cjpp-76-5-505 PMID: 9839076

43. Rowbottom DG, Green KJ. Acute exercise effects on the immune system. *Medicine & Science in Sports & Exercise* 32(7): 396–405, 2000.

4

Soccer heading and concussion are not associated with reduced brain volume or cortical thickness

Tiago Gil Oliveira[1,2], **Chloe Ifrah**[3], **Roman Fleysher**[3,4], **Michael Stockman**[3], **Michael L. Lipton**[3,4,5,6]*

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, **2** ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal, **3** Gruss Magnetic Resonance Imaging Center, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York, United States of America, **4** Department of Radiology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York, United States of America, **5** Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York, United States of America, **6** Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York, United States of America

* michael.lipton@einstein.yu.edu

Editor: Niels Bergsland, University at Buffalo, UNITED STATES

Abstract

Soccer is the most popular sport in the world and, since it is a contact sport, players are at risk for head injury, including concussion. Here, we proposed to investigate the association of heading and concussion with macroscopic brain structure among adult amateur soccer players. For this study, 375 amateur soccer players (median age 23 years) completed HeadCount-12m to estimate heading over the 12 months prior to MRI and lifetime concussion. T1-weighted 3D magnetization prepared rapid acquisition gradient echo (MP-RAGE) MRI was performed at 3 Tesla. Parcellation was performed using Freesurfer to extract regional gray and white matter volumes as well as regional cortical thickness and total intracranial volume. Regional cortical brain volumes were normalized by total intracranial volume. We categorized heading into quartiles and concussion as 0, 1 or 2 or more. Generalized linear regressions were used to test the association of heading or concussion with each brain morphometry metric, including age and sex, as covariates. Neither heading nor concussion were associated with reduced brain volume or cortical thickness. We observed that greater heading was associated with greater gray matter volume in the left inferior parietal area, which may reflect effects related to training.

Introduction

Soccer is the most popular sport in the world [1]. As a contact sport, soccer players are at risk for head injury including concussion [2, 3]. Unique to soccer, purposive heading, where the head is used to impact and direct the ball during play, is a fundamental part of the game, which raises concerns for adverse effects of repeated head impacts (RHI) in the context of soccer play. Soccer heading has thus become an area of concern as a potential source of brain

Competing interests: The authors have declared that no competing interests exist.

injury. Although recognized concussion is more prevalent in American football compared to soccer [4], high levels of heading are associated with concussive symptoms and worse neurocognitive performance (e.g., [5]). In addition, chronic traumatic encephalopathy (CTE) has been reported in former soccer players [6, 7].

Over time, repetitive head impacts, such as occurring with heading in soccer, have the potential to induce long-term structural effects. Indeed, imaging studies have identified adverse microstructural effects of heading, suggestive of traumatic axonal injury [5, 8]. Using diffusion-tensor imaging (DTI) to assess white matter integrity, professional soccer players have been found to exhibit higher radial and axial diffusivity compared to swimmers [8]. Heading has also been shown to be associated with lower fractional anisotropy (FA) in the temporo-occipital region in adult amateur players [5]. Moreover, ventricular enlargement indicative of central cerebral atrophy was shown using CT in a small cohort of former professional soccer players [9]. More recently, using structural magnetic resonance imaging (MRI), it was shown in a study with 15 subjects per group, that former professional soccer players had a steeper age-associated decrease in cortical thickness [10]. Apart from these, we are unaware of other studies that have addressed the potential effect of soccer RHI or concussion on brain macrostructure. The purpose of this study was to determine whether heading or concussion were associated with adverse effects on regional brain volume or cortical thickness in a large cohort of adult amateur soccer players.

Materials and methods

Soccer participants

Players were participants in a large longitudinal study of soccer play and its consequences. Players whose data are included in this report were adult amateur soccer players recruited between November 2013 and May 2018 by print and Internet advertisement and through soccer leagues, clubs and colleges in New York City and surrounding areas. Interested individuals were directed to an enrollment website, which, after informed consent, collected screening information. A research team member contacted qualifying individuals, confirmed eligibility, willingness to participate in the study and invited enrollment. Inclusion criteria were: age 18–55; at least 5 years of active amateur soccer play; current active amateur soccer play; at least 6 months of amateur soccer play annually; and English language fluency. Participants were asked to report neurological or other medical diagnoses. Exclusion criteria were: schizophrenia, bipolar disorder; current neurological disorder; pregnancy; or medical contraindication to MRI. At enrollment, each player completed HeadCount-12m (see below), to capture soccer activity, heading and concussion, as well as brain MRI. Details of the overall study design have been published previously [11, 12].

Exposure measures

HeadCount-12m, part of a suite of web-based assessments that estimate heading over distinct timeframes, was used to generate estimates of heading over the prior 12 months and recognized concussions over the lifetime. Prior publications have reported on HeadCount in detail (e.g., [11–15]). Briefly, participants were asked questions relative to their soccer activity, including the number of months played per year, the mean number of competitive soccer games per week, the mean number of headers per game, the mean number of practices per week, and the mean number of headers per practice. The total number of headers in the past year was estimated by multiplying the mean number of headers in each setting by the number of sessions per week in each setting, converted to month, and then multiplying by the number of months of play per year. Subtotals in each setting were summed to obtain an estimate of total 12-month heading. The HeadCount questionnaire, also asks the number of years that participants have played soccer at a similar frequency and their lifetime concussion history. Participants were instructed to consider a concussion as any head injury for which they sought or were asked to seek medical attention. Due to the high degree of right skew in the exposure measures, we treated each as a categorical variable. Heading was treated as an ordered categorical variable of approximately equal size quartiles and concussion was treated as three categories: “zero”, “1” and “2 or more” concussions over the participant’s lifetime.

Imaging data acquisition

Whole-brain MR imaging was performed with a 32-channel 3.0-T MR unit (Achieva TX; Philips Medical Systems, Best, the Netherlands) and a 32-channel head coil (Philips Medical Systems, Best, the Netherlands). T1-weighted 3D magnetization prepared rapid acquisition gradient echo (MP-RAGE) was acquired with axial slab selection. Imaging parameters were as follows: TR/TE/TI = 9.9/4.6/900ms, flip angle 8°, 1mm^3 isotropic resolution, 240 × 188 × 220 matrix and FOV.

Clinical image review

A board-certified neuroradiologist reviewed all images to detect structural abnormalities and evidence of prior trauma, including microhemorrhage.

Image processing

Segmentation of brain cortical and subcortical structures from T1-weighted image volumes was performed using the Freesurfer toolkit version 5.3 (https://surfer.nmr.mgh.harvard.edu). This software package implements a semi-automated segmentation workflow including skull removal, normalization of WM intensity, spatial registration to the Talairach standard space and tessellation of gray matter—white matter segmentation. For cortical parcellation, an atlas considering the gyral and sulcal components as separate regions was used [16]. We extracted and analyzed white matter gyral/sulcal volumes, cortical gyral/sulcal volumes, cortical thickness and deep brain structures. Intracranial volume (ICV) was measured for each subject [17]. All volume measures were normalized to intracranial volume (ICV) prior to analysis as follows: Each participant’s ICV was divided by the median ICV of the entire study cohort. Each brain region’s volume was then divided by this normalized ICV.

Data analysis

Statistical analysis was performed using IBM SPSS software, version 24 (IBM, New York, USA) and GraphPad Prism7 software (http://www.graphpad.com). We constructed separate general linear models to test the association of exposure (heading or concussion) with brain morphometry metrics (volume or cortical thickness) at each location. In each analysis we tested the significance of heading in the 2nd, 3rd and 4th quartiles compared to the 1st quartile and of 1 and 2+ concussions compared to 0 concussions. We included sex, age and handedness as covariates. ICV was included as a covariate in analyses of cortical volume, but not of cortical thickness. Bonferroni correction was used to mitigate Type 1 error to a corrected p-value of 0.05 (actual $p = 0.0002$), for 250 corrections. Visualization and representation of brain regions was accomplished using BrainNet Viewer 1.61 [18].

Participants consent

This study was reviewed and approved by the local institutional review board and complied with the Health Insurance Portability and Accountability Act. Players recruited to participate in the "Einstein Soccer Study", a multi-faceted longitudinal study of heading and its consequences in adult amateur soccer players, gave written informed consent prior to initiation of study procedures.

Results

375 amateur soccer players were included in the analysis. Median age was 23 years (mean = 25.7). Average heading for the 12 months preceding MRI was 2188 (median 691; range 0–139561). Players reported 0–6 prior concussions over their lifetimes (median 0, mean 0.71) (Table 1). History of a neurological diagnosis (such as headache or migraine, which were the most frequently reported) or other medical diagnosis was inquired, and due to the small number of participants with any individual medical or neurological history item (Table 1), we were not able to reliably test the effects of specific conditions.

Table 1. Demographic characteristics of 365 amateur soccer players.

	Soccer Players (N = 375)	Frequency
Sex	Male	70.9%
	Female	29.1%
Race	American Indian	0.8%
	Asian	7.2%
	Pacific Islander	1.3%
	Black	16.5%
	White	65.9%
	Chose not to report	7.5%
	Unknown	19.7%
Education		
Mean = 15.70	0–13	17.1%
Median = 16	14–15	28%
IQR = 3	16	23.2%
SD = 2.24	17+	31.7%
Age		
Mean = 25.67	18–21	36.3%
Median = 23	22–23	16%
IQR = 7	24–27	25%
SD = 7.54	28+	22.7%
Concussion Count		
Mean = 0.71	0	63.7%
Median = 0	1	16.3%
IQR = 1	2+	20%
SD = 1.17		
Heading Count / Year		
Mean = 2188.22	1	24.8%
Median = 691	2	25.3%
IQR = 1573	3	24.8%
SD = 10072.59	4	25.1%
ICV		
Mean = 1395568	0–1273356	24.8%
Median = 1400780	1273357–1400779	25.1%
IQR = 224996	1400780–1498352	25.3%

(*Continued*)

Table 1. (Continued)

	Soccer Players (N = 375)	Frequency
SD = 166578.78	1498353+	24.8%
History of neurological diagnosis	Yes	3.7%
	No	80.5%
	Unknown	15.7%
History of medical diagnosis	Yes	9.8%
	No	74.4%
	Unknown	15.7%

All frequencies are reported as percentage of the entire cohort (n = 375). Concussion is reported as 0, 1, 2 or more over the lifetime. Heading is reported as number of heading events during the prior 12 months. IQR: interquartile range; SD: standard deviation; ICV: intracranial volume.

Radiological review of the imaging studies revealed no evidence of prior trauma or other gross structural abnormalities. Thus, no players were excluded due to imaging findings.

Neither heading, at any level, nor concussion showed a significant association with either lower volume or thinner cortex of any brain region tested. We did find that heading at all levels was significantly associated ($p<0.0001$) with greater volume of the left inferior parietal cortex (Fig 1).

Discussion

The main finding of this study is that soccer heading or concussion are not associated with lower regional brain volume or cortical thickness in a large cohort of adult amateur players. The diagnosis of CTE in professional and amateur athletes with long histories of sport-related RHI, even in the absence of clinically diagnosed concussion [8], has raised concern regarding the role of RHI in accumulating brain injury and long-term risk for neurodegenerative disease [2]. It is important to consider the largely negative findings of this study of brain macrostructure in context of the nascent study of soccer effects on the brain as well as hypotheses regarding the role of subconcussive RHI and the evolution of brain pathology before gross tissue loss is detectable using volumetry [2].

Several studies have demonstrated microstructural changes related to soccer play and heading [5, 8, 14]. Moreover, studies have shown that subconcussive heading may be associated

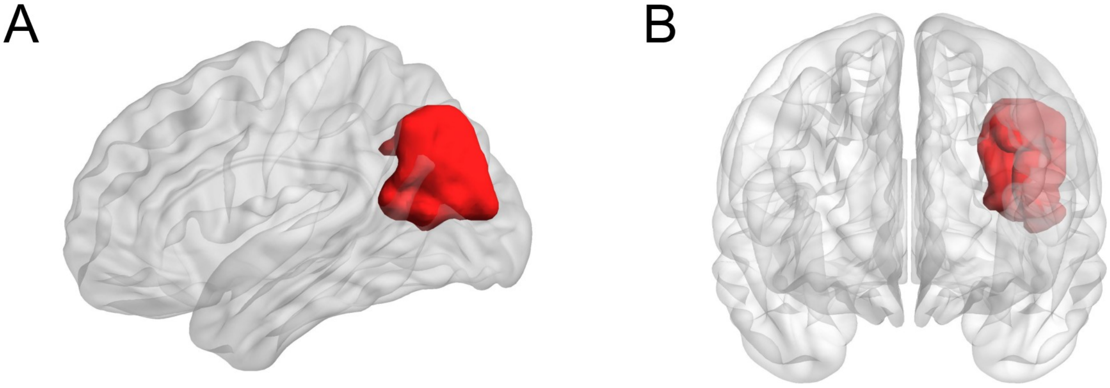

Fig 1. Heading was associated with greater volume of left inferior parietal cortex. (A) Lateral and (B) anterior 3D rendering showing the left inferior parietal cortex in red.

with concussion-like symptoms and worse cognitive function in the short and longer term [5]. Importantly, similar findings have been reported for professional soccer players [7, 8]. The fact that we do not detect gross structural changes related to amateur soccer RHI thus frames prior microstructural and functional findings as an early and potentially remediable point on a continuum, which precedes overt tissue loss due to frank neurodegeneration. Future work will be essential to better understand the nature of soccer-related RHI, potential for recovery of microstructural and functional effects and how long-term risk varies among individuals.

Heading is a complex motor skill, which requires precisely timed coordination of perception (the trajectory and speed of the incoming ball) and response (e.g., proprioception, muscle contraction). Neuroimaging correlates of neuroplasticity in healthy adults have been reported across a range of training paradigms [19]. Learning to juggle, for example, has been associated with localized gray matter expansion at multiple cortical locations including the mid-temporal area and the left intraparietal sulcus [20] as well as with localized increases in FA on DTI. Long-term practice of a musical instrument, has been associated with greater cortical thickness in the right frontal cortex [21]. In light of these and other examples (reviewed in [19]), we hypothesize that our finding of greater left inferior parietal volume in soccer players who head the ball more may represent an effect of heading skill acquisition. Further work would be required to elucidate pathways and mechanisms subserving the development and maintenance of heading skill. Understanding the potential neuroplastic effects of heading is therefore necessary to inform assessment of the effects of RHI. Moreover, increased levels of heading could be a surrogate marker for time playing soccer and, consequently, higher levels of fitness. Since fitness is associated with greater gray matter volume [22], it may represent an alternate explanation for the higher regional brain volumes we detect associated with heading.

Although we found no lower volume associated with RHI, these findings should be considered in light of several limitations. Even though our sample is the largest ever reported for the adult amateur soccer population, it is nonetheless a subset of that population and may not generalize to other groups such as children. We also studied a relatively young adult population. As such, our findings do not preclude brain volume loss due to neurodegenerative disease in later life. While our localized finding of greater brain volume associated with greater exposure to heading is consistent with a neuroplastic response to skill acquisition, our cross-sectional design precludes any explicit causal inference. We estimated exposure to RHI using HeadCount, which has been validated for this purpose [23]. Nonetheless, the possibility of reporting error or bias cannot be entirely excluded [5, 12]. Additionally, even though the population considered here is comprised predominantly of young adult athletes, we have not acquired data or corrected for body mass index effects, which is another potential limitation. Finally, due to the strong right skew of heading exposure, we treated it as a categorical variable (Table 1). Nonetheless, results were similar when analyses were repeated treating heading as a continuous variable.

In conclusion, we observed no adverse association of soccer-related RHI with brain macrostructure in a large sample of adult amateur players. Further studies and longer follow up will be required to determine whether previously reported adverse effects on brain microstructure and function are associated with such changes over the longer term. On the other hand, the highly localized elevation of brain volume we identified as associated with greater heading suggests a neural correlate for the skill acquisition inherent in heading.

Acknowledgments

We thank Dr. Ricardo Magalhães (University of Minho) for help producing the figure.

Author Contributions

Conceptualization: Michael L. Lipton.

Data curation: Tiago Gil Oliveira, Chloe Ifrah, Michael Stockman, Michael L. Lipton.

Formal analysis: Tiago Gil Oliveira, Chloe Ifrah, Roman Fleysher, Michael L. Lipton.

Funding acquisition: Michael L. Lipton.

Investigation: Tiago Gil Oliveira, Roman Fleysher, Michael L. Lipton.

Methodology: Roman Fleysher, Michael Stockman, Michael L. Lipton.

Project administration: Chloe Ifrah, Michael L. Lipton.

Resources: Michael L. Lipton.

Software: Roman Fleysher, Michael Stockman, Michael L. Lipton.

Supervision: Michael L. Lipton.

Validation: Tiago Gil Oliveira, Roman Fleysher, Michael L. Lipton.

Visualization: Tiago Gil Oliveira, Michael L. Lipton.

Writing – original draft: Tiago Gil Oliveira.

Writing – review & editing: Tiago Gil Oliveira, Chloe Ifrah, Roman Fleysher, Michael Stockman, Michael L. Lipton.

References

1. Kunz M. 265 million playing football. FIFA magazine. 2007 July 2007:10–5.
2. Rodrigues AC, Lasmar RP, Caramelli P. Effects of Soccer Heading on Brain Structure and Function. Front Neurol. 2016; 7:38. Epub 2016/04/06. https://doi.org/10.3389/fneur.2016.00038 PMID: 27047444; PubMed Central PMCID: PMC4800441.
3. Rice SG, American Academy of Pediatrics Council on Sports M, Fitness. Medical conditions affecting sports participation. Pediatrics. 2008; 121(4):841–8. https://doi.org/10.1542/peds.2008-0080 PMID: 18381550.
4. Delaney JS, Lacroix VJ, Leclerc S, Johnston KM. Concussions among university football and soccer players. Clin J Sport Med. 2002; 12(6):331–8. https://doi.org/10.1097/00042752-200211000-00003 PMID: 12466687.
5. Lipton ML, Kim N, Zimmerman ME, Kim M, Stewart WF, Branch CA, et al. Soccer heading is associated with white matter microstructural and cognitive abnormalities. Radiology. 2013; 268(3):850–7. https://doi.org/10.1148/radiol.13130545 PMID: 23757503; PubMed Central PMCID: PMC3750422.
6. Hales C, Neill S, Gearing M, Cooper D, Glass J, Lah J. Late-stage CTE pathology in a retired soccer player with dementia. Neurology. 2014; 83(24):2307–9. https://doi.org/10.1212/WNL.0000000000001081 PMID: 25378682; PubMed Central PMCID: PMC4277667.
7. Ling H, Morris HR, Neal JW, Lees AJ, Hardy J, Holton JL, et al. Mixed pathologies including chronic traumatic encephalopathy account for dementia in retired association football (soccer) players. Acta Neuropathol. 2017; 133(3):337–52. https://doi.org/10.1007/s00401-017-1680-3 PMID: 28205009; PubMed Central PMCID: PMC5325836.
8. Koerte IK, Ertl-Wagner B, Reiser M, Zafonte R, Shenton ME. White matter integrity in the brains of professional soccer players without a symptomatic concussion. JAMA. 2012; 308(18):1859–61. https://doi.org/10.1001/jama.2012.13735 PMID: 23150002; PubMed Central PMCID: PMC4103415.
9. Sortland O, Tysvaer AT. Brain damage in former association football players. An evaluation by cerebral computed tomography. Neuroradiology. 1989; 31(1):44–8. https://doi.org/10.1007/BF00342029 PMID: 2717003.
10. Koerte IK, Mayinger M, Muehlmann M, Kaufmann D, Lin AP, Steffinger D, et al. Cortical thinning in former professional soccer players. Brain Imaging Behav. 2016; 10(3):792–8. https://doi.org/10.1007/s11682-015-9442-0 PMID: 26286826.

11. Stewart WF, Kim N, Ifrah CS, Lipton RB, Bachrach TA, Zimmerman ME, et al. Symptoms from repeated intentional and unintentional head impact in soccer players. Neurology. 2017; 88(9):901–8. Epub 2017/02/06. https://doi.org/10.1212/WNL.0000000000003657 PMID: 28148633; PubMed Central PMCID: PMC5331870.

12. Levitch CF, Zimmerman ME, Lubin N, Kim N, Lipton RB, Stewart WF, et al. Recent and Long-Term Soccer Heading Exposure Is Differentially Associated With Neuropsychological Function in Amateur Players. J Int Neuropsychol Soc. 2018; 24(2):147–55. https://doi.org/10.1017/S1355617717000790 PMID: 28829004; PubMed Central PMCID: PMC6554717.

13. Stewart WF, Kim N, Ifrah C, Sliwinski M, Zimmerman ME, Kim M, et al. Heading Frequency Is More Strongly Related to Cognitive Performance Than Unintentional Head Impacts in Amateur Soccer Players. Frontiers in Neurology. 2018; 9(240). https://doi.org/10.3389/fneur.2018.00240 PMID: 29740384

14. Rubin TG, Catenaccio E, Fleysher R, Hunter LE, Lubin N, Stewart WF, et al. MRI-defined White Matter Microstructural Alteration Associated with Soccer Heading Is More Extensive in Women than Men. Radiology. 2018; 289(2):478–86. https://doi.org/10.1148/radiol.2018180217 PMID: 30063172; PubMed Central PMCID: PMC6209057.

15. Lipton ML, Ifrah C, Stewart WF, Fleysher R, Sliwinski MJ, Kim M, et al. Validation of HeadCount-2w for estimation of two-week heading: Comparison to daily reporting in adult amateur player. J Sci Med Sport. 2018; 21(4):363–7. https://doi.org/10.1016/j.jsams.2017.08.008 PMID: 28882435; PubMed Central PMCID: PMC5826783.

16. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage. 2006; 31(3):968–80. https://doi.org/10.1016/j.neuroimage.2006.01.021 PMID: 16530430.

17. Buckner RL, Head D, Parker J, Fotenos AF, Marcus D, Morris JC, et al. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. Neuroimage. 2004; 23(2):724–38. https://doi.org/10.1016/j.neuroimage.2004.06.018 PMID: 15488422.

18. Xia M, Wang J, He Y. BrainNet Viewer: a network visualization tool for human brain connectomics. PLoS One. 2013; 8(7):e68910. https://doi.org/10.1371/journal.pone.0068910 PMID: 23861951; PubMed Central PMCID: PMC3701683.

19. Valkanova V, Eguia Rodriguez R, Ebmeier KP. Mind over matter—what do we know about neuroplasticity in adults? Int Psychogeriatr. 2014; 26(6):891–909. Epub 2014/01/03. https://doi.org/10.1017/S1041610213002482 PMID: 24382194.

20. Draganski B, Gaser C, Busch V, Schuierer G, Bogdahn U, May A. Neuroplasticity: changes in grey matter induced by training. Nature. 2004; 427(6972):311–2. https://doi.org/10.1038/427311a PMID: 14737157.

21. Bermudez P, Lerch JP, Evans AC, Zatorre RJ. Neuroanatomical correlates of musicianship as revealed by cortical thickness and voxel-based morphometry. Cereb Cortex. 2009; 19(7):1583–96. https://doi.org/10.1093/cercor/bhn196 PMID: 19073623.

22. Erickson KI, Leckie RL, Weinstein AM. Physical activity, fitness, and gray matter volume. Neurobiol Aging. 2014; 35 Suppl 2:S20–8. https://doi.org/10.1016/j.neurobiolaging.2014.03.034 PMID: 24952993; PubMed Central PMCID: PMC4094356.

23. Catenaccio E, Caccese J, Wakschlag N, Fleysher R, Kim N, Kim M, et al. Validation and calibration of HeadCount, a self-report measure for quantifying heading exposure in soccer players. Res Sports Med. 2016; 24(4):416–25. https://doi.org/10.1080/15438627.2016.1234472 PMID: 27788599; PubMed Central PMCID: PMC5144162.

5

Salivary endocrine response following a maximal incremental cycling protocol with local vibration

Monèm Jemni[1,2]*, **Michel Marina**[3], **Anne Delextrat**[4], **Amy Tanner**[5], **Fabien A. Basset**[6], **Yaodong Gu**[1]*, **Qiuli Hu**[1], **Huiyu Zhou**[1], **Bessem Mkaouer**[7], **Ferman Konukman**[8]

1 Faculty of Sports Science, Ningbo University, Zhejiang, China, **2** The University of Cambridge—Institute of Continuing Education, Cambridge, United Kingdom, **3** INEFC, Barcelona, Spain, **4** Department of Sport, Health Sciences and Social Work, Oxford Brookes University, Oxford, United Kingdom, **5** School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom, **6** School of Human Kinetics and Recreation, Memorial University of Newfoundland, St. John's, Canada, **7** Higher Institute of Sport and Physical Education of Ksar Saïd, Manouba University, Manouba, Tunisia, **8** Sport Science Program, College of Arts and Science, Qatar University, Doha, Qatar

* monemj@hotmail.com, mj557@cam.ac.uk (MJ); guyaodong@hotmail.com (YG)

Abstract

The aim of this study was to compare the effects of vibration (Vib versus noVib) during a maximal graded cycling exercise on hormonal response, precisely on cortisol (C) and testosterone (T). Twelve active males (25 ± 5yrs; 181 ± 5cm; 80.7 ± 11.1kg) randomly performed two maximal incremental cycling tests on two separate days and at the same time of the day (09:00). The protocol consisted of incremental steps of 3 min duration performed on a PowerBIKE^{TM} that induces vibration cycling. The study was a repeated measures design and participants performed the test with and without vibration. Gas exchange and heart rate (HR) were continuously assessed and blood lactate (Bla) was recorded at the end of each incremental stage. Saliva samples were collected before and immediately after the test, and analysed for (C) and (T).

The results show that C and T increased in both cycling conditions; however, the C's magnitude of change was significantly higher by 83% after Vib cycling in comparison to the no Vib ($p = 0.014$), whereas the T's magnitude of change were not statistically different between trials ($p = 0.715$). Vibration induced a decrease of the T/C ratio ($p = 0.046$) but no significant changes were observed following noVib ($p = 0.476$). As a conclusion, the investigation suggests that adding mechanical vibration to cycling may potentiate a catabolic exercise-induced state, which could have potential clinical implications in rehabilitation and injury treatment. Sport experts should take this message home to carefully plan the recovery process and time during training and competitions.

Editor: Dalton Müller Pessôa Filho, São Paulo State University (UNESP), BRAZIL

Competing interests: The authors have declared that no competing interests exist.

Introduction

Local body vibration (LBV) during dynamic activity has only recently been applied to cycling exercise. Early and recent research on vibration cycling has reported a significant decrease in exercise duration compared to cycling-only trials [1]. However, more recent and meticulously controlled trials have indicated no differences in cardiorespiratory and metabolic variables between vibration cycling and cycling-only exercises, except a higher ventilation in favour of the vibration cycling [2]. These recent finding did not confirm prior studies who suggested higher rates of oxygen uptake, possibly due to vibration-induced activation of afferent neurons causing contraction of inactive muscle fibres [1, 3]. Note that there is a difference between whole body vibration exercise/training using platforms in comparison to vibration cycling which is the context of this study. Whole body vibration exercise showed consistent significant improvement of muscles' viscoelasticity and flexibility [4] whereas studies investigating its effect on strength are not unanimous, with some showing positive effects [4–6] and others showing the opposite [7] or no effect [4, 8].

Most of the studies examining the hormonal responses induced by vibration during training have been conducted on resistance training and have reported conflicting results, such as an elevation of the plasma testosterone [9], plasma cortisol [10] or no change [11–13]. These mixed results might stem from an insufficient exercise stimulus to elicit a change in hormonal response [14]. Therefore, it seems rational to investigate the hormonal response during high-intensity aerobic exercise. This study is indeed the first of a series of investigations that highlight several effects of different exercise regimes on the hormonal regulation with a particular focus on testosterone and cortisol.

During acute stress, activation of the sympathetic nervous system occurs, with the release of catecholamines and concomitantly, the hypothalamic-pituitary-adrenal (HPA) axis is stimulated and cortisol is released [15]. Cortisol plays a permissive role on catecholamines and glucagon in stimulation of gluconeogenesis and mobilisation of free fatty acids to initiate glucose maintenance [16], this is particularly important in response to stress and may reflect the metabolic demand of an exercise bout [17]. Furthermore, short term, acute stress has also been shown to increase circulating levels of testosterone [18]. Previous work has established that an incremental test to exhaustion elicits an increase in salivary cortisol [19, 20] and correlates with blood lactate measurements [19]; with suggestion that lactate may activate chemoreceptors in the working muscles and stimulates the HPA axis [21]. Furthermore, an increase in salivary cortisol and testosterone was observed after a short duration high intensity cycling bout [22, 23]. Consistently, studies looking at aerobic exercise have demonstrated increases in cortisol and testosterone; whereas, there is considerable variability in results following resistance based exercise for cortisol and power based exercise for testosterone [24]. As such, high-intensity exercises, such as speed endurance maintenance and speed endurance production lead to a significant acute increase in circulating cortisol levels [25]. However, the same authors did not confirm a long-term trend but rather a slow decrease of the cortisol production after repeated sprints training. Testosterone has been shown to increase few minutes post adequately stimulating resistance exercise. According to (Kraemer and Ratamess 2005 [26], high volume protocols, moderate to high in intensity, and exercise regimes that incorporate short rest intervals and involve a large muscle mass, tend to produce the greatest acute hormonal elevations (testosterone, Growth Hormone and Cortisol) when compared to low-volume, high-intensity protocols incorporating long rest intervals.

A recent study showed that vibration-induced cycling did not increase energy demands [2]. However, the question that remains unanswered is what are the effects of vibration on cortisol and other markers of metabolic stress during maximal exercise. Therefore, the aim of the study was to assess the impact of added vibration to cycling using an incremental exercise test on the salivary endocrine response compared to normal cycling. Our hypothesis states that added vibration stimuli to cycling would increase the body's endocrine response, in particular, the cortisol.

Methods

Participants

A call for participation in a research project was publicized across the University of Greenwich (London, UK) sports clubs via the social media network in June 2014. The inclusion criteria were: males only, recreationally active by performing at least three hours/week of sport for at least one year, age range between 18–28 years old, not suffering from any cardiorespiratory diseases, no family history of similar diseases. Exclusion criteria were: females, inactive and/or sedentary males, younger than 18 or older than 28 years old, suffering from any cardiorespiratory diseases, family history of similar diseases.

Twelve males agreed to take part in the study. Most of them were students but also staff members working across the campus. All have been seen by the main investigator and verbally briefed about the study and the risks associated. Written information and instructions were given to them to take away and written consent for participation was obtained from each participant. The study was approved by the University of Greenwich ethics committee (Ref: UoG-EC-FSE-SS-J-L-35-20/1/2014) and the research took place at the Faculty of Science and Engineering labs between July and September 2014.

Participants' anthropometrics characteristics were: age 25 ± 5 yrs; height 181 ± 5cm and weight 80.7 ± 11.9kg.

Testing procedure

Each participant performed two maximal incremental cycling exercise tests, one with vibration (Vib) and one without vibration (noVib) in a randomised order, on separate days and at similar times of the day (09:00). Tests were performed at the same time of the day, with a recovery period of 72 hours in between. Participants continued their habitual training regimen during the study period; however, they were asked to refrain from eating two hours prior to all trials, and from strenuous exercise, caffeine and alcohol consumption in the 24 hours before each trial. All participants were invited for a screening questionnaire as well as a familiarization session and to randomise the trials a few days prior the testing day.

All exercise tests were performed on the PowerBIKE (Power Plate, Netherlands), a stationary bike that induces Vib cycling or normal cycling. The protocol was a speed-based and similar to a previously described study [27]. After a 4 minute warm up at 70 RPM at (4th gear) cadence was increased 10 RPM every three minutes until volitional exhaustion (Fig 1). The PowerBIKE was set according to participants' anatomy: the height of the seat, distance between the handle bar and the seat, seat to centre of the crank, handle bar to the centre of the crank and handle bar to floor, recorded and applied identically at each trial.

Physiological measurements

Gas exchange was continuously assessed with an online gas analyzer Vacumed Metabolic Measurement System (Metamax, Cortex, Germany) monitored by a TurboFit software, V. 5.0 (USA). A five μL blood sample was collected from the fingertip at rest and during the last 30 seconds of each exercise stage. Samples were analysed for blood lactate (BLa, $\text{mmol}\bullet\text{l}^{-1}$) concentration using a lactate analyser (Biosen EKF diagnostic, Germany). Heart rate (HR, $\text{beats}\bullet\text{min}^{-1}$) was continuously monitored using a HR monitor (Polar, Finland) and averaged for the last 30 seconds of each stage; maximal HR was recorded at the end of each test (HR_{max}).

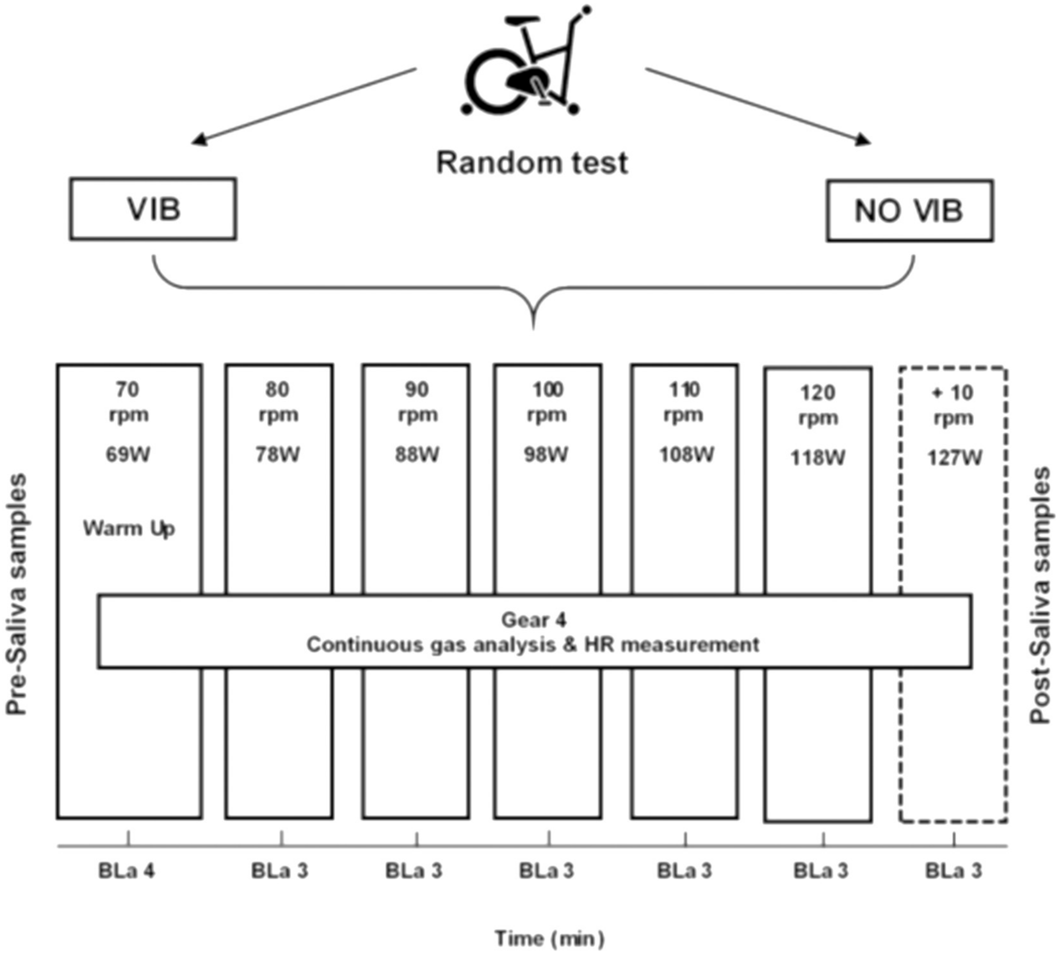

Fig 1. Flow chart of the experimental study. Graded exercise cycling tests to the maximum on PowerBike.

Saliva collection and cortisol and testosterone measurement

Saliva samples were collected pre-exercise and immediately post-exercise during each trial. Hence, each participant had four saliva samples. Each subject was required to attend his tests sessions at the same time of the day to avoid diurnal variation. Salivary measurement was chosen as there is evidence that salivary cortisol and teststerone levels offer a more sensitive assessment of the response to exercise than changes in blood concentration [22, 23]. Participants were required to stop drinking ten minutes before each sample collection to avoid dilution. Participants provided a stimulated saliva sample into a sterile container, with Parafilm to chew on to increase flow, since cortisol and testosterone are unaffected by saliva flow rate [28, 29]. Prior to collection participants were instructed to chew for one minute before swallowing any saliva in the oral cavity. The sampling time was three minutes to allow collection of a sufficient saliva volume. Samples were centrifuged at 3000rpm, divided into four aliquots and stored at -20˚C. Saliva was analysed for cortisol and testosterone with a commercially available ELISA kits (Salimetrics, State College, PA, USA). The sensitivity of the kit was 0.029 ng•ml^{-1} for cortisol and 1 pg•ml^{-1} for testosterone. The mean intra assay coefficients of variation were 8.3% for cortisol and 8.6% testosterone for duplicate samples.

Statistical analysis

The Shapiro-Wilk test confirmed the normal distribution of the data (p: 95% CI [0.422, 0.681]) enabling parametric analysis of the variables. Data is presented as mean and standard deviation. Cortisol, testosterone, HR and BLa differences, between pre-exercise and post-exercise as well as between Vib and noVib cycling conditions were assessed with a Two-Way (2 trials x 2 conditions) analysis of variance for repeated measures (ANOVA). The Greenhouse-Geisser's correction factor was applied if the sphericity test for proportionality of the dependent variable was significant ($p < 0.05$). Bonferroni *post hoc* adjustments were used for multiple comparisons and partial eta squared (η2) was used to report effect size.

Note that only nine participants were included in the hormonal analysis because of technical errors. The magnitude of changes induced by the cycling tests were calculated for cortisol, testosterone and T/C ratio by subtracting post-exercise values from pre-exercise. These were thereafter expressed as raw differences as well as in percentage relative to baseline (relative increments). Cohen's d test with Hedges' g correction were used to report effect size when pairwise comparing (Vib/NoVib). Statistical analysis was undertaken using SPSS statistics package software version 20 for Windows® (IBM, NY, USA). Statistical significance was accepted at $p \leq 0.05$.

Results

Heart rate

Resting HR was not different before both trials ($p = 0.98$). Maximal values (HR_{max}) did not statistically differ between the Vib and the no Vib trials (177 ± 13 and 181 ± 16 BPM respectively) at the end of the tests.

Maximal oxygen uptake ($\dot{V}O_{2max}$)

$\dot{V}O_{2max}$ did not significantly differ ($p = 0.1631$; $d = 0.60$) between Vib and no Vib trials (34.32 ± 9.7 and 40.11 ± 9.49 ml•min^{-1}•kg^{-1}, respectively) although they were reached at an average of 100 RPM with Vib and 120 RPM without Vib (98W after 13 min cycling and 118W after 19 min cycling respectively).

Blood lactate (BL)

The maximal BLa concentration (BLa_{max}) measured at the end of each trial was significantly higher ($p < 0.05$; $d = 0.87$) after the Vib trial (14.05 ± 2.86 mmol•l^{-1}) compared to no Vib (11.31 ± 3.44 mmol•l^{-1})

Salivary cortisol

Baseline cortisol levels were not significantly different before Vib and noVib ($p = 0.980$). ANOVA analysis revealed that the cortisol level increased significantly after both cycling tests (Vib and noVib) (Table 1) (Fig 2). Moreover, an interaction between the trial and vibration factors suggest a differentiated pattern of change when both factors are combined. The increase in cortisol concentration was significantly higher after the Vib cycling test in comparison to the no Vib. Cortisol increased by 83% after the Vib trial (Fig 2A). Individual comparative tests confirmed this observation for both, the absolute increment ($p = 0.014$) (Fig 1B; $p = 0.022$) and relative increment with respect to baseline condition (Fig 4B).

Table 1. Statistical analysis of the raw data for the cortisol, testosterone and their ratio pre- and post-cycling with or without vibration.

Variable	Effect	*F*	*df*	*P*	η2	Post-hoc	*P*
Cortisol	Oc x Vb	9.91	1, 8	0.014	0.55	Vb: $O_{c2} > O_{c1}$	0.001
						nVb: $O_{c2} > O_{c1}$	0.001
	Oc	43.09	1, 8	0.001	0.84	$O_{c2} > O_{c1}$	0.001
	Vb	1.54	1, 8	0.240	0.16		
Testosterone	Oc x Vb	3.16	1, 8	0.113	0.28		
	Oc	81.52	1, 8	0.001	0.91	$O_{c2} > O_{c1}$	0.001
	Vb	0.04	1, 8	0.839	0.01		
Ratio T/C	Oc x Vb	21.07	1, 8	0.002	0.73	Vb: $O_{c2} > O_{c1}$	0.046
						nVb: $O_{c2} \approx O_{c1}$	0.476
	Oc	1.38	1, 8	0.274	0.15		
	Vb	0.76	1, 8	0.408	0.09		

Oc : occasion; Vb : vibration; nVb : no vibration

Salivary testosterone

Baseline testosterone levels were not significantly different before Vib and noVib ($p = 0.392$). Salivary testosterone increased by 29% after Vib and 56% after noVib cycling test (Fig 3). Statistical analysis revealed that testosterone level increased significantly after both cycling tests (Vib and noVib) (Table 1); however, there was no statistical difference in magnitude of change between trials ($p = 0.715$) (Fig 3B).

Paired tests confirmed that the magnitude of the absolute ($p = 0.116$) and the relative increments with respect to baseline ($p = 0.187$) were similar after the two cycling tests (Fig 4B).

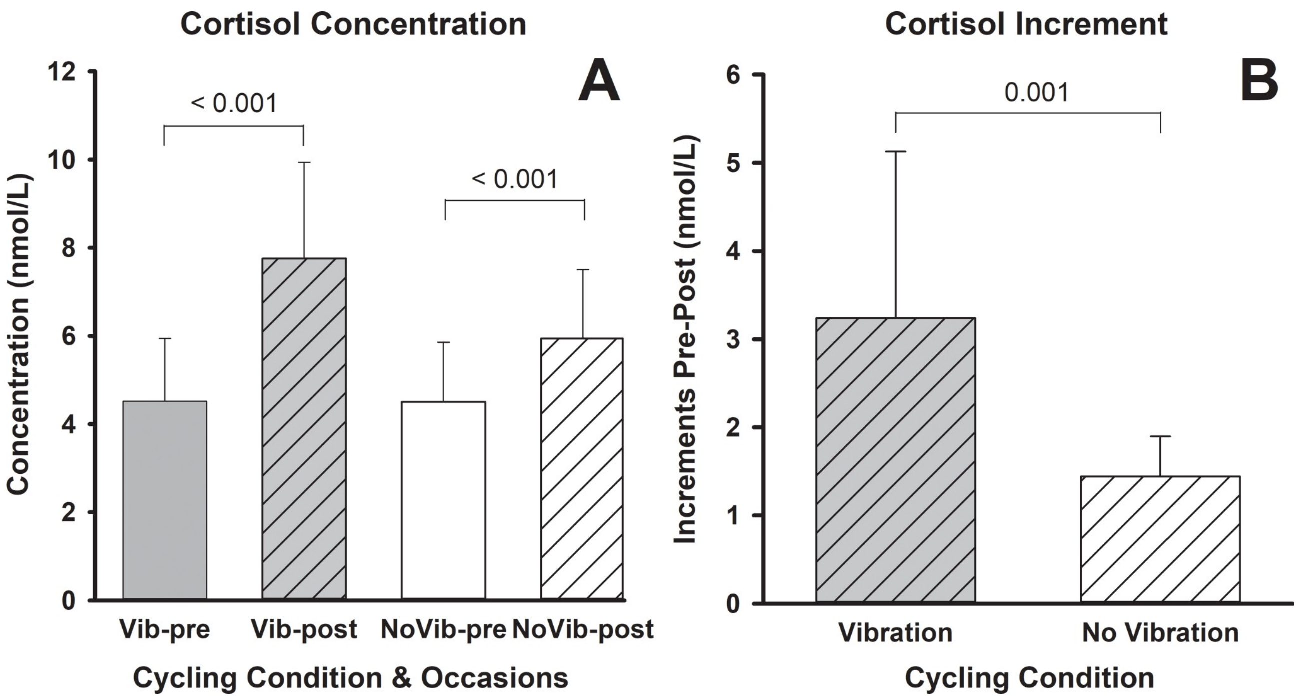

Fig 2. Salivary cortisol pre- and post-maximal graded exercise with and without vibration (A) and their respective magnitude of change (B). S: ($p \leq 0.05$).

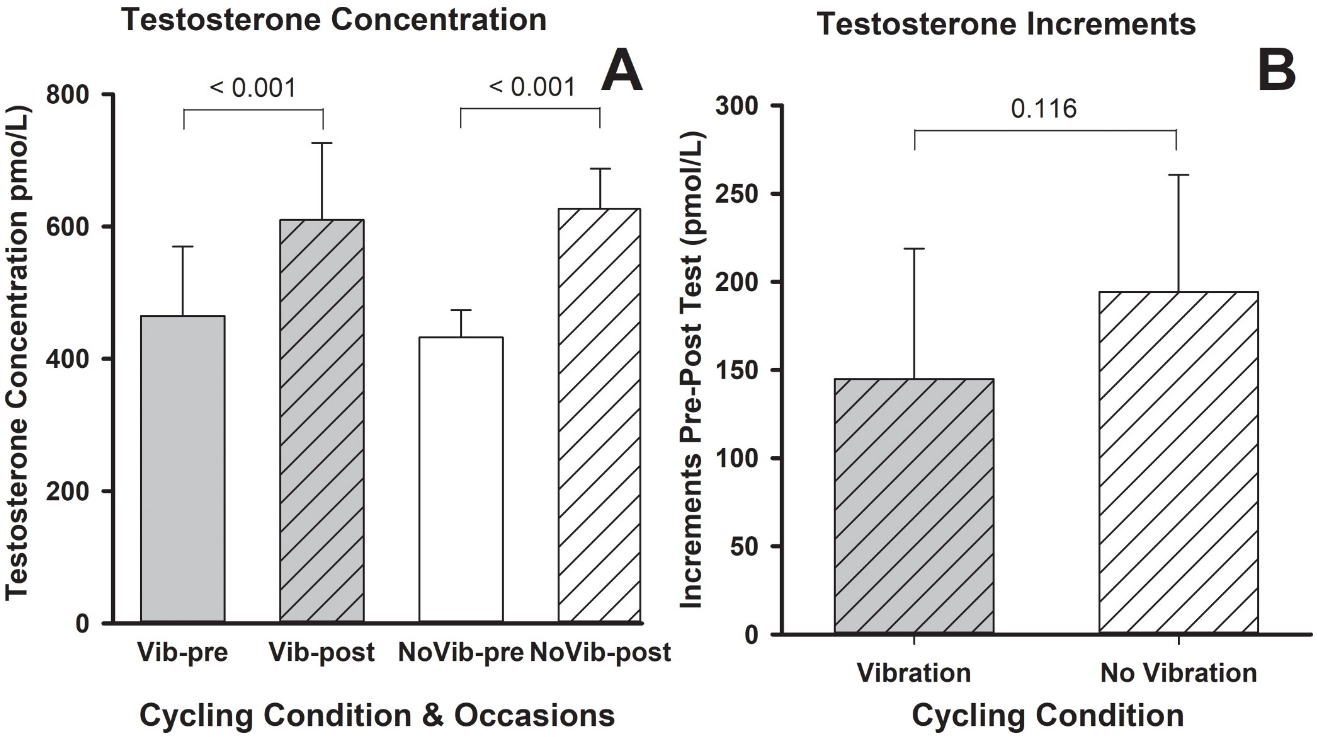

Fig 3. Salivary testosterone pre- and post-maximal graded exercise with and without vibration (A) and their respective magnitude of change (B). S: ($p \leq 0.05$).

Testosterone/cortisol (T/C) ratio

Baseline T/C ratios were not significantly different before Vib and noVib ($p = 0.825$). A significant main effect confirmed that Vib induced a decrease of the T/C ratio ($p = 0.046$) whereas no significant changes were observed following noVib ($p = 0.476$) (Table 1; Fig 4A).

There was a significant difference in the absolute ($p < 0.002$) and relative ($p \leq 0.001$) change in T/C ratio following the Vib trial compared to noVib (Fig 4).

Discussion

The main hypothesis of this study was that added vibration stimuli to cycling would increase the salivary endocrine response, in particular, the cortisol level. Larger and significant cortisol increase associated to a larger and significant T/C ratio decrease were noticed in the Vib trial compared to noVib. While some authors have reported decrease in constant-load cycling duration with the addition of a vibratory stimulus [1] others did not identified this trend [3]. Exercising with vibration has been hypothesised to recruit more motor neurons [30], and it has been suggested that full activation of the muscle may lead to a quicker motor unit fatigue [31, 32] and as a result contribute to an earlier onset of fatigue in Vibration exertions [33]. Bongiovanni and al. [34] argued that the decreased ability to generate high firing rates in high threshold motor units may cause the inability to sustain exercise. Another reason for this difference may be a higher energy demand with the addition of Vib, contributing to an increase in the ATP hydrolysis [35]. However, this study did not show any difference in the $\dot{V}O_{2\ max}$, hence re-enforces our latest findings that adding vibration to cycling did not induce a greater cardiorespiratory response compared to normal cycling [2]. Nonetheless, we acknowledge the non-negligible difference in $\dot{V}O_{2max}$ between the trials (more than—15%). A potential protocol

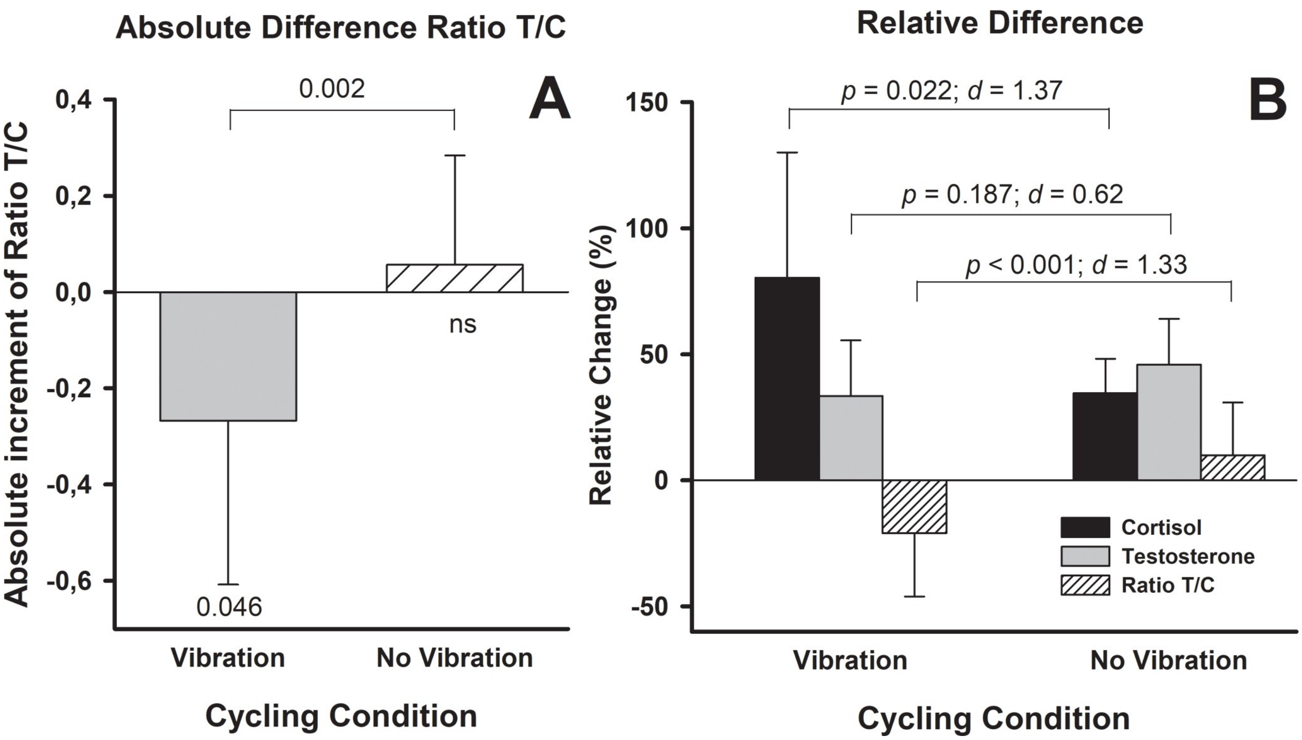

Fig 4. Absolute increments of the testosterone and cortisol ratios induced by the cycling graded exercise with and without vibration (A). Pairwise comparisons of the relative differences with respect to baseline (expressed in %) between the two cycling conditions (B).

effect could have been masked by not only the inter-participants' variability but also by the limited sample size (only 12 participants). The participants in the present study were recreationally active, undertaking a mixture of aerobic and weight training on average of three days per week; therefore, they do not represent an elite or competitive athlete population and as a consequence, these results should not be translated to a more highly trained cohort and neither to unfit or unhealthy indivuduals. Yet, the results could have some implications for a much wider and general population whose objective is to increase fitness for various reasons (e.g., quality of life). On the other side, one could question the higher BLa following the Vib trial compared to no Vib; this could undeniably be explained by the fact that the participants have perceived the Vib cycling stronger than no Vib as it was previously demonstrated by Filingerie et al in 2012 [27].

The results of this study show an increase in cortisol and testosterone in both trials. Similar high-intensity protocols have also triggered increased cortisol levels following acute exercise bouts [19, 22, 36]. Nevertheless, we do not know about the existence of previous research focused on cortisol levels in response to cycling with vibration. The present study supports observations of an increase in cortisol with vibration added up to resistance exercise [10]. Some scientists have hypothesised an increase in cortisol to be linked with blood lactate levels [19, 21, 37]. Given the observed higher blood lactate of the Vib trial in our study, this may have activated the HPA axis and led to an increase in cortisol concentration.

Salivary testosterone increased in both trials of the present study. It has been hypothesised that possible mechanisms for an increase in testosterone, as a result of exercise, include increased production by sympathetic stimulation of the testes [38]. Furthermore, activation of the sympathetic nervous system and increased lactate accumulation may have contributed to the increase in testosterone concentration, although supporting evidence is limited to rats

[39]. Protein binding affinity of the testosterone may be affected by changes in PH and temperature elicited by exercise, this in turn may lead to a higher free proportion of cortisol and testosterone in the blood and increased levels in saliva [40, 41]. However, a more recent study showed no binding affinity of testosterone changes after endurance exercise [38] and further research is surely required.

Only very few studies have examined the response to vibration on testosterone levels. Bosco et al. [9] demonstrated an increase in plasma testosterone following ten 60-second bouts on a vibration platform. However, as the present study showed no significant additive effect of vibration on salivary testosterone levels, hence these findings cannot be supported. It is worth to mention that Bosco and his colleagues [4] have used a whole body vibration whereas we only used localised vibration induced by the cycling gear through the lower limbs. The number of stimulated neuromuscular units could have made this difference.

There is suggestion that testosterone may also contribute to muscular repair and growth in response to training, however its role in this process has not been confirmed [42]. Owing to the absence of a bigger increment of testosterone as a result of vibration in the present study, and limited evidence to form a clear consensus, further research in this area is also warranted. Should we admit that testosterone is just less sensitive to small changes in demand?

The T/C ratio showed a trend towards a decrease in the Vib trial, this is likely to be due to the higher increase in cortisol after Vib. This decrease suggests that following Vib induced cycling the body was in a more catabolic state and perhaps a higher acute response was initiated with the addition of Vib. In support, a meta-analysis examining T/C in response to aerobic exercise showed a consistent decrease in the ratio which was primarily due to the magnitude of the salivary cortisol response [24]. However, the T/C ratio has proven to be more useful when considering the link with overreaching and overtraining; including absolute values, comparison of consecutive measurements during a season or changes in relation to baseline. Monitoring the T/C ratio in athletes has the potential to be used as a tool to diagnose overtraining syndrome while taking into account other clinical measurements [43]. Previous research has recognised that the mode of exercise and volume of stimulus is important in the hormonal response [17, 37]; therefore, it is currently difficult to make valid comparisons between vibration studies, given the use of many different protocols. Nonetheless, there is a increasing clinical interest about vibration mainly following the confirmed positive effects on muscles' viscoelasticity [4]. Combining vibration and exercise could see further applications in the medical sector where exercise alone has been suggested to prevent and to treat/rehabilitate certain conditions.

The study has few methodological considerations/limitations. Firstly, it has been carried out with recreational active subjects allowing statistical inference to a normal active and healthy population. These results cannot be extrapolated to neither elite oriented athletes nor unfit or unhealthy individuals, as it was not the aim of the present investigation. In addition, seeing the small sample size, it cannot be considered representative of a larger population. Secondly, saliva samples were taken only once before and just after the two trials (Vib and noVib cycling), while mostly the peak salivary cortisol and testosterone levels occur immediately post exercise, they have been shown to peak later in some individuals as a result of intermittent exercise [17]. Although our protocol was based on only one single long cycling bout, it could be worth investigating post-exercise peak hormone values in future studies. However, we have tried to double limit the C and T circannual variations by collecting the samples not only at the same time of the day for each participant but also within a minimum time (12 weeks). A recent study has indeed demonstrated that C, T and T/C incur significant circannual variations in professional footballer players, with the cortisol increasing during winter (February) while testosterone and T/C peak in the summer months (July) [44]. Lastly, it is important to state that the increment between the test stages was not the same in comparison to the study we published in 2019 [2]. Two different bikes were used in Jemni et al, 2019 (Lode Corvical and PowerBike), where 1 watt increment was equivalent to 18 ml/min. However, in this study the bike was the same, i.e the PowerBike, where 1 watt increment provoked an equivalent (27.5 ml/min). We presume that the higher value was due not only to the ergonomics difference between the bikes but also to the fitness levels of the different studies' cohorts. Jemni 2019 participants were moderately trained male subjects with six to eight hours of training per week (amongst them few cyclists), whereas this study involved few students and staff members from the university campus who were slightly less active.

The results discussed in the present investigation justify further research focused on vibration exertion to better understand the complex relationship between hormonal, metabolic, cardiovascular, respiratory and neurophysiological markers. Moreover, biomechanical and electromyographic assessments could help to better understand the pattern of these markers and their reciprocal influence with metabolic cost and muscle activation.

Finally, the fact that vibration cycling induced a higher acute catabolism in this investigation could lead to further practical implications either in the exercise training context or in the clinical sector where such state is required. Making the competition weight in certain sports or purposely elevating the catabolism state in certain health conditions could be of interest.

Conclusion

The main aim of this study was to examine the salivary endocrine response to vibration cycling exercise. We were expecting a higher cortisol response compared to the no vibration condition. Overall, it appears that a Vib cycling trial elicited a greater cortisol increase. Therefore, the null hypothesis is not accepted.

Cortisol increased dramatically after the Vib cycling and was significantly higher in comparison to the no Vib. Salivary testosterone has also increased after Vib cycling; however, there was no statistical difference in the magnitude of change between the trials. Vib induced a significant decrease of the T/C ratio whereas no significant changes were observed following noVib.

The study suggest that adding mechanical vibration to cycling may potentiate a catabolic exercise-induced state. Sport experts and mainly cyclists should take this message home to carefully plan the recovery process and time during training and competitions. The overall results could have potential clinical implications for older individuals and those undergoing rehabilitation from illness and injury. Future research should investigate the longer-term response to cycling exercise with added vibration.

Acknowledgments

We would like to thank all the participants who took part in this investigation. A particular thank to Mr Uchenna Nnadi and Ms Lavon Jackson who have substantially helped in the data collection. Also, our thank goes to the Sport Science Department at Greenwich University, London, UK who logistically supported the study together with K. C. Wong Magna Fund at Ningbo University for their continuing encouragement.

Author Contributions

Conceptualization: Monèm Jemni, Ferman Konukman.

Data curation: Michel Marina, Anne Delextrat, Amy Tanner, Fabien A. Basset, Qiuli Hu, Huiyu Zhou, Bessem Mkaouer.

Formal analysis: Michel Marina, Amy Tanner, Bessem Mkaouer.

Investigation: Monèm Jemni, Amy Tanner.

Methodology: Monèm Jemni.

Software: Amy Tanner.

Supervision: Monèm Jemni, Yaodong Gu, Ferman Konukman.

Validation: Monèm Jemni, Yaodong Gu, Ferman Konukman.

Visualization: Michel Marina, Anne Delextrat, Amy Tanner, Fabien A. Basset, Yaodong Gu, Qiuli Hu, Huiyu Zhou, Bessem Mkaouer, Ferman Konukman.

Writing – review & editing: Monèm Jemni, Michel Marina, Anne Delextrat, Fabien A. Basset, Yaodong Gu, Qiuli Hu, Huiyu Zhou, Bessem Mkaouer, Ferman Konukman.

References

1. Samuelson B, Jorfeldt L, Ahlborg B. Influence of Vibration on Work Performance during Ergometer Cycling. Ups J Med Sci. 1989; 94(1):73–9.
2. Jemni M, Gu Y, Hu Q, Marina M, Fessi MS, Moalla W, et al. Vibration cycling did not affect energy demands compared to normal cycling during maximal graded test. Frontiers in physiology. 2019; 10:1083.
3. Sperlich B, Kleinoeder H, De Marées M, Quarz D, Linville J, Haegele M, et al. Physiological and perceptual responses of adding vibration to cycling. J Exerc Physiol Online. 2009; 12:40–6.
4. Jemni M, Mkaouer B, Marina M, Asllani A, Sands WA. Acute vibration-induced stretching enhanced hamstring and quadriceps viscoelasticity but did not affect maximal voluntary contractions in footballers. J Strength Cond Res. 2014; 28(11):3105–14.
5. Cochrane DJ and Stannard SR. Acute whole-body vibration training increases vertical jump and flexibility performance in elite female field hockey players. Br J Sports Med 2005; 39: 860–866.
6. Marshall L.C., and Wyon M.A. The effect of whole-body vibration on jump height and active range of motion in female dancers. J Strength Cond Res. 2012; 26(3): 789–793.
7. Herda T.J., Ryan E.D., Smith A.E., Walter A.A., Bemben M.G., Stout J.R., JT Cramer. Acute effects of passive stretching vs vibration on the neuromuscular function of the plantar flexors. Scand J Med Sci Sports. 2009; 19: 703–713.
8. Kinser AM, Ramsey MW, O'Bryant HS, Ayres CA, Sands WA, and Stone MH. Vibration and stretching effects on flexibility andexplosive strength in young gymnasts. Med Sci Sports Exerc. 200; 40: 133–1408.
9. Bosco C, Iacovelli M, Tsarpela O, Cardinale M, Bonifazi M, Tihanyi J, et al. Hormonal responses to whole-body vibration in men. Eur J Appl Physiol. 2000; 81(6):449–54.
10. Kvorning T, Bagger M, Caserotti P, Madsen K. Effects of vibration and resistance training on neuromuscular and hormonal measures. Eur J Appl Physiol. 2006; 96(5):615–25.
11. Erskine J, Smillie I, Leiper J, Ball D, Cardinale M. Neuromuscular and hormonal responses to a single session of whole body vibration exercise in healthy young men. Clin Physiol Funct Imaging. 2007; 27 (4):242–8.
12. Cardinale M, Leiper J, Erskine J, Milroy M, Bell S. The acute effects of differentwhole body vibration amplitudes on the endocrine system of young healthy men: a preliminary study. Clin Physiol Funct Imaging. 2006; 26(6):380–4.
13. Chen W, Wu C, Cai Z. Effect of one bout of local vibration exercise with blood flow restriction on neuromuscular and hormonal responses. Physiology international. 2018; 105(2):166–76.
14. Vuorimaa T, Ahotupa M, Hakkinen K, Vasankari T. Different hormonal response to continuous and intermittent exercise in middle-distance and marathon runners. Scand J Med Sci Sports. 2008; 18 (5):565–72.
15. Bowden JA, Colosi DM, Mora-Montero DC, Garrett TJ, Yost RA. Enhancement of chemical derivatization of steroids by gas chromatography/mass spectrometry (GC/MS). Journal of Chromatography B. 2009; 877(27):3237–42.
16. Salway JG. Medical Biochemistry at a Glance (2nd ed.). Oxford: Blackwell.; 2006.

17. Tanner AV, Nielsen BV, Allgrove J. Salivary and plasma cortisol and testosterone responses to interval and tempo runs and a bodyweight-only circuit session in endurance-trained men. Journal of Sports Sciences. 2014; 32(7):680–9.

18. Sutton J, Coleman M, Casey J, Lazarus L. Androgen responses during physical exercise. Br Med J. 1973; 1(5852):520.

19. Port K. Serum and saliva cortisol responses and blood lactate accumulation during incremental exercise testing. Int J Sports Med. 1991; 12(5):490–4.

20. Rahman ZA, Abdullah N, Singh R, Sosroseno W. Effect of acute exercise on the levels of salivary cortisol, tumor necrosis factor-alpha and nitric oxide. J Oral Sci. 2010; 52(1):133–6.

21. Farrell PA, Garthwaite TL, Gustafson AB. Plasma adrenocorticotropin and cortisol responses to submaximal and exhaustive exercise. J Appl Physiol. 1983; 55(5):1441–4.

22. Crewther BT, Lowe TE, Ingram J, Weatherby RP. Validating the salivary testosterone and cortisol concentration measures in response to short high-intensity exercise. J Sports Med Phys Fitness. 2010; 50 (1):85–92.

23. Obminski Z, Stupnicki R. Comparison of the testosterone-to-cortisol ratio values obtained from hormonal assays in saliva and serum. J Sports Med Phys Fitness. 1997; 37(1):50–5.

24. Hayes LD, Sculthorpe N, Herbert P, Baker JS, Hullin DA, Kilduff LP, et al. Resting Steroid Hormone Concentrations in Lifetime Exercisers and Lifetime Sedentary Males. Aging Male. 2015; 18(1):22–6.

25. Vitale JA, Povìa V, Belli E, Lombardi G, Banfi G, La Torre A. Are two different speed endurance training protocols able to affect the concentration of serum cortisol in response to a shuttle run test in soccer players? Res Sports Med. 2020; 28(2):293–301.

26. Kraemer WJ, Ratamess NA. Hormonal responses and adaptations to resistance exercise and training– A review. Sports Med. 2005; 35(4):339–61.

27. Filingeri D, Jemni M, Bianco A, Zeinstra E, and Jimenez A. The effects of vibration during maximal graded cycling exercise: A pilot study. J. Sport Sci. Med. 2012; 11:423–429.

28. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoendocrine research: recent developements and applications. Psychoneuroendocrinology. 1994; 19:313–33.

29. Tabata I, Ogita F, Miyachi M, Shibayama H. Effect of low blood glucose on plasma CRF, ACTH, and cortisol during prolonged physical exercise. J Appl Physiol. 1991; 71(5):1807–12.

30. Rittweger J, Mutschelknauss M, Felsenberg D. Acute changes in neuromuscular excitability after exhaustive whole body vibration exercise as compared to exhaustion by squatting exercise. Clin Physiol Funct Imaging. 2003; 23(2):81–6.

31. Sale DG. Influence of exercise and training on motor unit activation. Exerc Sport Sci Rev. 1987; 15:95– 151.

32. Gozansky WS, Lynn JS, Laudenslager ML, Kohrt WM. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic–pituitary–adrenal axis activity. Clin Endocrinol (Oxf). 2005; 63(3):336–41.

33. Martin BJ, Park HS. Analysis of the tonic vibration reflex: influence of vibration variables on motor unit synchronization and fatigue. Eur J Appl Physiol Occup Physiol. 1997; 75(6):504–11.

34. Bongiovanni L, Hagbarth K, Stjernberg L. Prolonged muscle vibration reducing motor output in maximal voluntary contractions in man. J Physiol. 1990; 423(1):15–26.

35. Hoffman U, Leyk D, Mester J, Mader A, Spitzenpfeil P, Wackerhage H, editors. Effects of vibration on muscle energy turnover. XVIIth Congress of the International Society of Biomechanics,; 1999; Calgary.

36. Allgrove JE, Gomes E, Hough J, Gleeson M. Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. J Sports Sci. 2008; 26(6):653–61.

37. Viru M, Hackney A, Janson T, Karelson K, Viru A. Characterization of the cortisol response to incremental exercise in physically active young men. Acta Physiologica Hungarica. 2008; 95(2):219–27.

38. Fahrner CL, Hackney AC. Effects of Endurance Exercise on Free Testosterone Concentration and the Binding Affinity of Sex Hormone Binding Globulin (SHBG). Int J Sports Med. 1998; 19(1):12,5.

39. Lu SS, Lau CP, Tung YF, Huang SW, Chen YH, Shih HC, et al. Lactate and the effects of exercise on testosterone secretion: evidence for the involvement of a cAMP-mediated mechanism. Med Sci Sports Exerc. 1997; 29(8):1048–54.

40. Obminski Z, Stupnicki R. Effect of temperature and pH on the magnitude of the free fraction of cortisol in serum. Exp Clin Endocrinol Diabetes. 1996; 104(4):350–2.

41. Rosner W. The Functions of Corticosteroid-Binding Globulin and Sex Hormone-Binding Globulin: Recent Advances. Endocrine Reviews 1990; 11(1):80–91.

42. Obmiński Z, Stupnicki R. Comparison of the testosterone-to-cortisol ratio values obtained from hormonal assays in saliva and serum. The Journal of sports medicine and physical fitness. 1997; 37(1): 50–5.

43. Banfi G, Dolci A. Free Testosterone/Cortisol Ratio in Soccer: Usefulness of a Categorization of Values. J Sports Med Phys Fitness. 2006; 46(4):611–6.

44. Lombardi G, Vitale JA, Logoluso S, Logoluso G, Cocco N, Cocco G, et al. Circannual Rhythm of Plasmatic Vitamin D Levels and the Association With Markers of Psychophysical Stress in a Cohort of Italian Professional Soccer Players. Chronobiol Int. 2017; 34(4):471–479.

6

Caffeine increases motor output entropy and performance in 4 km cycling time trial

Bruno Ferreira Viana[1,2,3☯]**, Gabriel S. Trajano**[4,5☯]**, Carlos Ugrinowitsch**[6☯]**, Flávio Oliveira Pires**[3☯*]

1 Physical Education course, Augusto Motta University Center (UNISUAM), Rio de Janeiro, RJ, Brazil, **2** Physical Education course, Estácio de Sá University (UNESA), Rio de Janeiro, RJ, Brazil, **3** Exercise Psychophysiology Research Group, School of Arts, Sciences and Humanities, University of São Paulo, SP, Brazil, **4** School of Exercise and Nutrition Sciences, Queensland University of Technology, Kelvin Grove, QLD, Australia, **5** Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, QLD, Australia, **6** School of Physical Education and Sport, University of São Paulo, SP, Brazil

☯ These authors contributed equally to this work.
* piresfo@usp.br

Editor: Chris Harnish, Mary Baldwin University Murphy Deming College of Health Sciences, UNITED STATES

Abstract

Caffeine improves cycling time trial performance through enhanced motor output and muscle recruitment. However, it is unknown if caffeine further increases power output entropy. To investigate the effects of caffeine effects on cycling time trial performance and motor output entropy (MOEn), nine cyclists (VO_{2MAX} of 55 ± 6.1 $mL{\cdot}kg^{-1}min^{-1}$) performed a 4 km cycling time trial (TT_{4km}) after caffeine and placebo ingestion in a counterbalanced order. Power output data were sampled at a 2 Hz frequency, thereafter entropy was estimated on a sliding-window fashion to generate a power output time series. A number of mixed models compared performance and motor output entropy between caffeine and placebo every 25% of the total TT_{4km} distance. Caffeine ingestion improved power output by 8% ($p = 0.003$) and increased MOEn by 7% ($p = 0.018$). Cyclists adopted a U-shaped pacing strategy after caffeine ingestion. MOEn mirrored power output responses as an inverted U-shape MOEn during the time trial. Accordingly, a strong inverse correlation was observed between MOEn and power output responses over the last 25% of the TT_{4km} ($p < 0.001$), regardless of the ingestion, likely reflecting the end spurt during this period ($p = 0.016$). Caffeine ingestion improved TT_{4km} performance and motor output responses likely due to a greater power output entropy.

Introduction

According to the dynamic system theory, the variability presented by a given physiological system, a concept that is known as complexity, may reflect its flexibility to face natural perturbations [1,2]. For example, the neuromuscular system is characterized by regular fluctuations in electrophysiological responses (i.e. complexity) which enable the central nervous system (CNS) to adapt to environment-induced perturbations [3]. Assuming that every single body motion is a dynamic acceleration-deceleration interplay [4], the level of complexity in motor

Competing interests: The authors have declared that no competing interests exist.

output responses may indicate the CNS ability to face a physical task-induced perturbation. Studies have reported an association between motor output complexity and fatigue, as a reduced knee extensor torque entropy has been observed as a fatiguing single-joint isometric exercise progresses [5,6]. In this regard, it has been hypothesized that a "loss of complexity" is likely present in fatiguing exercises, so that variations in neuromuscular complexity such as in motor output entropy (MOEn), may indicate the neuromuscular system ability to face the exercise-induced fatigue [7].

Studies investigating the MOEn-fatigue relationship have used highly controlled isometric muscle tasks as an exercise mode [5,6]. Despite providing a well-controlled intensity and power output response, this exercise mode has a low ecological validity as it reflects an unnatural form of exercise. Consequently, isometric muscle task-derived results cannot provide enough to understand the MOEn in more usual forms of exercise. For example, exercises such as a cycling time trial may be insightful to understand the MOEn-fatigue relationship in strenuous whole-body self-paced exercises, as this exercise mode requires a more complex, moment-to-moment regulation when regulating pacing and exercise performance [8–15]. For example, power output fluctuations during a cycling time trial may indicate the CNS ability to deal with the central-peripheral fatigue interplay during a more natural form of exercise [8,15], thereby offering insights on the role of the neuromuscular complexity in exercise regulation and pacing strategy.

Whether both central and peripheral fatigue increase as a cycling time trial progresses, one may argue that the neuromuscular complexity decreases as a function of the trial distance [16]. Consequently, a likely U-shape pacing strategy during a short cycling trial [17] may indicate a reduction in MOEn, what could be related to the trial fatigue status. Importantly, a shorter cycling time trial may be preferable to emphasize the CNS complexity when regulating the motor output during exercise, given that the magnitude of neural drive required to complete a short time trial such as 4km (TT_{4km}) is greater than the neural drive necessary to complete longer ones (e.g. 40km). In this sense, a higher power output could suggest an enhanced motor unit firing synchronization during exercise, as the electromyography (EMG) entropy is lower in higher (i.e. 330 W) than lower (i.e. 150 W) power output values [18]. Therefore, considering that trained cyclists produce a higher mean power output in short (TT_{4km}) than in long cycling time trials (i.e. 40km) [16], analysis of MOEn in TT_{4km} could elucidate the MOEn-fatigue interplay in a high ecological validity exercise.

Some ergogenic aids could add valuable information to the neuromuscular complexity-cycling paradigm, as some ergogenics have the ability to change neuromuscular properties. For example, caffeine may be an interesting approach to investigate the MOEn-fatigue interplay, indicating if fluctuations in power output responses during cycling time trials may be related to changes in neuromuscular response complexity. It has been suggested that caffeine increases MOEn through amplification of the synaptic inputs to α-motor neurons [19]. Caffeine increases the monoamines synthesis and turnover [20], thereby amplifying the synaptic input and motoneuronal gain [21,22] as indicated by a steeper H-reflex curve and greater self-sustained motor unit firing frequency [22,23]. Consequently, assuming that a higher motor neuron gain is associated with a greater muscle force variability as suggested elsewhere [24], one may hypothesize that caffeine increases MOEn through increased neuromuscular complexity. Assuming this hypothesis is right, one may also expect that caffeine may further attenuate the fatigue-induced reduction in MOEn as the trial progresses, thereby likely improving power output and performance [25].

Therefore, the present study aimed to characterize MOEn in a TT_{4km} and verify if caffeine ingestion increases power output complexity and performance in this trial. We hypothesized that caffeine would attenuate a fatigue-induced reduction in power output complexity, improving power output and performance during TT_{4km}.

Methods

Participants and experimental design

Nine endurance-trained male cyclists (32.0 ± 7.5 years, body mass of 74.9 ± 8.6 kg, height of 1.73 ± 5.2 m, VO_{2MAX} of 55.0 ± 6.1 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$,) having a minimum 3 years training experience competing at regional competitions, classified as performance level 3 [26] and experienced in cycling time trials, volunteered to participate in this study. They were non-smokers and had no neuromuscular or cardiopulmonary disorder that could affect the study outcomes. Most cyclists (n = 7) were low-to-moderate consumers of caffeine (50–250 mg of caffeine per day) and two were classified as non-consumers ($\leq$50 mg of caffeine per day), according to classification used elsewhere [27,28]. The experimental procedures were previously approved by the Research Ethics Committee of the University of São Paulo (#0023.0.342.000–10) and explained to participants before the informed consent form signature.

After a preliminary visit to obtain anthropometric measures and assess the VO_{2MAX} through a maximal incremental cycling exercise performed with a 80 rpm pedal cadence (25 $W{\cdot}min^{-1}$ increases until exhaustion), cyclists attended to 3 sessions in a counterbalanced order; 1) a baseline 4 km cycling time trial (TT_{4km}); 2) a TT_{4km} after caffeine ingestion; 3) a TT_{4km} after placebo ingestion. All visits were interspersed by a ~7 days interval. The cyclists were encouraged to maintain the training schedule (intensity and volume) throughout the study period and avoid vigorous exercise, alcohol, and stimulant or caffeine beverages for the last 24 h before the sessions. Briefly, we chose a TT_{4km} as a strenuous whole-body self-paced exercise and assumed that endurance-trained cyclists complete this trial having a mean power output higher than 300 W [15,16], therefore potentiating a likely reduction in MOEn [18]. In contrast, caffeine ingestion may increase MOEn and TT_{4km} performance.

Caffeine and placebo ingestion

Caffeine and placebo capsules (6 $mg{\cdot}kg^{-1}$ of body mass) were ingested ~ 60 min before the TT_{4km} commencement. Caffeine and sucrose-based (i.e. placebo) substances were formulated in opaque capsules of equal size, color and taste to prevent that participants rightly guessed the treatment. Importantly, instead of a double-blind, randomized placebo-controlled clinical trial, we used a placebo-deceived design, as some have argued that the use of double-blind designs is a possible source of bias in clinical trials [29,30]. To ensure that eventual differences between caffeine and placebo were solely due to caffeine pharmacological effects, cyclists were led to believe they ingested caffeine in both sessions and the study was investigating the reproducibility of caffeine effects on TT_{4km} performance. They were informed about the presence of a placebo condition at the study completion, as reported elsewhere [31]. Informal and anecdotal communication revealed that participants were blinded about the presence of a true placebo pill.

Instruments, measures, and analysis

All cyclists performed the TT_{4km} on the same road bike (Giant®, Thousand Oaks, CA, USA) attached to a cycle-simulator calibrated before every test (Racer Mate®, Computrainer, Seattle, WA, EUA), individually fitted with crank, pedals and saddle. This equipment provided power output measures (W) at a 2Hz sampling rate. The validity and reliability of this system have been previously reported [32,33]. Cyclists performed a standard 7 min warm-up, consisting of a 5 min self-paced (gear and cadence freely adjusted) and a 2 min controlled-pace cycling (fixed gear at 100 W and 80 rpm pedal cadence). When they were still cycling at the end of the controlled-pace warmup, they immediately started the TT_{4km}. The cyclists were oriented to rate their perceived exertion (RPE) at each 0.5 km, according to the 6–20 Borg's scale [34], so that the mean RPE during the TT_{4km} was calculated. A researcher unaware of the substance ingested encouraged the cyclists to complete the distance as fast as possible, while distance feedback was available to cyclists to pace themselves.

Entropy calculation

The entropy could be interpreted as a non-linear analysis that provides a measure of the complexity of a system [35]. Based on the information theory, entropy is a measure that reflects the level of uncertainty of a dataset or time series. Entropy can be obtained as the probability (p_k) of each possible event multiplied by log of the inverse probability of each event ($\log\left(\frac{1}{p_k}\right)$) [36] as described in Eq 1.

$$H = \sum_{i=1}^{N} p_k \log\left(\frac{1}{p_k}\right) \tag{1}$$

However, the prior knowledge of the probability (p_k) for the occurrence of all events is impossible in stochastic processes, therefore, adequate methodologies such as the sample entropy (SampEn) have been suggested [37]. The SampEn (Eq 2) fits the approximate entropy [38] to generate less time series length-dependence and self-matching-reduced bias (Eq 2).

$$SampEn(m, r, N) = -\ln\left(\frac{A_{m+1}(r)}{A_m(r)}\right) \tag{2}$$

Where m is the length of sequences to be compared, r is the tolerance for accepting matches and N is the length of the time series. In the present study, the input parameters were set as $r = 0.2$, $m = 2$, $N = 120$. In the SampEn algorithm, r is multiplied by the standard deviation (SD) of N, providing a matching threshold and allowing comparisons among sequences of m points. Readers are referred to a seminal work by Richman et al. [37] for a comprehensive SampEn demonstration.

Data analysis and statistics

In this study MOEn was estimated applying SampEn algorithm in the mechanical power output signal obtained during TT_{4km}. A custom code (Matlab v.2013a, The Mathworks, EUA) was used to estimate MOEn over time, by applying a sliding-window over 120 samples epochs having 10 samples overlap. Thereafter, absolute power output data, as well as MOEn vectors, were expressed at each 25% of the total TT_{4km} distance (i.e. 25%, 50%, 75% and 100% of the trial).

Data were reported as mean (± SD) and 95% confidence limits (CI 95%). Power output and MOEn obtained at each 25% of the cycling trial were compared through a number of mixed models, having substance (caffeine and placebo) and distance (25%, 50%, 75% and 100% of the TT_{4km}) as fixed factors, and cyclists as the random factor. The Pearson correlation coefficient was calculated between mean values of power output and MOEn for each 25% of the TT_{4km}, as we expected that MOEn would decrease if cyclists significantly increased the power output. Significant results were accepted as $p < 0.05$ (SPSS software, version 17.0, SPSS Inc., Chicago, IL, USA).

Results

Ingestion of caffeine resulted in a 8% increase in mean power output ($p = 0.003$, $F = 9.69$) when compared to placebo, as mean power output was 331.4 ± 53 W (CI 95% [306.5–356.3]) in caffeine vs 306.2 ± 40 W (CI 95% [281.3–331.1]) in placebo (Fig 1A). This improved power output in caffeine was reflected in ~1.8% shorter times ($p > 0.05$) in caffeine (350.0 ± 14.6 s) than placebo (357.0 ± 13.2 s). Additionally, cyclists presented comparable mean RPE during the TT_{4km} in both supplementations with caffeine (16 ± 0.62 a.u.) and placebo (16 ± 0.63 a.u.).

Cyclists adopted a U-shaped pacing strategy (Fig 2) so that a distance main effect was detected ($p = 0.002$, $F = 5.70$), and power output decreased by 14% from 25% to 75% of the TT_{4km} (-41.6 ± 11.4 w; CI 95% [-10.3, -72.9], $p = 0.004$), but increased by 11% from 75% to 100% of the TT_{4km} (35.9 ± 11.4 w; CI 95% [4.6, 67.2], $p = 0.016$). No substance by distance interaction effects were found in power output responses ($p = 0.178$, $F = 1.697$).

We observed a substance main effect on MOEn results, as MOEn was 7% greater in caffeine than placebo ($p = 0.018$, $F = 5.983$; CI 95% [0.019, 0.190]) (Fig 1B). We observed a distance main effect in MOEn ($p < 0.001$, $F = 10{,}118$; CI 95% [0.032, 0.339]), so that MOEn increased by ~ 20% from 25% to 50% of the trial (0.284 ± 0.060 A.U.; CI 95% [0.120, -0.449], $p < 0.001$), but remained unchanged between 50% and 75% (- 0.005 ± 0.06 A.U.; CI 95% [- 0.170, 0.160], $p < 0.05$) and between 75% and 100% (- 0.115 ± 0.060 A.U.; CI 95% [- 0.280, 0.050], $p = 0.368$). No substance by distance interaction effect was found in MOEn ($p = 0.337$, $F = 1.151$). Fig 3 depicts MOEn responses during the cycling trial, and Table 1 shows individual power output and MOEn responses over the TT_{4km} in both supplementations.

Correlations analysis revealed that MOEn was inversely correlated with power output in the first 25% ($r = - 0.82$; $p < 0.001$) of placebo condition, but not in caffeine. Negative correlations were also founded between 25% and 50% of the TT_{4km} in caffeine ($r = - 0.76$; $p = 0.03$), but not in placebo, perhaps as a result of the steady power distribution in placebo during this part of the trial. Furthermore, MOEn was inversely correlated with power output in the last 25% of the TT_{4km} in both caffeine ($r = - 0.92$, $p < 0.001$) and placebo trials ($r = - 0.83$, $p < 0.001$), being coincident with a ~11% increase in power output at the end of the trial, regardless of the supplementation. Table 2 shows all correlation coefficients between MOEn and power output.

Discussion

This study aimed to characterize the MOEn during a TT_{4km} and investigate if caffeine could change the MOEn-fatigue interplay during this strenuous, whole-body short cycling exercise. Our results showed a progressive reduction in motor output complexity as the TT_{4km} progressed, however caffeine increased TT_{4km} performance through an altered MOEn-fatigue interplay. These results may support the notion that caffeine increases power output responses and attenuates the fatigue-induced reduction in MOEn during TT_{4km}.

This is the first study characterizing the MOEn during a natural exercise mode with high ecological validity such as a strenuous, whole-body short cycling time trial. In the present study, cyclists used a U-shaped pacing strategy to complete the TT_{4km}, as they yielded an end spurt in the last 25% of the trial, after an increased power output in the initial 25% and unaltered power output in the intermediate 50%. In contrast, there was a progressive reduction in MOEn in the last 25% of the TT_{4km}, regardless of the ingested substance, thereby supporting the fatigue-induced loss of entropy hypothesis as suggested in single-joint isometric exercises [6,39]. Briefly, MOEn responses could involve changes in neuromuscular complexity such as in CNS areas such as cortical, subcortical and spinal areas, as well as in motor neuron conduction to skeletal muscles. In this regard, the 20% increase in SampEn during the first half of the

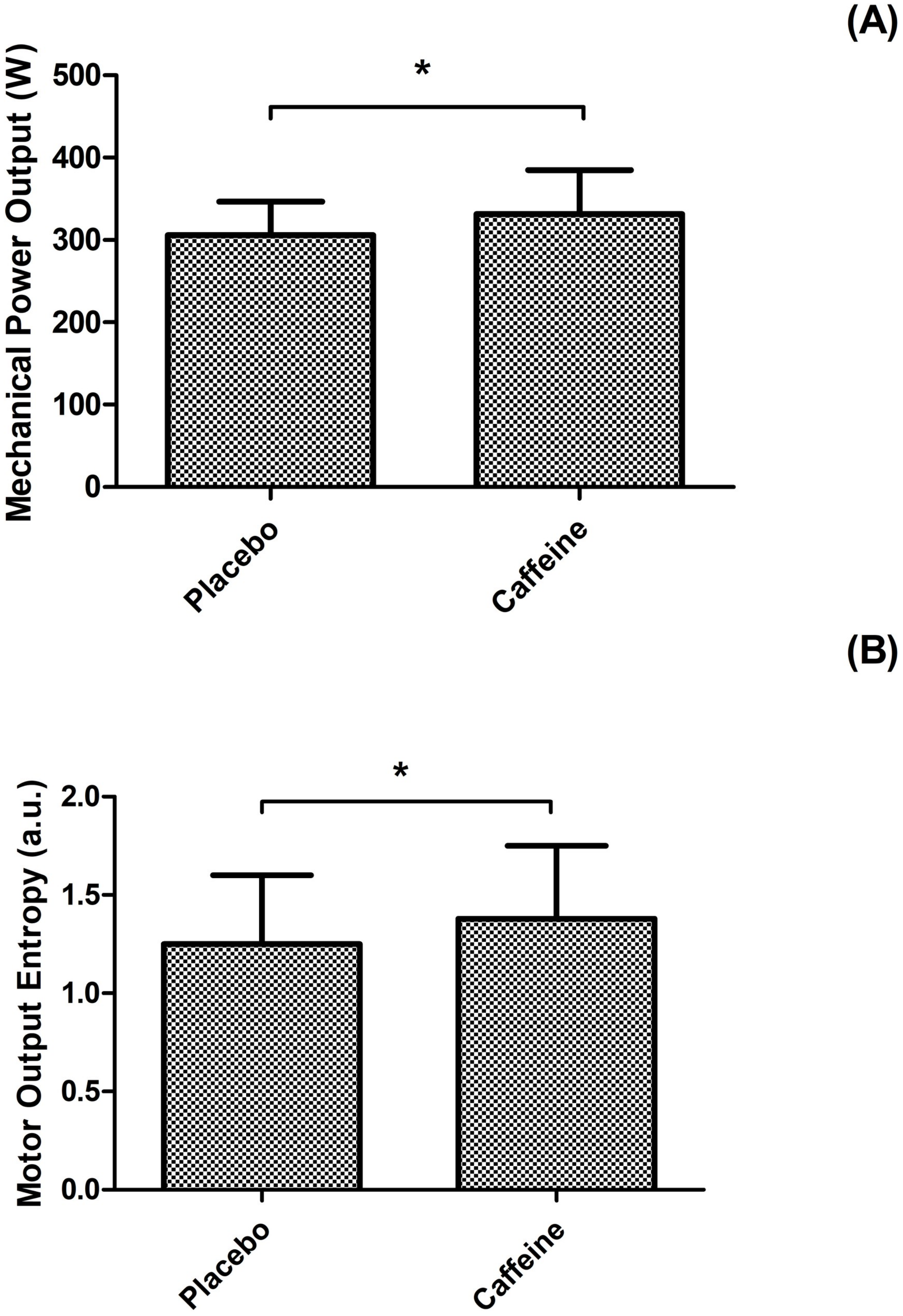

Fig 1. Cycling time trial performance and entropy. Mechanical power output (panel A) and motor output entropy (panel B) in placebo and caffeine trials. * indicates supplementation main effect in power output ($p = 0.003$, $F = 9.69$) and motor output entropy ($p = 0.018$, $F = 5.983$).

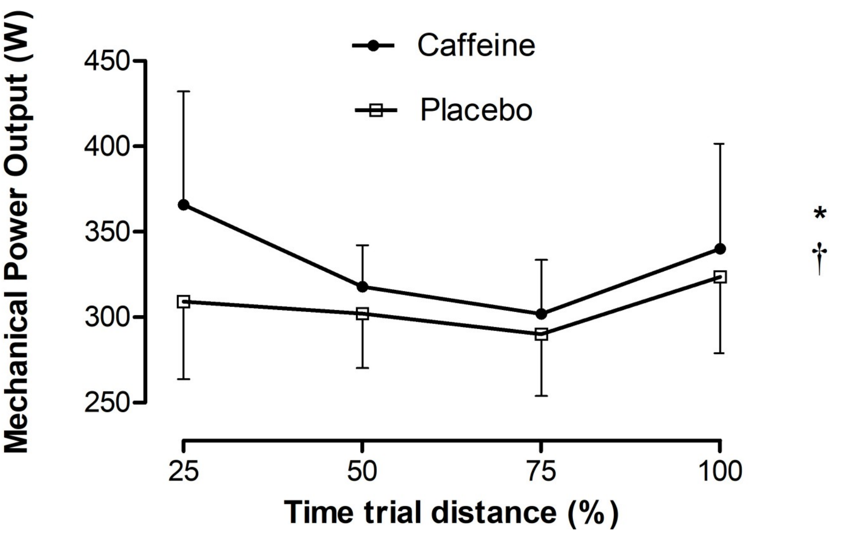

Fig 2. Pacing strategy. Mechanical power output relative to a percentage of the TT_{4km} distance. * indicates distance main effect (p = 0.002, F = 5.70) and † indicates supplementation main effect (p = 0.003, F = 9.69).

TT_{4km} was likely due to an enhanced exercise-induced perturbation, given that most relevant increases in psychophysiological responses take place in this part of the trial [15]. However, despite the increasing exercise-induced perturbation, neuromuscular fatigue was likely low over this half of the trial and probably allowed an increased MOEn when regulating the motor output during this part [40].

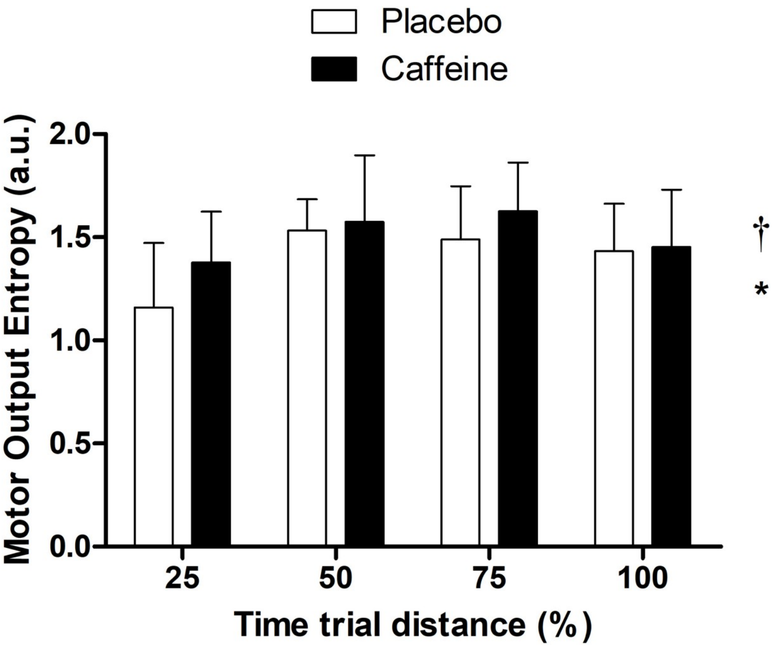

Fig 3. Motor output entropy during the cycling time trial. Motor output entropy was expressed relative to a percentage of the TT_{4km} distance. * indicates distance main effect ($p < 0.001$, F = 10.11) and † indicates supplementation main effect (p = 0.018, F = 5.98).

Table 1. Individual power output (PO) and motor output entropy (MOEn) responses were reported as a percentage of the total cycling time trial distance.

		Time trial distance (%)							
		25		50		75		100	
Cyclists	**Caffeine**	**PO**	**MOEn**	**PO**	**MOEn**	**PO**	**MOEn**	**PO**	**MOEn**
1		342.34	1.19	290.04	1.31	270.39	1.56	288.26	1.31
2		315.38	1.19	344.43	1.1	301.1	1.64	324.77	1.37
3		336.24	1.62	322.99	1.95	309.11	1.84	308.23	1.68
4		305.98	1.39	243.81	2.08	234.07	2.03	238.69	1.96
5		379.99	1.11	299.51	1.45	274.32	1.31	262.33	1.39
6		322.42	1.37	339.16	1.45	366.02	1.32	386.87	1.49
7		376.9	1.13	311.7	1.46	299.67	1.53	302.76	1.03
8		341.9	1.78	300.49	1.88	280.63	1.78	246.09	1.64
9		344.6	1.62	326.72	1.48	335.18	1.61	364.72	1.35
	Placebo	**PO**	**MOEn**	**PO**	**MOEn**	**PO**	**MOEn**	**PO**	**MOEn**
1		308.22	1.21	291.75	1.48	267.53	1.32	268.5	1.63
2		298.72	1.24	339.59	1.44	319.98	0.91	342.24	1.03
3		349.09	1.19	283.82	1.7	268.22	1.67	256.75	1.61
4		253.83	1.28	235.22	1.75	224.6	1.69	240.1	1.68
5		365.88	0.58	297.12	1.38	275.27	1.59	291.38	1.36
6		364.19	0.9	370.11	1.38	359.13	1.51	364.02	1.38
7		369.75	0.99	295.63	1.57	281.31	1.5	287.63	1.55
8		311.61	1.7	308	1.7	310.87	1.77	320.73	1.54
9		365.57	1.35	311.37	1.39	314.31	1.45	370.08	1.12

In the present study, we observed that cyclists attacked the first 25% of the TT_{4km} more aggressively when they ingested caffeine rather than placebo, somehow influencing the significant inverse correlation between power output and MOEn observed only with caffeine for this part of the trial. One may argue that neuromuscular fatigue is low during this initial part of the trial, thus likely allowing an adequate response of the neuromuscular system to the exercise-imposed perturbation through an increased motor unit firing variability. Moreover, the power output reduction observed from 25% to 50% of the caffeine TT_{4km} resulted in an inverse correlation between MOEn and power output during this part of the trial. In contrast, such a correlation between power output and MOEn was not observed in placebo TT_{4km} during these parts. In particular, the lowest MOEn and power output values were observed from 50% to 75% of the trials, so that no correlation between MOEn and power output was observed during this part, regardless of the ingested substance. Importantly, MOEn was inversely correlated with power output during the last 25% of the TT_{4km}, regardless the ingested substance. This is a part of the cycling trial usually characterized by a sharp increase in power output (i.e. end spurt), so that one may hypothesize that the loss of MOEn during this latter part of the TT_{4km}

Table 2. Pearson's correlation coefficient between power outptut (PO) and motor output entropy (MOEn) over the 4km cycling time trial (TT4km) expressed as a percentage of the trial distance, in both caffeine and placebo supplementations.

%TT_{4km}	**Caffeine**	**p-value**	**Placebo**	**p-value**
0–25	-0.25	0.33	-0.82	< 0.001
25–50	-0.76	0.03	-0.36	0.15
50–75	-0.36	0.16	-0.30	0.25
75–100	-0.92	< 0.001	-0.83	< 0.001

was possibly related to a higher motor unit firing frequency, as neuromuscular fatigue is higher in the second half of a cycling trial [40].

A short cycling time trial having an end spurt may be a challenging scenario for the neuromuscular system, as this may represent fewer chances to vary muscle recruitment during pedaling mainly at the final stages of the trial [18], thereby reducing the mechanical power output variability (i.e. power output bandwidth) and MOEn. This hypothesis is based on a previous study that reported a different neuromuscular strategy as indicated by EMG analysis when contrasting fixed-load cycling at 150 W vs 300 W [18]. The authors of that study concluded that the lower EMG entropy observed during higher cycling power output was likely due to a higher synchronism of motor units firing.

The present study hypothesized that caffeine may increase MOEn by increasing motoneuronal gain and changing the input-output relationship in the motor pathway, thereby resulting in a greater variability in motor output. Although caffeine effects on skeletal muscles cannot be ruled out [41], the most convincing caffeine mechanism involves its action on neuronal A_1 adenosine receptors, as improvements in exercise performance after caffeine ingestion have been associated with increases in spinal and supraspinal excitability [42,43]. Accordingly, the 7% increase observed in MOEn during the TT_{4km} after caffeine ingestion may be related to the caffeine's action on neuronal tissue. Considering the 8% increase in mean power output in caffeine, one may argue that the higher power output observed in this condition was also related to a higher synchronism of motor units firing [18].

Analysis of movement variability have been used in different research fields [1,2,44], so that such analysis have been recently incorporated in neuromuscular fatigue studies [5,6]. In an exercise performance scenario, nonlinear measures such as MOEn may be a useful mean to estimate exercise-induced neuromuscular fatigue and its repercussion on motor control and performance responses [5]. Therefore, such a nonlinear measure could be helpful to improve the understanding of exercise performance and fatigue in different fields of sports sciences.

Limitations and methodological considerations

The increased motoneuronal gain suggestion should be interpreted with caution, as no specific measures were performed to indicate motoneuronal gain. Insights to a motoneuronal gain mechanism could be obtained with advanced EMG techniques, such as the motor unit decomposition algorithms from electrode matrices-derived signal [45]. However, this technique is still restricted to low-intensity isometric contractions so that the dynamic whole-body exercise used in the present study limited the use of these measures to provide motoneuronal gain mechanisms insights after caffeine ingestion. Future studies comparing recruitment and derecruitment frequencies of pairs of motor units could shed-light on caffeine effects on motoneuronal gain during voluntary contractions [46].

The present study is descriptive rather than mechanistic, and its design and methods may not elucidate if losses in power output entropy during cycling time trial were due to central or peripheral fatigue factors. In this sense, the power output was sampled at a 2 Hz frequency, a sampling rate that may not detect all variability in power output data, given the possible aliasing effect resulted from sampling the data in different pedal positions at each revolution. Another limitation was the absence of EMG responses, a measure that could have assessed the neuromuscular system and power output entropy, simultaneously.

Furthermore, we disregarded eventual subgroup comparisons based on the habitual caffeine consumption effects on performance, given that a recent well-designed study [28] and an important sports nutrients position stand challenged [27] the myth that habituation to caffeine consumption affects the caffeine's potential as an ergogenic aid. However, considering that habitual caffeine consumption may change physiological responses to caffeine supplementation such as heart rate and ventilation, future studies may want to investigate potential habitual caffeine consumption effects on MOEn and EMG during cycling time trial.

Conclusion

Results of the present study showed a progressive reduction in MOEn during the TT_{4km}, thus revealing a progressive loss of motor output complexity as the trial progressed, mainly during the last 25% of the TT_{4km}. However, caffeine ingestion improved TT_{4km} performance and MOEn. These results reinforce a likely fatigue-induced loss of complexity hypothesis.

Supporting information

S1 Raw data.
(XLSX)

Author Contributions

Conceptualization: Bruno Ferreira Viana, Gabriel S. Trajano, Carlos Ugrinowitsch, Flávio Oliveira Pires.

Data curation: Flávio Oliveira Pires.

Formal analysis: Bruno Ferreira Viana, Gabriel S. Trajano, Flávio Oliveira Pires.

Funding acquisition: Carlos Ugrinowitsch, Flávio Oliveira Pires.

Investigation: Flávio Oliveira Pires.

Methodology: Bruno Ferreira Viana, Gabriel S. Trajano, Carlos Ugrinowitsch, Flávio Oliveira Pires.

Project administration: Carlos Ugrinowitsch, Flávio Oliveira Pires.

Resources: Flávio Oliveira Pires.

Software: Bruno Ferreira Viana, Flávio Oliveira Pires.

Supervision: Flávio Oliveira Pires.

Validation: Flávio Oliveira Pires.

Visualization: Bruno Ferreira Viana, Gabriel S. Trajano, Flávio Oliveira Pires.

Writing – original draft: Bruno Ferreira Viana, Gabriel S. Trajano, Carlos Ugrinowitsch, Flávio Oliveira Pires.

Writing – review & editing: Bruno Ferreira Viana, Gabriel S. Trajano, Carlos Ugrinowitsch, Flávio Oliveira Pires.

References

1. Lipsitz L. A. and Goldberger A. L. Loss of; complexity; and aging: Potential applications of fractals and chaos theory to senescence. JAMA 1992; 267(13): 1806–1809 https://doi.org/10.1001/jama.1992.03480130122036 PMID: 1482430
2. Pincus S. M. Assessing Serial Irregularity and Its Implications for Health. Annals of the New York Academy of Sciences 2001; 954(1): 245–267 https://doi.org/10.1111/j.1749-6632.2001.tb02755.x PMID: 11797860
3. Hogan M. J., O'Hora D., Kiefer M., Kubesch S., Kilmartin L., Collins P., et al. The effects of cardiorespiratory fitness and acute aerobic exercise on executive functioning and EEG entropy in adolescents.

Frontiers in Human Neuroscience 2015; 9(538) https://doi.org/10.3389/fnhum.2015.00538 PMID: 26539093

4. Merfeld D. M., Zupan L. and Peterka R. J. Humans use internal models to estimate gravity and linear acceleration. Nature 1999; 398(6728): 615–618 https://doi.org/10.1038/19303 PMID: 10217143
5. Pethick J., Winter S. L. and Burnley M. Fatigue reduces the complexity of knee extensor torque fluctuations during maximal and submaximal intermittent isometric contractions in man. The Journal of Physiology 2015; 93(8): 2085–2096 https://doi.org/10.1113/jphysiol.2015.284380 PMID: 25664928
6. Pethick J., Winter S. L. and Burnley M. Loss of knee extensor torque complexity during fatiguing isometric muscle contractions occurs exclusively above the critical torque." American Journal of Physiology—Regulatory, Integrative and Comparative Physiology 2016; 310(11): R1144–R1153 https://doi.org/10.1152/ajpregu.00019.2016 PMID: 27101290
7. Hong S. L. and Newell K. M. Entropy compensation in human motor adaptation. Chaos 2008 18(1): 013108 https://doi.org/10.1063/1.2838854 PMID: 18377059
8. St Clair Gibson A. and Noakes T. D. Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. Br J Sports Med 2004; 38(6): 797–806 https://doi.org/10.1136/bjsm.2003.009852 PMID: 15562183
9. St Clair Gibson A., Lambert E. V., Rauch L. H., Tucker R., Baden D. A., Foster C., et al. The role of information processing between the brain and peripheral physiological systems in pacing and perception of effort. Sports Med 2006; 36(8): 705–722 https://doi.org/10.2165/00007256-200636080-00006 PMID: 16869711
10. Tucker R., Bester A., Lambert E. V., Noakes T. D., Vaughan C. L. and St Clair Gibson A. Non-random fluctuations in power output during self-paced exercise. Br J Sports Med 2006; 40(11): 912–917; discussion 917 https://doi.org/10.1136/bjsm.2006.026435 PMID: 16980537
11. Marino F. The limitations of the constant load and self-paced exercise models of exercise physiology. Comparative Exercise Physiology 2010; 7(4): 6 https://doi.org/10.1017/S1755254012000013
12. Angus S. D. and Waterhouse B. J. Pacing strategy from high-frequency field data: more evidence for neural regulation? Med Sci Sports Exerc 2011; 43(12): 2405–2411 https://doi.org/10.1249/MSS.0b013e3182245367 PMID: 21606868
13. Marino F. E., Gard M. and Drinkwater E. J. The limits to exercise performance and the future of fatigue research. Br J Sports Med 2011; 45(1): 65–67 https://doi.org/10.1136/bjsm.2009.067611 PMID: 19955162
14. Smits B. L. M., Pepping G.-J. and Hettinga F. J. Pacing and Decision Making in Sport and Exercise: The Roles of Perception and Action in the Regulation of Exercise Intensity. Sports Medicine 2014; 44(6): 763–775 https://doi.org/10.1007/s40279-014-0163-0 PMID: 24706362
15. Pires F. O., Dos Anjos C. A., Covolan R. J., Pinheiro F. A., St Clair Gibson A., Noakes T. D., Magalhaes F. H. and Ugrinowitsch C. Cerebral Regulation in Different Maximal Aerobic Exercise Modes. Front Physiol 2016; 7: 253 https://doi.org/10.3389/fphys.2016.00253 PMID: 27458381
16. Thomas K., Goodall S., Stone M., Howatson G., Gibson A. S. C. and Ansley L. Central and Peripheral Fatigue in Male Cyclists after 4-, 20-, and 40-km Time Trials. Medicine & Science in Sports & Exercise 2015; 47(3): 537–546 https://doi.org/10.1249/mss.0000000000000448 PMID: 25051388
17. Abbiss C. R. and Laursen P. B. Describing and understanding pacing strategies during athletic competition. Sports Med 2008; 38(3): 239–252 https://doi.org/10.2165/00007256-200838030-00004 PMID: 18278984
18. Enders H., V V. O. N. T. and Nigg B. M. Neuromuscular Strategies during Cycling at Different Muscular Demands. Med Sci Sports Exerc 2015; 47(7): 1450–1459 https://doi.org/10.1249/MSS.0000000000000564 PMID: 25380476
19. Hounsgaard J., Hultborn H., Jespersen B. and Kiehn O. Intrinsic membrane properties causing a bistable behaviour of α-motoneurones. Experimental Brain Research 1984; 55(2): 391–394 https://doi.org/10.1007/BF00237290 PMID: 6086378
20. Nehlig A., Daval J.-L. and Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Research Reviews 1992; 17(2): 139–170 https://doi.org/10.1016/0165-0173(92)90012-b PMID: 1356551
21. Gorassini M. A., Bennett D. J. and Yang J. F. Self-sustained firing of human motor units. Neuroscience Letters 1998; 247(1): 13–16 https://doi.org/10.1016/s0304-3940(98)00277-8 PMID: 9637398
22. Johnson M. D. and Heckman C. J. Gain control mechanisms in spinal motoneurons. Frontiers in Neural Circuits 2014; 8: 81 https://doi.org/10.3389/fncir.2014.00081 PMID: 25120435
23. Walton C., Kalmar J. M. and Cafarelli E. Effect of caffeine on self-sustained firing in human motor units. The Journal of Physiology 2002; 545(2): 671–679 https://doi.org/10.1113/jphysiol.2002.025064 PMID: 12456842

24. Vestergaard M. and Berg R. W. Divisive Gain Modulation of Motoneurons by Inhibition Optimizes Muscular Control. The Journal of Neuroscience 2015; 35(8): 3711–3723 https://doi.org/10.1523/JNEUROSCI.3899-14.2015 PMID: 25716868

25. Shen J. G., Brooks M. B., Cincotta J. and Manjourides J. D. Establishing a relationship between the effect of caffeine and duration of endurance athletic time trial events: A systematic review and meta-analysis. J Sci Med Sport 2019; 22(2): 232–238 https://doi.org/10.1016/j.jsams.2018.07.022 PMID: 30170953

26. De Pauw K., Roelands B., Cheung S. S., de Geus B., Rietjens G. and Meeusen R. Guidelines to classify subject groups in sport-science research. Int J Sports Physiol Perform 2013; 8(2): 111–122 https://doi.org/10.1123/ijspp.8.2.111 PMID: 23428482

27. Goldstein E.R., Ziegenfuss T., Kalman D. et al. International society of sports nutrition position stand: caffeine and performance. J Int Soc Sports Nutr 2010; 7(5). https://doi.org/10.1186/1550-2783-7-5

28. Gonçalves LS, Painelli VS, Yamaguchi G, et al. Dispelling the myth that habitual caffeine consumption influences the performance response to acute caffeine supplementation. J Appl Physiol (1985). 2017; 123(1):213-220. https://doi.org/10.1152/japplphysiol.00260.2017 PMID: 28495846

29. Kirsch I. and Weixel L. J. Double-blind versus deceptive administration of a placebo.Behavioral Neuroscience 1988; 102(2): 5 http://dx.doi.org/10.1037/0735-7044.102.2.319.

30. Saunders B., et al. Placebo in sports nutrition: a proof-of-principle study involving caffeine supplementation. Scandinavian Journal of Medicine & Science in Sports 2017; 27(11): 1240–1247 https://doi.org/10.1111/sms.12793 PMID: 27882605

31. Foad A. J., Beedie C. J. and Coleman D. A. Pharmacological and Psychological Effects of Caffeine Ingestion in 40-km Cycling Performance. Medicine & Science in Sports & Exercise 2008; 40(1): 158–165 https://doi.org/10.1249/mss.0b013e3181593e02 PMID: 18091009

32. Peveler W. W. The Accuracy of Simulated Indoor Time Trials Utilizing a CompuTrainer and GPS Data. The Journal of Strength & Conditioning Research 2013; 27(10): 2823–2827 https://doi.org/10.1519/JSC.0b013e318280ce76 PMID: 23287833

33. Sparks S. A., Williams E., Jones H., Bridge C., Marchant D. and McNaughton L. Test-retest reliability of a 16.1 km time trial in trained cyclists using the CompuTrainer ergometer. Journal Of Science And Cycling, 2016; 5(3), 35–41. https://doi.org/10.28985/jsc.v5i3.272

34. Borg G. A. (1982). Psychophysical bases of perceived exertion. Med. Sci. Sports Exerc. 14, 377–381. https://doi.org/10.1249/00005768-198205000-00012 PMID: 7154893

35. Singh V. P. Entropy theory and its application in environmental and water engineering. 2013; New York: Wiley-Blackwell.

36. Shannon C. E. A Mathematical Theory of Communication. Bell System Technical Journal 1948; 27(3): 379–423 https://doi.org/10.1002/j.1538-7305.1948.tb01338.x PMID: 30854411

37. Richman J. S. and Moorman J. R. Physiological time-series analysis using approximate entropy and sample entropy. Am J Physiol Heart Circ Physiol 2000; 278(6): H2039–2049 https://doi.org/10.1152/ajpheart.2000.278.6.H2039 PMID: 10843903

38. Pincus S. M. Approximate entropy as a measure of system complexity. Proceedings of the National Academy of Sciences 1991; 88(6): 2297–2301 https://doi.org/10.1073/pnas.88.6.2297 PMID: 11607165

39. Pethick J., Winter S. L. and Burnley M. Caffeine Ingestion Attenuates Fatigue-induced Loss of Muscle Torque Complexity. Medicine & Science in Sports & Exercise 2018; 50(2): 236–245 https://doi.org/10.1249/mss.0000000000001441 PMID: 28991045

40. Lepers R., Maffiuletti N. A., Rochette L., Brugniaux J. and Millet G. Y. Neuromuscular fatigue during a long-duration cycling exercise. J Appl Physiol 2002 92(4): 1487–1493 https://doi.org/10.1152/japplphysiol.00880.2001 PMID: 11896014

41. Tarnopolsky M. and Cupido C. Caffeine potentiates low frequency skeletal muscle force in habitual and nonhabitual caffeine consumers. Journal of Applied Physiology 2000; 89(5): 1719–1724 https://doi.org/10.1152/jappl.2000.89.5.1719 PMID: 11053318

42. Kalmar J. M. The influence of caffeine on voluntary muscle activation. Med Sci Sports Exerc 2005; 37 (12): 2113–2119 https://doi.org/10.1249/01.mss.0000178219.18086.9e PMID: 16331138

43. Meyers B. M. and Cafarelli E. Caffeine increases time to fatigue by maintaining force and not by altering firing rates during submaximal isometric contractions. J Appl Physiol 2005; 99(3): 1056–1063 https://doi.org/10.1152/japplphysiol.00937.2004 PMID: 15879163

44. Stergiou N, Decker LM. Human movement variability, nonlinear dynamics, and pathology: is there a connection?. Hum Mov Sci. 2011; 30(5):869-888. https://doi.org/10.1016/j.humov.2011.06.002 PMID: 21802756

45. Farina D., Negro F., Muceli S. and Enoka R. M. Principles of Motor Unit Physiology Evolve With Advances in Technology. Physiology 2016; 31(2): 83–94 https://doi.org/10.1152/physiol.00040.2015 PMID: 26889014

46. Johnson M. D., Thompson C. K., Tysseling V. M., Powers R. K. and Heckman C. J. The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. Journal of Neurophysiology 2017; 118(1): 520–531 https://doi.org/10.1152/jn.00018.2017 PMID: 28356467

7

A 2-year longitudinal follow-up of performance characteristics in Chinese male elite youth athletes from swimming and racket sports

Kewei Zhao[1,2,3], **Andreas Hohmann**[4]*, **Irene Faber**[5], **Yu Chang**[6], **Binghong Gao**[7]*

1 School of Kinesiology, Shanghai University of Sport, Shanghai, China, **2** Shandong Sport University, Jinan, China, **3** China Institute of Sport Science, Beijing, China, **4** Institute of Sports Science, University of Bayreuth, Bayreuth, Germany, **5** Institute of Sports Science, University of Oldenburg, Oldenburg, Germany, **6** Shanghai Sports School, Shanghai, China, **7** School of Physical Education and Sport Training, Shanghai University of Sport, Shanghai, China

* Binghong.gao@hotmail.com (BG); Andreas.hohmann@uni-bayreuth.de(AH)

Editor: Yih-Kuen Jan, University of Illinois at Urbana-Champaign, UNITED STATES

Abstract

Training in elite sport aims at the optimization of the athletic performance, and to control the athletes'progress in physiological, anthropometrical and motor performance prerequisites. However, in most sports, the value of longitudinal testing is unclear. This study evaluates the longitudinal development and the influence of intense training over 2-years on specific physiological performance prerequisites, as well as certain body dimensions and motor abilities in elite youth athletes. Recruited between 11–13 years of age at Shanghai Elite Sport school, the sample of student-athletes (N = 21) was categorized as the swimming group (10 athletes), and the racket sports group (11 players: 7 table tennis and 4 badminton players). The performance monitoring took place over two years between September 2016 and September 2018 and included 5 test waves. In all the test waves, the athletes were assessed by means of three physiological measurements (vital capacity, hemoglobin concentration, heart rate at rest), three anthropometric parameters (body height, body weight, chest girth), and two motor tests (back strength, complex reaction speed). Seven out of eight diagnostic methods exhibit medium to high validity to discriminate between the different levels of performance development in the two sports groups. The investigated development of the performance characteristics is attributed partly to the inherited athletic disposition as well as to the different sport-specific training regimens of the two sports groups.

Introduction

Worldwide, the predominant policies and structure of elite sport systems, and specifically in the youth departments, reflect the 'talent account' at least to a certain extent [1]. This covers, among other things, the search for talented players already at a young age who show natural abilities for a certain sport (discipline). Although the debate about validness of innate talent is lively and still ongoing, national sports associations are searching for the key-indicators for

Competing interests: The authors have declared that no competing interests exist.

performance that can predict future successes. This early talent identification is considered crucial to keep up with the global medal race [2]. Nevertheless, even without over- or underestimating the role of innate talent, research focusing on the value of specific profiles of performance characteristics in elite youth athletes and the influence of training with respect to elite sports seems sensible. It will provide a better insight in the characteristics needed to perform at a high level and how efficient and effective trainings are to get there. Thus, it is paramount to diagnose the typical characteristics of the athletic profile and progress of the participants of different types of sports.

At this moment, it is clear that performance profiles are multi-dimensional and various characteristics should be taken into account [3–6]. Moreover, there is evidence that growth, development and specific training can influence a player's profile especially at adolescence [7, 8]. Until now, most of the existing research concerning this topic is cross-sectional and focused on European and/or North-American athletes. These results might not be generalizable for adolescent athletes with a different origin. Players' profiles and their response to training might be different across continents or even countries. Since Asia, and specifically China, can be considered as an ambitious competitor in sports, this study focused on the profiles of Chinese youth elite players of two different sports groups (i.e. swimming and racket sports) and their response to training. In long-term athletic development programs, training aims at the systematic optimization of performance prerequisites [9]. To allow for a close monitoring of the performance development of youth athletes in Shanghai elite sport school, the general sport administration and school officials introduced an assessment covering physiological, anthropometric, and motor tests during the whole time-course of school attendance of the talented youngsters. Although that these tests were suggested to monitor the development of performance characteristics over certain training stages, the development profiles can also help to discriminate players of different types of sports. In this study, these follow-up tests are used to investigate whether in the endurance sport swimming and the two speed-oriented racket sports table tennis and badminton, the performance development is also responsive to the intensive sport-specific training program. The insight in the discriminative ability and responsiveness to training of the different performance characteristics will support both concerns, talent identification and athletic development programs.

Elite endurance athletes as well as elite game sport participants are characterized by a markedly increased O_2 transport capacity, hemoglobin concentration [Hb] and hemoglobin mass (Hbmass) [10, 11]. Hbmass and hemoglobin concentration [Hb] belong to the major limiting factors of maximum endurance performance. In various studies in endurance sports, a strong correlation between maximum oxygen uptake (VO2max) and Hbmass has been shown at all ages [12–14]. Furthermore, also in certain game sports, like e.g. field hockey, Hbmass is correlated with running endurance [15]. Therefore, swimmers, but also racket sports athletes aim to develop a high hemoglobin concentration [Hb]. However, even in primarily by aerobic endurance dominated sports, like e.g. cycling, no training effects were found after a 12-month training intervention in 11–15 yrs aged players [16] or after an 18 month-s'period of intensive endurance training in elite athletes aged 15–17 yrs [17]. These results lead to the assumption that erythropoietic adaptation might occur at a very young age or during late adolescence or that Hbmass is genetically determined. In contrast, some authors found a small (3%) increase in elite athletes during intensive training [16] or between training and recovery periods [18]. Thus, the question arises whether the high hemoglobin concentration [Hb]/high Hbmass observed in elite endurance athletes is primarily inherited and/or possibly achievable through long-term endurance training already during childhood and adolescence.

One marker of the physical fitness, that is sensitive to training during adolescence, is the heart rate at rest [19]. Thus, it is of major interest whether already at the preadolescent age there were differences between elite youth participants from the endurance-oriented sport swimming, and the more speed-oriented racket sports table tennis and badminton.

Additionally, the vital capacity (VC) is normally increased in endurance athletes, especially in swimmers [20]. Doherty and Dimitriou [21] found an 11% higher functional VC in male swimmers than in land-based athletes at the mean age of 15 years (M = 4,010 ml; SD = 1,200). As aerobic endurance does not play a dominant role in the individual game sports, table tennis and badminton, it is not surprising that even young, preadolescent swimmers exhibit a VC that comes close to that of adult table tennis (M = 4,420 ml, SD = 790) and badminton players (M = 4,000 ml, SD = 630) [22]. The superiority of swimmers over athletes from the individual game sports in VC was confirmed by Bloomfield et al. [23] as they found better values for 11–12 years old swimmers in comparison to tennis players of the same age. Also Zhao et al. [24] found VC values of M = 5,071 ml (SD = 863) in Chinese U15/U16 age group swimmers, which were significantly higher than in a group, comprising youth athletes from five different other sports. In contrast, in that study VC was systematically lower in table tennis athletes (M = 3,823 ml; SD = 423). In combination with the VC, the chest girth (CG) is an adaptation effect of the respiratory muscles in well-trained swimmers [25]. For a Chinese U15/U16 age group of swimmers, Zhao et al. [24] reported a chest circumference of M = 90.3 (SD = 4.3), which was significantly greater than in the group of the youth athletes from the other five sports.

In racket sports, speed parameters, like the reaction time (RT), are suggested to play an important role [26]. As a general result, one can confirm the assumption that elite athletes in most sport disciplines similar to racket sports differ from non-athletes when performing generic, single, and elementary reaction tasks (for table tennis, see [27]). Nevertheless, there is but little evidence for the validity of a simple eye-hand RT assessment to distinguish between elites from different sports [28].

Finally, in jumping disciplines like basketball [29] or volleyball [30] maximal dynamic back strength (BS) turned out to be a relevant predictor of sport performance. This is explained by that the deadlift exercise contributes to the activation of the m. semitendinosus during leg extension and the push-off movement [31]. In swimming [32], the power of the squat movement, which is highly dependent on dynamic BS, is a relevant predictor for the lunge speed and swimming power, respectively. Zhao et al. [24] reported on a higher level of dynamic BS in U15/U16 Chinese table tennis athletes compared to a group of other sports. Furthermore, the high reliability of the deadlift test (ICC = 0.99; [33]) allowed for the use of this measurement in all three sports groups included in this study.

On the basis of such findings, the purpose of this study was to investigate the profile and its response to training of adolescent Chinese elite athletes of swimming and racket sports. It is hypothesized that these youth players already show a sport specific development profile of certain physiological, anthropometric and/or motor performance characteristics which is in line with the specific requirements of each of the particular sports. Moreover, it is hypothesized that the response to the different sport-specific training regimens will also lead to increasing differences in the development of the athletic make-up.

Materials and methods

Participants

The Shanghai Elite Sport School focuses on five different sports branches, of which the investigated two sports groups are the largest at the respective age. Whereas the investigated endurance sports group consists of the open water and bassin swimmers, the group of speed-oriented sports embraces the comparatively homogeneous racket sports group of the investigated table tennis and badminton players. Besides these two groups, the Elite Sport School also promotes combat sports which includes the more heterogeneous judo and fencing athletes, as well as the "big" sports games which consist of baseball, basketball, and volleyball players, and a selection of somewhat older sprint running, hurdling, high and long jump, pole vault and decathlon athletes.

In total, 39 youth male Chinese elite athletes aged between 12–14 years started to take part in the 2-years follow-up assessment. Due to injuries or illness 18 athletes could not take part in all five waves of the repeated measurements, so that n = 21 participants completed the full study (swimming = 10, and racket sports = 11 that is 7 table tennis and 4 badminton players; mean age 12.14 ± 0.62 years, age range 11–13 years) participated in this study. All participants were part of the Shanghai Elite Sport School, and regularly participated in the sports investigated in one 3-hours training session from Monday to Friday and two 3-hours training sessions on Saturday which amount to 21 hours total training time per week, as well as in frequent competitions. The mean training time per week of the youth athletes was M = 20.8 hrs/w (not including school sports) over the entire study period. According to the coaches‘-training log the swimmers weekly training volume of 60–75 km is apportioned to six days per week and equivalent to about 15 hrs/wk of swimming. Over the course of the training year the swimming program consisted of 67 percent of aerobic endurance training, 28 percent of mixed aerobic-anaerobic, and 5 percent of anaerobic-lactic training. In addition to the swimming sessions, the athletes conducted 2–3 of strength and conditioning sessions per week (4–6 hrs/wk). Although the training load of the in-water and dryland training could be differentiated according to more precise categories of training intensity, at least the total training volume in both forms of training is in line with the data reported by Pollock et al. [34] for British national caliber swimmers. Furthermore, the swimmers weekly training volume complies with the time standard of 21 training hours of the racket sports players. The highly comparable total amount of training load and a training quality on international level of the swimmers and racket sports groups is secured by the official timetable of the Elite sport school training and education program, and the professional expert staff, respectively. Like in the swimmers, the racket sports groups‘training program included 15 hrs per week of 67 percent sport-specific technical-tactical training and 33 percent match play activities, accompanied by 2–3 strength and conditioning sessions amounting to 4–6 hrs/wk. In both sports groups the high volume of sport-specific training should enhance the development of the aerobic endurance which in swimming is needed for sustaining swimming speed [35], and in table tennis and badminton for quick recovery (see [36, 37], as well as [38–40], resp). The strength and conditioning exercises in both sports groups take place at the same high standard level and is focused predominantly on core strength and the explosive power of arms and legs. So, in both sports groups the young elite athletes should demonstrate considerable training adaptations in the compared physiological performance characteristics, body dimensions, and motor abilities [41, 42].

The mean training experience at the beginning of the study was two years. All athletes were performing at a high level in their respective sport, representing China and/or the Shanghai province in international competitions.

As non-athletes are not part of the Shanghai Elite Sport School, a control group was not at hand. To allow for the conclusion that the investigated development of the 12–14 years old youth athletes could not be related alternatively to the natural grow of Chinese teenagers, we could refer our initial assessment parameters at least to actual data of representative Chinese surveys on body height and weight [43], vital capacity [44], and hemoglobin concentration [45].

The participants were recruited according to the ethical standards of the Shanghai University of Sports (SUS). This study was carried out in accordance with the recommendations of

"Science Research Ethics Committee at the Shanghai University of Sport" with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the "Science Research Ethics Committee at the Shanghai University of Sport." All athletes' parents were informed about the protocol of this study, which was outlined in an information letter. No data collection took place without parents' consent.

Design of the study

In our study, a mixed cross-sectional and longitudinal design was used to allow for the detection of differences in the athletic development pathways exhibited by the two different sports groups, and to inform about selection and/or training effects. Both sports groups were monitored for two years by means of 5 test waves, with 2 visits (March and September) per year. All 21 athletes completed the study and performed all 8 tests.

Procedures and protocols

At each visit, all athletes completed a questionnaire that asked about their training volume and frequency, injuries, and illnesses within the previous three months. None of the participants taking part in all five test waves showed any severe injuries or illnesses that would have been a reason for exclusion. For both groups, the same parameters were measured during one visit by expert sport school staff members; body height (BH) and body weight (BW), resting heart rate (HR), CG, Hb concentration [Hb] and VC, maximum dynamic BS and eye-hand RT. All tests were conducted on the same day in both the gym and sports science laboratory on campus. The testing started at 10 a.m., and all athletes refrained from strenuous exercise one day prior to the test session.

Physiological characteristics. VC (in ml; high precision digital electronic spirometer, Donghuateng Sports Apparatus Ltd, Beijing, CN) and hemoglobin concentration [Hb] (in mg per l; HemoCue Hb 201; HemoCue AB, Angelholm, Sweden) were diagnosed by medical personnel of the Shanghai University of Sport. The typical error for the expiratory VC measurement is 1.7% [46]. Arterialized blood samples were taken from a hyperemized earlobe to determine the [Hb]. [Hb] was assessed photometrically (HemoCue® HB201+, HemoCue AB, Sweden). The typical error for [Hb] measurement with this device is as accurate as CV $< 1.0\%$ (limits of agreement −1.28 to 0.20 gr/dL; [47]). The HR at rest was recorded in all five periodical measurements for 2 min immediately after awakening in the morning by positioning an H7 Bluetooth HR strap (Polar Electro, Kempele, Finland) on the chest.

Anthropometrics. BH were measured to the nearest 0.1 cm with a mechanical height tester (Donghuateng Sports Apparatus Ltd, Beijing, CN), BW to the nearest 0.1 kg (calibrated Seca Alpha 770), and CG to the nearest 0.1 cm with a circumference ruler (Donghuateng Sports Apparatus Ltd, Beijing, CN) were measured according to standardized test prescriptions that ensure a high reproducibility [48, 49].

Motor performance prerequisites. Maximum dynamic BS (in kg; measured by a power deadlift) and simple RT (in ms; PsyTech Sports; Xinyi Electronic Technology Company, Shanghai, CN) were tested by expert staff members from Shanghai elite sport school. Before the dynamic BS test, subjects performed a warm-up that consisted of cycling and dynamic stretching, followed by the standardized one repetition maximum (1 RM) deadlift [50]. During the test, the standardized procedures for the one repetition maximum (1 RM) deadlift was followed [50]. A low-intensity set of 5–10 repetitions was performed using 40–60% of the perceived 1 RM. After a 1-min rest, subjects performed a set of 2–3 repetitions at 60–80% of the perceived 1 RM. Subsequently, subjects performed 3–5 maximal trials, followed by an assessment of 1 RM deadlift strength. For the power deadlift, Dorrell et al. [51] reported typical errors varying for all variables between two visits from low to moderate (range 0.6–8.8%).

In the simple RT assessment, the test device was prepared to measure the time of a simple finger movement response to light stimulation. The participant sat in front of the test instrument, placed his right index finger on the button, and pressed the button when the red light was on. The measurement included 20 repetitions, and the average value was calculated and used for all further data analysis.

Data analyses

All data were analyzed with SPSS (Version 25.0; SPSS Inc., Chicago, IL, USA). To compare the data from the two sports groups, the group means of each parameter were calculated for each of the five test waves. Subsequently, these data were used in a General Linear Model (GLM) with a 2*5 repeated measurements ANOVA to determine the differences between the five subsequent test waves (factor time), and possible differences in the development of the 8 parameters between the two groups (factor sports), as well as interactions between groups and development. In all analysis the significance level was set at $p < 0.05$. According to the rules of thumb proposed by Miles and Shevlin [52], effect sizes (partial η^2) were interpreted as being small ($< .06$), medium ($< .14$) and large ($> .14$). Although there were no systematic statistical differences among the two sports groups in regard to calendar age, in the GLM analysis the age of the participants at the beginning of the study (test wave 1) was used as a covariate to exclude even slight influences of this factor on the parameters investigated.

Results

Descriptive statistics for all 8 variables of the 5 test waves used in the GLM can be found in Table 1.

Physiological characteristics

The results of the General Linear Model (GLM) with age as a covariate for [Hb] in the athletes from both sports groups are presented in Fig 1. When the two sports groups were compared, [Hb] developed in a slightly different manner, and appears to have increased in a more linear pathway in the swimmers, whereas the racket sports participants exhibit a time-lagged sharp increase in the development of [Hb] over time, which took place on a significantly lower level ($F_{1;18} = 7.983$; $p < 0.05$ partial $\eta^2 = 0.307$). Nevertheless, no significant interaction effect between the time course of [Hb] development and the sport performed could be detected ($F_{4;15} = 1.383$; $p = 0.249$; partial $\eta^2 = 0.071$).

A greater development of the VC took place in the group of swimmers, which was found to be significantly different from that of the racket sports groups ($F_{1;18} = 24.521$; $p < 0.001$; partial $\eta^2 = 0.577$), which also showed an increase, but again on a lower level (Fig 2). Overall, the investigated ten swimmers improved their VC by 31.5%. This large effect led to a maximum VC at the end of the study period of almost five liters (M = 4,911 ml). Although the increase in the racket sports athletes' group by 32.5% was almost the same, the endurance athletes‘maximum VC was more than 1.5 liters higher (Table 1). As the development in the swimmers‘group took place on a higher level, a significant time by group effect ($F_{4;15} = 2.665$; $p = 0.039$; partial $\eta^2 = 0.129$) could be detected.

The resting HR in both sports groups did not decrease over the investigated 2-years preadolescent time span, and remained more or less on the same level (Fig 3). The significantly lower HR at rest of the Chinese swimmers persisted over the whole investigation period ($F_{1;18} = 13.674$; $p < 0.01$; partial $\eta^2 = 0.432$).

Table 1. Physiological, anthropometric, and motor performance data.

	Initial test (9/2016)	Test wave 2 (3/2017)	Test wave 3 (9/2017)	Test wave 4 (3/2018)	Test wave 5 (9/2018)
Swimming group (n = 10)					
Age (months)	145.8 ± 7.2	151.8 ± 7.2	157.8 ± 7.2	163.8 ± 7.2	169.8 ± 7.2
Hemoglobin concentration (gr/l)	128.5 ± 11.1	129.8 ± 12.5 #	132.3 ± 8.3	* 137.2 ± 8.7 ###	* 138.1 ± 11.0
Vital capacity (ml)	3796 ± 645 ###	** 4200 ± 857 ##	** 4486 ± 1064 ##	*** 4823 ± 1081 ##	*** 4911 ± 11.30 ##
Heart rate at rest (bpm)	61.9 ± 7.0	63.8 ± 7.5	61.7 ± 7.3 ##	62.6 ± 4.7 ###	61.7 ± 5.9 ##
Body height (cm)	165.1 ± 9.4 ##	*** 168.5 ± 10.6 ##	*** 171.9 ± 10.7 #	*** 175.4 ± 10.2 #	*** 177.9 ± 9.5 #
Body weight (kg)	57.2 ± 14.3 ##	57.9 ± 13.2 ##	58.9 ± 14.6 #	62.4 ± 15.0 #	63.7 ± 14.2 #
Chest girth (cm)	81.0 ± 7.5 ##	** 83.7 ± 7.3 ##	** 83.9 ± 8.0 #	*** 86.4 ± 8.9 ##	*** 87.5 ± 8.2 ##
Dynamic back strength (kg)	68.6 ± 10.1	* 74.7 ± 15.3	** 79.6 ± 17.3	** 89.8 ± 21.7	** 90.3 ± 19.8
Eye-hand reaction time (ms)	210 ± 27	199 ± 18	197 ± 19 #	222 ± 26	200 ± 23
Racket sports group (n = 11)					
Age (months)	145.6 ± 8.0	151.6 ± 8.0	157.6 ± 8.0	163.6 ± 8.0	169.6 ± 8.0
Hemoglobin concentration (gr/l)	119.7 ± 12.7	117.1 ± 9.7 #	122.3 ± 17.1	119.6 ± 10.4 ###	** 132.8 ± 14.5
Vital capacity (ml)	2686 ± 335 ###	** 2946 ± 399 ##	*** 3059 ± 478 ##	*** 3345 ± 414 ##	*** 3432 ± 477 ##
Heart rate at rest (bpm)	70.5 ± 10.1	68.1 ± 5.7	71.2 ± 6.4 ##	70.4 ± 3.8 ###	69.4 ± 4.0 ##
Body height (cm)	154.9 ± 8.7 ##	*** 157.7 ± 9.1 ##	*** 161.4 ± 9.6 #	*** 165.1 ± 9.4 #	*** 169.0 ± 8.9 #
Body weight (kg)	42.4 ± 9.1 ##	** 46.3 ± 9.2 ##	** 47.5 ± 9.7 #	*** 51.1 ± 9.0 #	*** 53.2 ± 9.2 #
Chest girth (cm)	71.7 ± 7.6 ##	** 73.9 ± 8.0 ##	*** 76.0 ± 7.1 #	*** 78.1 ± 6.6 ##	*** 79.8 ± 6.7 ##
Dynamic back strength (kg)	57.1 ± 17.0	** 67.9 ± 19.3	*** 71.1 ± 14.0	*** 77.2 ± 18.6	*** 85.2 ± 17.3
Eye-hand reaction time (ms)	223 ± 34	213 ± 32	219 ± 21 #	229 ± 17	223 ± 32

Values are means and standard deviations (M ± SD). Significance of differences within the two sports groups between initial and following values

$^{*}p < 0.05$

$^{**}p < 0.01$

$^{***}p < 0.001$.

Significance of differences between the swimming and racket sports groups

$^{\#}p < 0.05$

$^{\#\#}p < 0.01$

$^{\#\#\#}p < 0.01$.

Anthropometrics

Changes in the anthropometric parameters of the two groups during the study period are shown in Table 1. The increase of BH in the pubertal athletes of both sports groups by approximately 6–7 cm per year did not reach significance ($F_{4;15} = 2.348$; $p = 0.062$; partial $\eta^2 = 0.115$), although the swimming group was significantly taller ($F_{1;18} = 11.765$; $p < 0.01$; partial $\eta^2 = 0.395$). In the generally heavier swimmers ($F_{1;18} = 8.212$; $p < 0.05$; partial $\eta^2 = 0.313$) body mass showed a small increase in the first year, followed by a higher increase in both groups of more than 5 kg during the second year (Table 1).

CG increased by 3–4 cm in the first, and also in the second year (see Table 1), thus the overall increase in the total group was significant ($F_{4;15} = 2.989$; $p < 0.05$; partial $\eta^2 = 0.142$). As Fig 4 shows, the CG of the ten swimmers was already greater in the beginning of the monitoring and their lead remained stable over the whole two-year follow-up period. Although the swimmers presented a much larger upper body than their racket sports counterparts ($F_{1;18} = 9.790$; $p < 0.05$; partial $\eta^2 = 0.352$), the shape of the course of the developmental pathway did not vary significantly between the two sports groups.

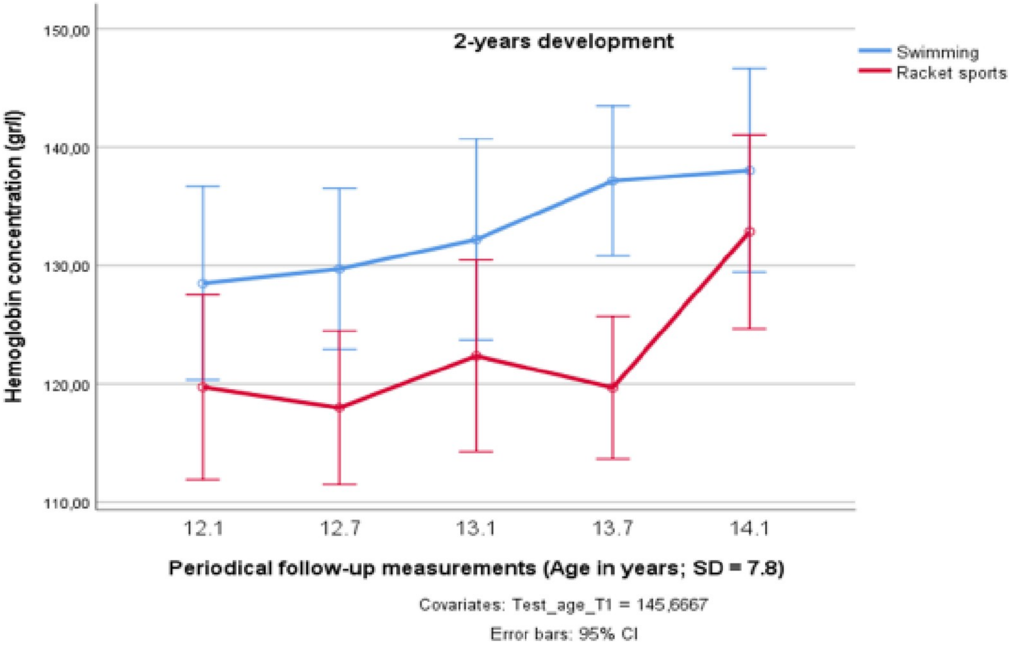

Fig 1. Changes in Hb concentration over two years in preadolescent male elite athletes from swimming and racket sports.

Motor performance prerequisites

The swimmers'group performed the BS test constantly on a higher level, but this difference was not significant ($F_{1;18} = 2.844$; $p = 0.109$; partial $\eta^2 = 0.139$). Also, the development of the

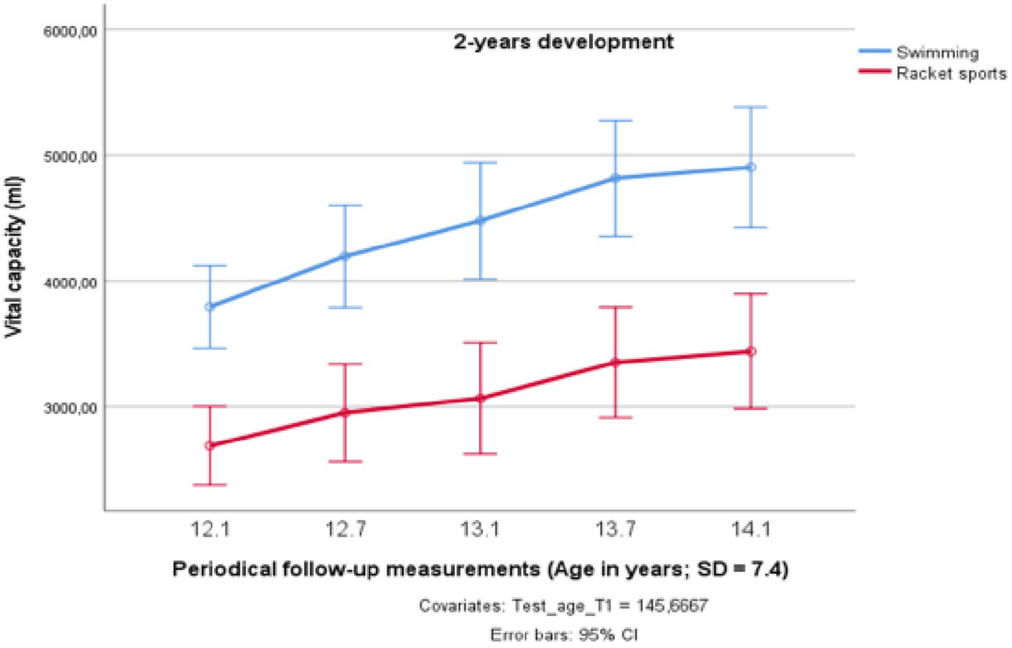

Fig 2. Changes in vital capacity over two years in preadolescent male elite athletes from swimming and racket sports.

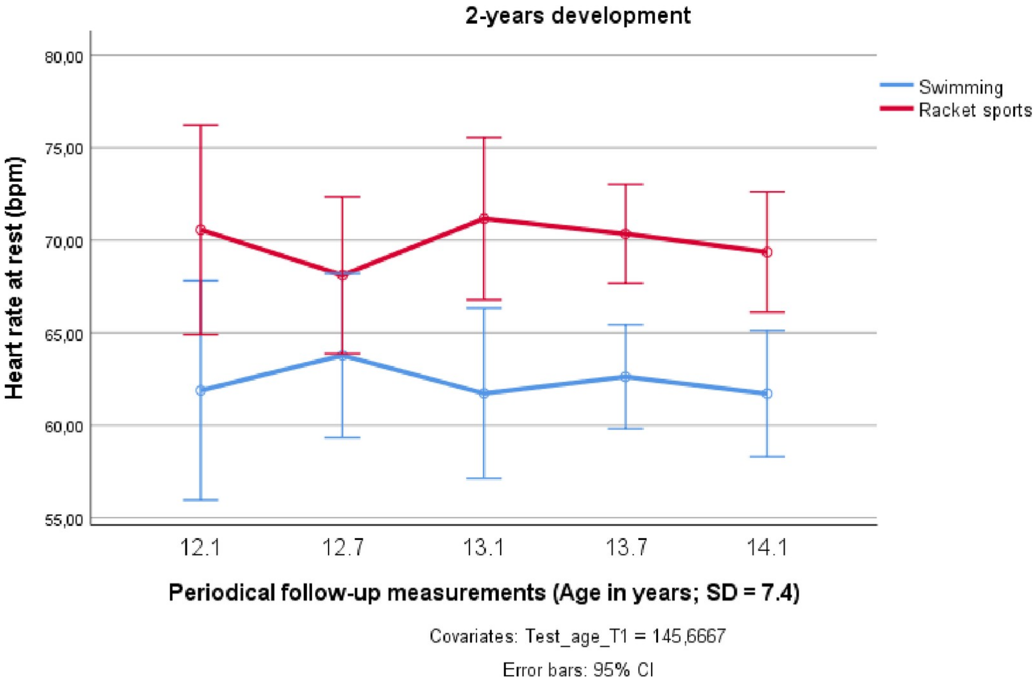

Fig 3. Changes in heart rate at rest over two years in preadolescent male elite athletes from swimming and racket sports.

maximum dynamic BS was not systematically different between the racket sports and swimming groups, although there seemed to exist a certain levelling of the BS development during the last half year in the swimming group. In the racket sports group, the dynamic BS increased

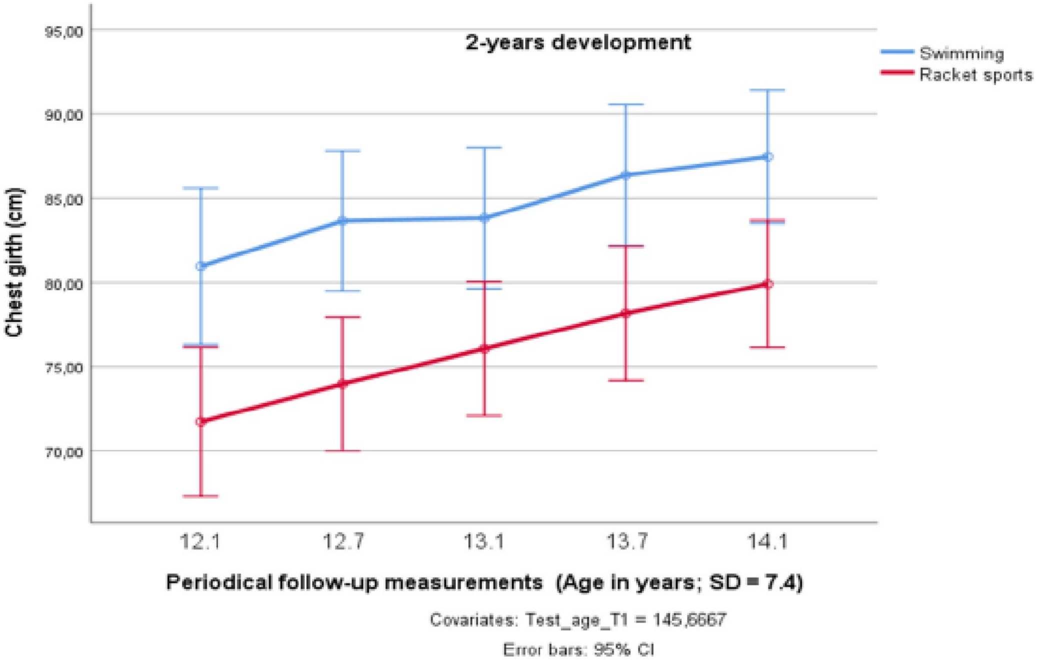

Fig 4. Changes in chest girth over two years in preadolescent male elite athletes from swimming and racket sports.

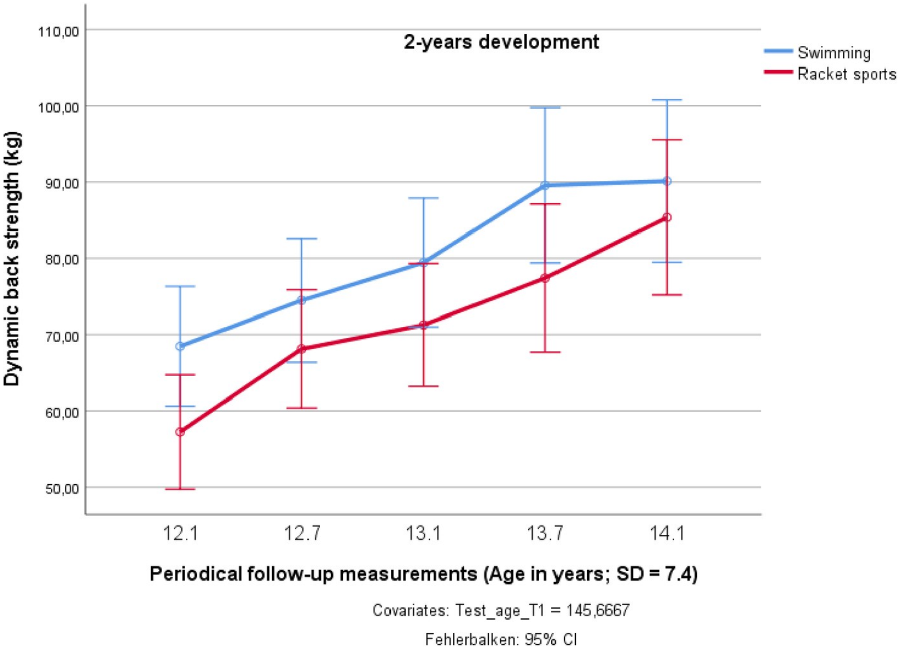

Fig 5. Changes in dynamic back strength over two years in preadolescent male elite athletes from swimming and racket sports.

over the two years by 44.0%. Although the ten swimmers exhibited the highest maximum BS over the whole investigation period, their relative gain in BS was less (34.2%; see Fig 5).

The development of the single RT exhibited a rather unsystematic pattern (see Fig 6). It came as a surprise that the swimmers performed significantly better in the eye-hand RT test ($F_{1;18} = 7.718$; $p < 0.05$; partial $\eta^2 = 0.300$), even though these athletes did not improve much over time (Table 1). As Fig 6 demonstrates, there was no significant interaction between the RT development and the sports performed ($F_{4;15} = 0.444$; $p = 0.776$; partial $\eta^2 = 0.024$).

Discussion

The aim of the study was to monitor the development of anthropometric, physiological and motor performance prerequisites in elite youth athletes for a period of two years to investigate the influence of systematic and sport-specific training on these variables. Based on the results of this current study the development pathways of the anthropometric, physiological, and motor performance prerequisites assessed in the elite sport school in Shanghai, China, are not systematically different from Caucasian athletes investigated in comparable studies in the literature.

In regard to body height and body weight, the swimmers‘group was at the age of 12 years about 11 cm taller and 11 kg heavier than untrained male children of the same age, when compared to the representative data of Zong et al. [43] from the large coastal cities in China. On the other hand, the racket sports players did not exhibit a difference in body height, but were about 4 kg lighter than non-athletes of the same age. Body height in swimming is widely recognized as an important talent factor and performance characteristic [53]. In table tennis, a low body weight as well as a short lower leg length [24] might enhance primarily the players‘agility which plays an important role in elite table tennis performance [54].

The mean values for the [Hb] of the athletes of the three sports groups showed an average yearly increase of 4.4% in the swimmers, and 6.5% in the racket sports players, which was

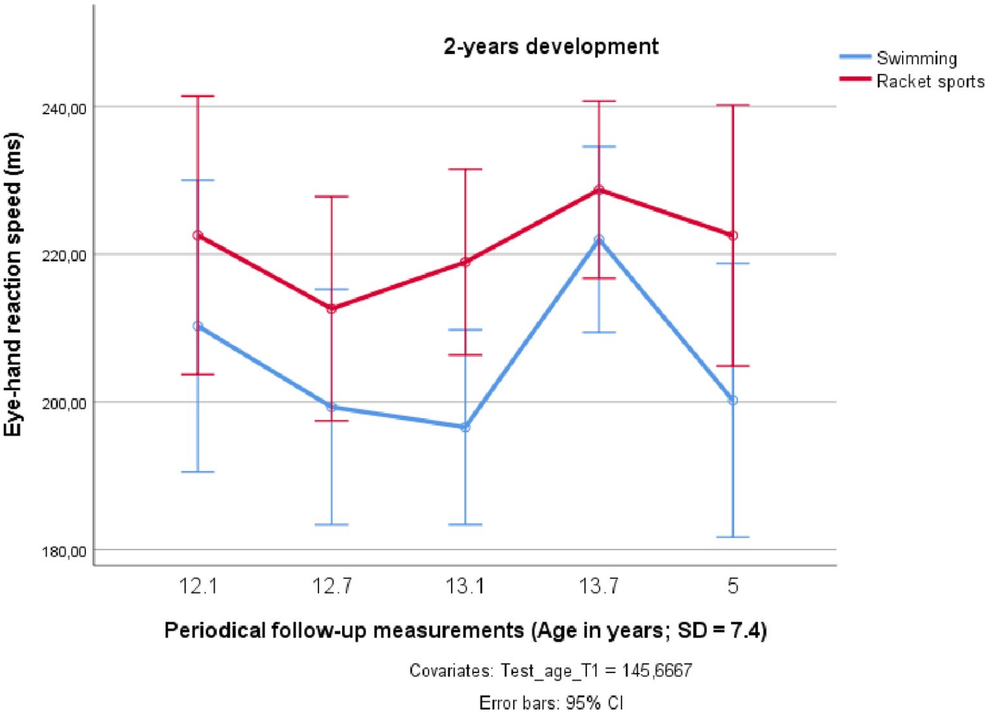

Fig 6. Changes in eye-hand reaction time over two years in preadolescent male elite athletes from swimming and racket sports.

similar to published data for Hbmass, showing a mean value of ~430 g in 11–13-yr-old boys and ~370 g in 12–13-yr-old girls and boys [12]. Despite the general increase in hemoglobin mass with age, Prommer et al. [11] did not find an independent statistical effect of age on Hbmass because, in their study, the effects fully overlapped with those of lean body mass (LBM) in such way that a 1 kg increase in LBM was associated with a 14.7 g increase in Hbmass. As literature has stressed the close relationship between the circulating testosterone levels and [Hb] [55], this leads to the hypothesis, that also the increase of [Hb] in young athletes is primarily affected by androgens [56–58]. This supports the assumption that the sharp increases in [Hb] of 15.8 percent in the racket sports group between the age of 13.7 and 14.1 years observed in our study seem to reflect puberty-associated changes in erythropoiesis and were directly linked to an increase in testosterone levels in the male athletes. In contrast, it remains unclear why such a nonlinear development of [Hb] was not found in the swimmers of the endurance group who showed an almost linear increase over the two-years period.

The impact of the endurance training, itself, on accelerated erythropoiesis has been discussed and the findings are controversial. According to Steiner and Wehrlin [59], who compared the Hbmass values of elite endurance athletes at various adolescent ages and concluded that training at an age younger than 16 years appeared to have had negligible effects with respect to enhanced erythropoiesis, our findings support the hypothesis that, during the investigated 2-years training period during adolescence, genetic predispositions more than the different sports-specific training regimens had an essential impact on [Hb]. Our results are also in line with Ulrich, Bartsch, and Friedmann-Bette [17], who monitored the Hbmass of 15–17-yr-old boys and girls during a 1.5-yr training period, and found a 15% higher Hbmass in the trained subjects than in the untrained subjects, but no systematic training effects. Very similar results were demonstrated by Eastwood et al. [16] for 11–15-yr-old boys and girls after a 1-year training period, showing 10% higher values in the athletic group (cyclists) compared to non-athletes, which the authors attributed to the normal maturation process and not to the training, itself. These results coincide well with the increase of 10–13 gr/l in [Hb] observed in this study for the 12.1–14.1 years old age group (Table 1), corresponding to a change of 7.5% in the swimmers‘, and 10.9% in the racket sports group over the 2-years training period. This training effect exceeds the development of 5.1% of [Hb] in untrained Chinese children between 12–14 years of age, that was reported by Song et al. [45] in his national overview.

However, when comparing the various training regimens during the training period, we cannot determine whether this increase represents a sports-specific training effect, or whether the higher values of the young swimmers are due to a selection process that favors children with a naturally high [Hb] in this endurance sport [16, 17]. As a similar increase of [Hb] occurred in the racket sports group during the pubertal phase investigated here, we hypothesize a high basic genetic impact and a genetically determined influence of endurance training on [Hb], as the gains per year have not been higher in the swimming group. Alternatively, it can be speculated that a higher effect of the swimmers endurance training regimen on [Hb] had been masked by a parallel increase of blood plasma volume due to erythropoiesis [60].

In the HR at rest, similar results have been observed in former studies on preadolescent elite athletes in sports that require particularly extended cardiac demands in response to an overall endurance-oriented training program. So, it is interesting to note that in the investigated Chinese age group swimmers, the average resting HR was about 7 bpm lower than in the national calibre athletes of the same age (*M* = 14.3 yrs; *SD* = 1.0) from a Lithuanian elite sports school, where Kamandulis et al. [61] diagnosed M = 68.6 ± 6.9 bpm. As the resting HR is one marker of the functional status of the organism, it can be assumed that the lower values of the Chinese youth athletes resulted from a more demanding training regimen that exceeded the weekly training volume of the European swimmers by 2–3 training sessions that is about 5–6 hours per week.

A very similar picture as that for [Hb] and resting HR development was found for VC with respect to the developmental change during the age period of 12.1 to 14.1 years investigated in this study. As expected, the greatest development of the VC took place in the endurance group. Here, the investigated ten swimmers improved their VC by 31.5%, which led to a maximum VC at the end of the study period of almost five liters (M = 4,911 ml). Although, the increase in the racket sports athletes by 32.5% was almost the same, the endurance athletes‘-maximum VC was about 1.5 liters higher (Table 1). The greater development found in the swimmers agrees with the previous studies of Lazovic et al. [25], who reported higher lung volumes in endurance sports compared to skill, mixed, and power sports. Furthermore, Mercier et al. [62] stressed that, in 10–14 years old swimmers, the increase of $V02_{max}$ is strongly linked to training volume. As the training volume of the circumpubertal swimmers investigated in our study is higher than the amount of 14 h per week reported for the group with the highest gain in $V02_{max}$ by Mercier et al. [62], it can be assumed that the superior values of the Chinese swimmers compared to their racket sports counterparts resulted, primarily, from their sports-specific training regimen. The main influence factor that might be responsible for the higher gains in VC in the swimmers compared to the group of the racket sports participants, exhibiting a quite similar development of their VC on a lower level, can be seen in the strengthening effect of the underwater pressure on the respiratory muscles of the swimmers [63]. So, it is not a surprise that the VC of the swimmers‘group already at the beginning of our investigation period exceeded the mean value of 12.5 years old untrained subjects from the city of Shanghai (M = 2517 ml; [44]) by 50.8%., while in the racket sports group this difference was 6.7%.

The strengthening effect of water pressure on the respiratory muscles of swimmers not only enhances swimming performance but might also contribute to the greater CG in the swimmers of the endurance group. This mechanism is not only typical for swimmers but can also be detected in fin swimming [64] and scuba diving [65]. The CG of the ten swimmers of the endurance group was already greater in the beginning of the study and remained stable over the whole 2-years follow-up period.

The relevance of dynamic BS was reported in a variety of different sports. In game sports like basketball [29] or volleyball [30], maximal dynamic BS turned out to be a relevant predictor of sport performance, and also reduced injury prevalence [66]. In swimming, Morouco et al. [32] found that the power of the squat movement is a relevant predictor not only for lunge speed and swimming power, but also enhances swimming endurance via a more stable prone position which reduces drag resistance considerably for all swimming velocities. Besides its validity, the high reliability of the deadlift test (ICC = 0.99; [33]) allowed for the use of this measurement in both sports groups of this study.

The finding of this study, which concludes shorter RT in a single eye-hand coordination task for the group of the swimmers needs further investigation as it cannot be confirmed by the existing literature.

Study limitations

Our study has several limitations. The first limitation is the relatively small sample size, which was a consequence of the inclusion of only elite athletes. This implies that members of the investigated age group of 12-14-year-old male athletes cannot be numerous. Besides that, the small number of athletes from the two sports groups, resulted also from the short-term dropout rate of 18 participants shortly before any one of the five single measuring dates due to injuries, illness or other causes.

The second limitation is the study's narrow focus on only male youth athletes. This was due to feasibility reasons. At the moment of testing, the number of elite female athletes was even smaller compared to the males. This would have caused further statistical restrictions. oreover, we expected a different influence of the gender-specific athletic make-up on the sports-specific performances of male and female youth athletes in the two sports groups. For this reason, it seems invalid to group male and female athletes together.

Furthermore, the lack of a control group participating in a more general training program on a non-elite level, monitored over the same time period and with the same frequency as the elite youth athletes‘groups, makes it difficult to distinguish between real training effects and genetic predispositions. Nevertheless, the data collected in this study provide valuable insights into the changes in [Hb], VC, and CG, as well as BS development, under the relatively intensive training regimen in an elite youth sport school in China. Although that the elite sport school system secures a comparable maximum of total training load and a training quality on the international standard level in the players from different sports, in future studies a deeper insight into the details of the sports-specific training load set-up and the progression and periodization of the training volume and intensity over this important age period in the long-term athletic development is desirable. Besides more precise information on the kilometers swum in different intensity categories in the swimmers group, and on the exercise duration in specific training content categories, like e.g. technical and tactical training drills and match play in the racket sports group, further information on the individual training response is warranted. So, besides measures of resting, exercise, and recovery heart rate during the training sessions [67], also daily training logs, psychometric questionnaires [68], and frequent performance testing of endurance, speed and strength parameters [69] may offer a more complete solution to diagnose the long-term performance development in athletes participating in aerobic- and strength-oriented sports.

Conclusion

In general, elite youth athletes of the different sports groups (swimming and racket sports) from China between 12–14 years of age exhibit similar development profiles of physiological, anthropometric, and motor performance prerequisites profiles, compared to North American or European athletes. [Hb] and VC of Chinese male youth athletes linearly increase between the ages of 12 and 14 years, showing a mean increase of 5% per year, not only reflecting their sports-specific response to training, but also the impact of testosterone production during the onset of puberty. These age-related changes in [Hb] are mainly promoted by the development of body mass, although long-term training exerts additional effects. However, this study provides evidence that the higher [Hb] found in the endurance-trained swimmers between the age of 12 and 14 years is not primarily due to the sports-specific training regimen alone, but also to genetic preselection. From our study, there is no evidence that the talent recruitment strategies or the sports-specific training regimens administered in a typical elite sport school in China cause different development pathways in Chinese youth athletes when compared to North-American or European players, and thus need to be changed. Nevertheless, the heterogeneity of the intra-group samples in the two racket sports disciplines table tennis and badminton summed-up as similar sports types, require a cautious interpretation of our results. Also, the focus on solely male athletes point to a need for further investigations in the talent development process-up of elite youth sport cadres. Furthermore, a greater variety of motor tests including speed, endurance and flexibility tests in the youth athletes‘assessments were reasonable.

Supporting information

S1 Data.
(XLSX)

Acknowledgments

The authors thank all the children and parents for their excellent compliance.

Author Contributions

Conceptualization: Binghong Gao.

Data curation: Kewei Zhao, Yu Chang.

Funding acquisition: Binghong Gao.

Methodology: Irene Faber.

Project administration: Andreas Hohmann.

Writing – original draft: Kewei Zhao.

Writing – review & editing: Andreas Hohmann.

References

1. Baker J. & Wattie N. (2018). Innate talent in sport: Separating myth from reality. *Current Issues in Sport Science (CISS).*
2. Faber I.R. (2019). The talent quest–comment on Baker. Accepted for publication in: *Current Issues in Sport Science (CISS)*. https://doi.org/10.15203/CISS_2019.103

3. Elferink-Gemser M.T., Visscher C., Lemmink K.A.P.M. & Mulder T. (2007). Multidimensional performance characteristics and standard of performance in talented youth field hockey players: A longitudinal study. *J Sports Sci*., 25(4):481–489. https://doi.org/10.1080/02640410600719945 PMID: 17365535
4. Opstoel K., Pion J., Elferink-Gemser M., Hartman E., Willemse B., Philippaerts R., Visscher C., et al. (2015). Anthropometric Characteristics, Physical Fitness and Motor Coordination of 9 to 11 Year Old Children Participating in a Wide Range of Sports. *PLoS One*, 10(5): e0126282. Published online 2015 May 15. https://doi.org/10.1371/journal.pone.0126282 PMID: 25978313
5. Sieghartsleitner R., Zuber C., Zibung M. & Conzelmann A. (2019). Science or Coaches' Eye?—Both! Beneficial Collaboration of Multidimensional Measurements and Coach Assessments for Efficient Talent Selection in Elite Youth Football. *J Sports Sci Med*., 18(1):32–43. PMID: 30787649
6. Vaeyens R., Lenoir M., Williams A.M. & Philippaerts R.M. (2008). Talent identification and development programmes in sport: current models and future directions. *Sports Med*., 38(9):703–14. https://doi.org/10.2165/00007256-200838090-00001 PMID: 18712939
7. Fransen J., Pion J., Vandendriessche J., Vandorpe B., Vaeyens R., Lenoir M., et al. (2012). Differences in physical fitness and gross motor coordination in boys aged 6–12 years specializing in one versus sampling more than one sport. *J Sport Sci*., 30(4):379–386. https://doi.org/10.1080/02640414.2011.642808 PMID: 22214429
8. Fransen J., Deprez D., Pion J., Tallir I.B., D'Hondt E., Vaeyens R., et al. (2014). Changes in Physical Fitness and Sports Participation Among Children With Different Levels of Motor Competence: A 2-Year Longitudinal Study. *Pediatr Exerc Sci*., 26(1):11–21. https://doi.org/10.1123/pes.2013-0005 PMID: 24018944
9. Balyi I., and Hamilton A. (2004). *Long-Term Athletic Development*: *Trainability in Childhood and Adolescence*. *Windows of Opportunity*. *Optimal Trainability*. Victoria: National Coaching Institute & British Columbia Advanced Training and Performance Institute.
10. Brocherie F., Millet G.P., Hauser A., Steiner T., Wehrlin J.P., Rysman J., et al. (2015). Association of Hematological Variables with Team-Sport Specific Fitness Performance. *PLoS One*. 10(12):e0144446. https://doi.org/10.1371/journal.pone.0144446 eCollection 2015. PMID: 26641647.
11. Prommer N., Wachsmuth N., Thieme I., Wachsmuth C., Mancera Soto E. M., Hohmann A., and Schmidt W. F. (2018). Influence of endurance training during childhood on total hemoglobin mass. *Frontiers in Physiology*. 9, 251. https://doi.org/10.3389/fphys.2018.00251 PMID: 29618981
12. Astrand P.-O. (1952). *Experimental studies of physical working capacity in relation to sex and age*. Copenhagen: Ejnar Munksgaard.
13. Schmidt W., and Prommer N. (2010). Impact of alterations in total hemoglobin mass on VO2max. *Exercise and Sport Science Review*. 38, 68–75.
14. Montero D., and Lundby C. (2017). Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. *Journal of Physiology*. 595, 3377–3387. https://doi.org/10.1113/JP273480 PMID: 28133739
15. Hinrichs T., Franke J., Voss S., Bloch W., Schänzer W., and Platen P. (2010). *J Strength Cond Res*. 24 (3):629–38. https://doi.org/10.1519/JSC.0b013e3181a5bc59 PMID: 19704383.
16. Eastwood A., Bourdon P. C., Withers R., and Gore C. J. (2009). Longitudinal changes in hemoglobin mass and VO2max in adolescents. *European Journal of Applied Physiology*. 105, 715–721. https://doi.org/10.1007/s00421-008-0953-x PMID: 19084989
17. Ulrich G., Bartsch P., and Friedmann-Bette B. (2011). Total haemoglobin mass and red blood cell profile in endurance-trained and non-endurance-trained adolescent athletes. Europ*ean Journal of Applied Physiology*, 111, 2855–2864. https://doi.org/10.1007/s00421-011-1920-5 PMID: 21431423
18. Garvican L. A., Martin D. T., McDonald W., and Gore C. J. (2010). Seasonal variation of haemoglobin mass in internationally competitive female road cyclists. *European Journal of Applied Physiology*. 109, 221–231. https://doi.org/10.1007/s00421-009-1349-2 PMID: 20058020
19. Silva D.A.S., de Lima T.R. & Tremblay M.S. (2018). Association between Resting Heart Rate and Health-Related Physical Fitness in Brazilian Adolescents. *Biomedical Research International*, 2018:3812197. Published online 2018 Jun 28. https://doi.org/10.1155/2018/3812197 PMID: 30050928
20. Noriega-Sanchez S.A., Legaz-Arrese A., Suarez-Arrones L., Santalla A., Floria P. & Munguia-Izquierdo D. (2015). Forced inspiratory volume in the first second as predictor of front-crawl performance in young sprint swimmers. *Journal of Strength and Conditioning Research*, 29(1), 188–194. https://doi.org/10.1519/JSC.0000000000000634 PMID: 25051007.
21. Doherty M. and Dimitriou L. (1997). Comparison of lung volume in Greek swimmers, land-based athletes, and sedentary controls using allometric scaling. *British Journal of Sports Medicine*. 31, 337–341. https://doi.org/10.1136/bjsm.31.4.337 PMID: 9429014

22. Ghosh A. K., Ahuja A., and Khanna G. L. (1985). Pulmonary capacities of different groups of sportsmen in India. *British Journal of Sports Medicine*. 19, 232–234. https://doi.org/10.1136/bjsm.19.4.232 PMID: 4092147

23. Bloomfield J., Blanksby B. A., Beard D. F., Ackland T. R., Badau A., and Elliott B. C. (1984). Biological characteristics of young swimmers, tennis players and non-competitors. *British Journal of Sports Medicine*. 18, 97–103. https://doi.org/10.1136/bjsm.18.2.97 PMID: 6466937

24. Zhao K., Hohmann A. A., Chang Y., Zhang B., Pion J., and Gao B. (2019). Physiological, anthropometric, and motor characteristics of elite Chinese youth athletes from six different sports. *Frontiers in Physiology*. https://doi.org/10.3389/fphys.2019.00405 PMID: 31105576

25. Lazovic B., Mazic S., Suzic-Lazic J., Djelic M., Djordjevic-Saranovic S., Durmic T., et al. (2015). Respiratory adaptations in different types of sport. *European Review of Medicine and Pharmacological Science*. 19, 2269–2274.

26. Hülsdünker T., Ostermann M. & Mierau A. (2019). The Speed of Neural Visual Motion Perception and Processing Determines the Visuomotor Reaction Time of Young Elite Table Tennis Athletes. *Frontiers in Behavioral Neuroscience*, *19*; 13:165. https://doi.org/10.3389/fnbeh.2019.00165 PMID: 31379535. PMCID: PMC6659573. eCollection 2019.

27. Nakamoto H. and Mori S. (2008). Sport-specific decision-making in a Go/NoGo reaction task: difference among nonathletes and baseball and basketball players. *Perceptual and Motor Skills*. 106, 163–70. https://doi.org/10.2466/pms.106.1.163-170 PMID: 18459365

28. Badau D., Baydil B., and Badau A. (2018). Differences among three measures of reaction time based on hand laterality in individual sports. *Sports* (Basel). 6, 45. https://doi.org/10.3390/sports6020045 PMID: 29910349

29. Chaouachi A., Brughelli M., Chamari K., Levin G. T., Ben-Abdelkrim N., Laurencelle L., et al. (2009). Lower limb maximal dynamic strength and agility determinants in elite basketball players. *Journal of Strength and Conditioning Research*, 23(5), 1570–1577). https://doi.org/10.1519/JSC.0b013e3181a4e7f0 PMID: 19620905

30. Bunn J. A., Ryan G. A., Button G.R., and Zhang S. (2017). Evaluation of strength and conditioning measures with game success in Division I collegiate volleyball: A retrospective study. *Journal of Strength and Conditioning Research*, 2017, Aug 4, https://doi.org/10.1519/0000000000002181 (E-pub ahead of print).

31. Tsaklis P., Malliaropoulos N., Mendiguchia J., Korakakis V., Tsapralis K., Pyne D., et al. (2015). Muscle and intensity-based hamstring exercise classification in elite female track and field athletes: implications for exercise selection during rehabilitation. Open Access *Journal of Sports Medicine*. 26, 209–217. https://doi.org/10.2147/OAJSM.S79189 eCollection 2015. PMID: 26170726

32. Morouço P., Neiva H., González-Badillo J. J., Garrido N., Marinho D. A., and Marques M. C. (2011). Associations between dry land strength and power measurements with swimming performance in elite athletes: a pilot study. *Journal of Human Kinetics*. 29A:105–112. https://doi.org/10.2478/v10078-011-0065-2 Epub 2011 Oct 4. PMID: 23486734

33. Comfort P. and McMahon J.J. (2015). Reliability of maximal back squat and power clean performances in inexperienced athletes. *Journal of Strength and Conditioning Research*. 19, 3089–3096.

34. Pollock S., Gaoua N., Johnston M.J., Cooke K., Girard O. & Mileva K.N. (2019). Training Regimes and Recovery Monitoring Practices of Elite British Swimmers. *Journal of Sports Science and Medicine*, 18 (3), 577–585. eCollection 2019 Sep. PMID: 31427881. PMCID: PMC6683628.

35. Reis J.F., Alves F.B., Bruno P.M., Vleck V. & Millet G.P. (2012). Oxygen uptake kinetics and middle distance swimming performance. *Journal of Science and Medicine in Sport*, 15(1), 58–63. https://doi.org/10.1016/j.jsams.2011.05.012 Epub 2011 Jul 28. PMID: 21802360

36. Phomsoupha M. & Laffaye G. (2015). The science of badminton: game characteristics, anthropometry, physiology, visual fitness and biomechanics. *Sports Medicine*, 45(4), 473–495. https://doi.org/10.1007/s40279-014-0287-2 PMID: 25549780.

37. Rampichini S., Limonta E., Pugliese L., Cè E., Bisconti A.V., Gianfelici A., et al. (2018). Heart rate and pulmonary oxygen uptake response in professional badminton players: comparison between on-court game simulation and laboratory exercise testing. *European Journal of Applied Physiology*, 118(11), 2339–2347. https://doi.org/10.1007/s00421-018-3960-6 Epub 2018 Aug 18. PMID: 30121883.

38. Kondric M., Zagatto A.M. & Sekulic D. (2013). The physiological demands of table tennis: a review. Journa*l of Sports Science and Medicine*, 12(3), 362–370. eCollection 2013. PMID: 24149139. PMCID: PMC3772576.

39. Zagatto A.M., Morel E.A. & Gobatto C.A. (2010). Physiological responses and characteristics of table tennis matches determined in official tournaments. Jour*nal of Strength and Conditioning Research*, 24 (4), 942–949. https://doi.org/10.1519/JSC.0b013e3181cb7003 PMID: 20300034.

40. Zagatto A.M., Kondric M., Knechtle B., Nikolaidis P.T. & Sperlich B. (2018). Energetic demand and physical conditioning of table tennis players. A study review. *Journal of Sports Sciences*, 36(7), 724–731. https://doi.org/10.1080/02640414.2017.1335957 Epub 2017 Jun 5. PMID: 28582628.

41. Berryman N., Mujika I., Arvisais D., Roubeix M., Binet C. Bosquet L. (2018). Strength Training for Middle- and Long-Distance Performance: A Meta-Analysis. *International Journal of Sports Physiology and Performance*, 13(1), 57–63. https://doi.org/10.1123/ijspp.2017-0032 PMID: 28459360.

42. Ozmen T. & Aydogmus M. (2016). Effect of core strength training on dynamic balance and agility in adolescent badminton players. *Journal of Bodywork and Movement Therapy*, 20(3), 565–570. https://doi.org/10.1016/j.jbmt.2015.12.006 Epub 2015 Dec 19. PMID: 27634079.

43. Zong X.N. & Li H. (2014). Physical growth of children and adolescents in China over the past 35 years. *Bull World Health Organ.*, 92(8):555–564. https://doi.org/10.2471/BLT.13.126243 PMID: 25177070

44. Wang H.J., Li Q., Guo Y., Song J.Y., Wang Z. & Ma J. (2017). Geographic variation in Chinese children' forced vital capacity and its association with long-term exposure to local PM10: a national cross-sectional study. *Environ Sci Pollut Res Int.*, 24(28):22442–22449. https://doi.org/10.1007/s11356-017-9812-9 PMID: 28803437

45. Song Y., Wang H.J., Dong B., Wang Z., Ma J. & Agardh A. (2017).National Trends in Hemoglobin Concentration and Prevalence of Anemia among Chinese School-Aged Children, 1995–2010. *J Pediatr.*, 183:164–169.e2. https://doi.org/10.1016/j.jpeds.2017.01.012 PMID: 28153479

46. Hutchison D.C.S., Barter C.E., and Martelli N.A. (1973). Errors in the measurement of vital capacity. A comparison of three methods in normal subjects and in patients with pulmonary emphysema *Thorax. 1973 Sep;* 28(5): 584–587. PMCID: PMC470082. https://doi.org/10.1136/thx.28.5.584 PMID: 4784379.

47. Jaggernath M., Naicker R., Madurai S., Brockman M. A., Ndung'u T., and Gelderbloom H. C. (2016). Diagnostic Accuracy of the HemoCue Hb 301, STAT-Site MHgb and URIT-12 Point-of-Care Hemoglobin Meters in a Central Laboratory and a Community Based Clinic in Durban, South Africa. *PLoS One. 2016;* 11(4): e0152184. Published online 2016 Apr 5. https://doi.org/10.1371/journal.pone.0152184 PMCID: PMC4821624. PMID: 27046200.

48. Hawes M.R., and Martin A.D. (2001). Human Body Composition. In *Kinanthropometry and Exercise Physiology Laboratory Manual*: *Tests*, *Procedures and Data* (Vol I, pp. 00–00). London: Routledge.

49. Stewart A., Marfell-Jones M., Olds T., and de Ridder H. (2011). *International standards for anthropometric assessment*. New Zeland: ISAK; LowerHutt.

50. Hoffman J. R. (2006). *Norms for fitness*, *performance*, *and health*. Champaign, IL: Human Kinetics.

51. Dorrell H. F., Moore J. M., Smith M. F., and Gee T. I. (2018). Validity and reliability of a linear positional transducer across commonly practised resistance training exercises. *Journal of Sports Sciences*, https://doi.org/10.1080/02640414.2018.1482588 PMID: 29851551

52. Miles J., and Shevlin M. (2001) App*lying Regression and Correlation*: *A Guide for Students and Researchers*. Sage: London.

53. Moura T., Costa M., Oliveira S., Júnior M.B., Ritti-Dias R. & Santos M. (20199. Height and body composition determine arm propulsive force in youth swimmers independent of a maturation stage. *J Hum Kinet.*, 42:277–284. https://doi.org/10.2478/hukin-2014-0081 PMID: 25414760

54. Nikolić I., Nikolić I., Furjan-Mandić G. & Kondric M. (2014). The relationship of morphology and motor abilities to specific table tennis tasks in youngsters. *Coll Antropol.*, 38(1):241–245. PMID: 24851624

55. Handelsman D.J., Hirschberg A.L., and Bermon S. (2018). Circulating Testosterone as the Hormonal Basis of Sex Differences in Athletic Performance. *Endocr. Rev.* 2018 Oct 1; 39(5):803–829. https://doi.org/10.1210/er.2018-00020 PMID: 30010735

56. Coviello A.D., Kaplan B., Lakshman K.M., et al. (2008). Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. *J Clin Endocrinol Metab*. 93:914–919. https://doi.org/10.1210/jc.2007-1692 PMID: 18160461

57. Hero M., Wickman S., Hanhijärvi R., Siimes M.A., and Dunkel L. (2005). Pubertal upregulation of erythropoiesis in boys is determined primarily by androgen. *J Pediatr*. 146(2):245–52. https://doi.org/10.1016/j.jpeds.2004.09.002 PMID: 15689918

58. Thomsen K., Riis B., Krabbe S., and Christiansen C. (1986). Testosterone regulates the haemoglobin concentration in male puberty. *Acta Paediatr Scand*. 75(5):793–6. https://doi.org/10.1111/j.1651-2227.1986.tb10292.x PMID: 3564947

59. Steiner T., and Wehrlin J. P. (2011). Does hemoglobin mass increase from age 16 to 21 and 28 in elite endurance athletes? *Medicine and Science in Sports and Exercise*. 43, 1735–1743. https://doi.org/10.1249/MSS.0b013e3182118760 PMID: 21311364

60. Sitkowski D., Szygula Z., Pokrywka A., Turowski D., and Malczewska-Lenczowska J. (2018). Interrelationships between changes in erythropoietin, plasma volume, haemoglobin concentration, and total haemoglobin mass in endurance athletes. *Res Sports Med*. 26(3):381–389. https://doi.org/10.1080/15438627.2018.1447936 Epub 2018 Mar 8. PMID: 29516744

61. Kamandulis S., Juodsnukis A., Stanislovaitiene J., Zuoziene I.J., Bogdelis A., Mickevicius M., et al. (2020). Daily Resting Heart Rate Variability in Adolescent Swimmers during 11 Weeks of Training. *International Journal of Environmental Research and Public Health*, 17(6), 2097. Published online 2020 Mar 22. https://doi.org/10.3390/ijerph17062097 PMID: 32235693

62. Mercier J., Vago P., Ramonatxo M., Bauer C., and Prefaut C. (1987). Effect of aerobic training quantity on the VO2 max of circumpubertal swimmers. *International Journal of Sports Medicine*. 8, 26–30. https://doi.org/10.1055/s-2008-1025635 PMID: 3557779

63. Lemaitre F., Coqart J.B., Chavallard F., Castres I., Mucci P., Costalat G., et al. (2013). Effect of additional respiratory muscle endurance training in young well-trained swimmers. *Journal of Sport Science and Medicine*. 12, 630–638. eCollection 2013.

64. Vasickova J., Neumannova K., and Svozil Z. (2017). The effect of respiratory muscle training on fin-swimmers' performance. *Journal of Sport Science and Medicine*. 16, 521–526. eCollection 2017 Dec.

65. Simpson A.L., Ray A.D., Lundgren C.E., and Pendergast D.R. (2012). Energy cost of breathing at depth: effect of respiratory muscle training. *Undersea Hyperbaric Medicine*. 39, 829–834. PMID: 22908839

66. Leetun D., Ireland M., and Wilson J. (2004). Core stability measures as risk factors for lower extremity injury in athletes. *Medicine and Science in Sports and Exercise*, 36: 926–934. https://doi.org/10.1249/01.mss.0000128145.75199.c3 PMID: 15179160

67. Buchheit M. (2014). Monitoring training status with HR measures: do all roads lead to Rome? *Frontiers in Physiology*, 5, 73. Published online 2014 Feb 27. https://doi.org/10.3389/fphys.2014.00073 PMCID: PMC3936188. PMID: 24578692.

68. Main L.C., Warmington S.A., Korn E. & Gastin P. (2016). Utility of the multi-component training distress scale to monitor swimmers during periods of training overload. *Research in Sports Medicine*, 24(3), 269–80. https://doi.org/10.1080/15438627.2016.1202828 Epub 2016 Jul 1. PMID: 27368060.

69. McGuigan M. (2017). *Monitoring Training and Performance in Athletes*. Champaign, IL: Human Kinetics.

8

The effect of an airflow restriction mask (ARM) on metabolic, ventilatory, and electromyographic responses to continuous cycling exercise

João Francisco Barbieri[1☯]*, Arthur Fernandes Gáspari[1☯], Cassia Lopes Teodoro[1☯], Leonardo Motta[1☯], Luz Albany Arcila Castaño[1☯], Romulo Bertuzzi[2], Celene Fernandes Bernades[1‡], Mara Patrícia Traina Chacon-Mikahil[1‡], Antonio Carlos de Moraes[1‡]

1 Department of Sport Science, School of Physical Education, University of Campinas, Campinas, Brazil,
2 Endurance Performance Research (GEDAE-USP), School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* joao.francisco.barbieri@gmail.com

Editor: Laurent Mourot, University of Bourgogne France Comté, FRANCE

Abstract

This study analyzed the physiological adjustments caused by the use of the Elevation training mask® (2.0), an airflow restriction mask (ARM) during continuous exercise. Eighteen physically active participants (12 men and 6 women) were randomized to two protocols: continuous exercise with mask (CE-ARM) and continuous exercise without mask (CE). Exercise consisted of cycling for 20 minutes at 60% of maximum power. Metabolic variables, lactate, and gas concentration were obtained from arterialized blood samples at pre and post exercise. Continuous expired gases and myoelectric activity of the quadriceps were performed at rest and during the test. We observed no reduction in oxygen saturation in CE-ARM, leading to lower pH, higher carbon dioxide, and greater hematocrit (all $p < 0.05$). The expired gas analysis shows that the CE-ARM condition presented higher oxygen uptake and expired carbon dioxide concentrations ($p < 0.05$). The CE-ARM condition also presented lower ventilatory volume, ventilatory frequency, and expired oxygen pressure ($p < 0.05$). No changes in electromyography activity and lactate concentrations were identified. We conclude that using ARM does not induce hypoxia and represents an additional challenge for the control of acid-base balance, and we suggest the use of ARM as being suitable for respiratory muscle training.

Introduction

The benefits of hypoxic training are well documented in the literature and this type of training is known to act upon various body systems, including the central nervous, cardiorespiratory, and muscular systems [1,2]. Because training in high altitudes or using the normobaric

Competing interests: The authors have declared that no competing interests exist.

hypoxia strategy is not easily accessible, other methods to simulate hypoxic training were developed in the last few years. The Elevation training mask® is one of these products. Nowadays known as Training Mask®, this mask is characterized as an airflow restriction mask (ARM). In the marketing material for the product (trainingmask.com), suggestions are made comparing the ARM use and hypoxia exposure (see Training mask Works–Scientifically Proven Performance section). The site states that the use of the mask would increase the circulation Growth Hormone induced by hypoxic conditions [3]. The resistance to airflow would provide this hypoxia condition during breathing via an adjustable valve system that have different "altitude" levels (Elevation Training Mask 2.0). In addition, the ARM is postulated to increase respiratory muscle work leading to improvements in endurance performance, via respiratory muscle training (RMT), as shown in previous literature [4].

The effectiveness of ARMs is not well understood because of the limited number of studies, and limited access to more direct and precise physiologic measurements. Studies using ARMs in long-term training (6 weeks or more) found no major changes in oxygen saturation and cardiovascular adaptations such as maximum oxygen intake ($\dot{V}O_{2\ max}$) or time to exhaustion test [5,6]. Porcari et al. [7] observed that aerobic performance improved after 6 weeks of training, which was attributed to the potential action of ARMs influencing the respiratory muscles, although there were no significant differences in the results of pulmonary function tests. Furthermore, Porcari et al [7] demonstrated that ARMs significantly improved $\dot{V}O_2$ and power output at the respiratory compensation point and ventilatory threshold. However, the metabolic and neuromuscular responses to exercise in the presence or absence of ARMs were not investigated by these authors, and remain unknown.

In a randomized, counterbalanced, and within-subjects study, Romero-Arenas [8] demonstrated that the use of ARMs during a maximal incremental exercise test on a cycle ergometer reduced maximal power and lactate concentration. The use of ARMs in the test did not significantly affect oxygen saturation (SaO_2) in the muscle, but did increase the delivery of O_2 to the frontal cortex measured with near-infrared spectroscopy. The authors attributed this phenomenon to a hypercapnic state induced by the mask, but did not measure this variable (pCO_2) in the blood. Granados et al. [9] also investigated the use of ARM, showing that acute treadmill exercise using a modified version of ARM reduces ventilation (VE) and respiratory rate (RR), which can lead to an increase in CO_2 blood level, although this variable was not directly assessed in the study.

The purpose of this study was to compare blood gases, blood acid-base balance, ventilatory, and electromyographic (EMG) responses during continuous acute cycling exercise using ARM. We hypothesized that ARMs are effective in inducing a decrease in SaO_2 when measured directly in the blood, as well as an increase in blood CO_2. We hypothesized that the mask use will cause changes in metabolites and EMG signals due to alterations in acid-basic balance variables.

Materials and methods

Participants

The study included 18 healthy subjects (12 men and 6 women; age, 25.1 ± 4.6 years; BMI, 22.4 ± 2 kg/cm^2; height, 170 ± 10 cm; $\dot{V}O_{2max}$, 46.8 ± 4.0; maximum power, 247 ± 44 watts) who were physically active (engaged regularly in at least 3 days of physical activity per week). Before the study, the participants were informed about the protocols, possible risks and benefits, and the schedule of activities. The participants were instructed not to perform strenuous physical exercises for 48 hours and not to consume alcohol or stimulant drugs for 24 hours before the tests. Also all participants was instructed to maintenance the supplements that their used and avoid new supplements. The study was approved by the Research Ethics Committee of the University of Campinas (Protocol No. 1.376.230) and was conducted in accordance with the Declaration of Helsinki.

Experimental design

Subjects completed six visits to the laboratory. The first visit was intended to familiarize subjects with the environment, procedures, and exercise protocols. All participants signed the consent form during the first visit. In the second and third visits, a maximal incremental exercise test was performed on a cycle ergometer in order to obtain the maximum power output (Pmax). Two maximal incremental exercise tests were carried out to improve the reliability of the evaluation. The highest Pmax in the two tests was used for training intensity prescription.

The Pmax was used for exercise prescription in the experimental sessions. The fourth visit was intended to familiarize the subjects with the exercise protocol using ARMs. During visits five and six, the participants performed a continuous exercise bout with ARMs (CE-ARM) or a continuous exercise without ARMs (CE) in a crossover, counterbalanced, randomized and within-subject design.

The maximal incremental exercise tests and the two exercise protocols were performed on the same cycle ergometer (RacerMate®, CompuTrainer^{TM}, Seattle, USA) at the same time of day using the same seat, handlebar, and gait settings. The ARMs were configured to simulate an altitude of 15,000 feet (4,572 m) in the CE-ARM condition. The CE condition wore the mask to maintain the same dead space volume but did not use the valves to allow normal VE. The ARM configuration, test protocols, and interval between visits (at least 48h) were determined previously using a pilot study in our laboratory.

Experimental session

For the EMG analysis, electrodes were placed in the quadriceps muscles. After that, the participants remained seated for 10 min, and blood was collected from the earlobe and the distal phalanges of the fingers for metabolic (see Table 1) and blood gas analyses. Participants were positioned on the cycle ergometer at the beginning of the test, and expired gases were collected for seven min at rest as baseline parameters. After this collection, the test began.

The cycling load of the CE-ARM and CE conditions was set at 60% of the Pmax, and was held constant throughout the test at a cadence of 65–75 revolutions per minute (rpm). Both experimental sessions lasted 20 min.

Expired breath-to-breath gases and the EMG activity of quadriceps muscles were collected during all the test. Blood samples were drawn from the distal phalanges of the fingers immediately and at three, five, and seven min after exercise. Arterialized blood was drawn from the left ear lobe for metabolite analysis at the end of the test.

ARM adaptation piece to gas analyzer

ARM was coupled to the gas analyzer using a mouthpiece made with the polymer acrylonitrile-butadiene-styrene in an FDM-type 3D printer (RepRap Prusa Mendel I3, Sethi Indústria e Comércio de Produtos Eletrônicos, São Paulo, Brazil). The total volume of the ARM and mouthpiece was approximately 350 ml (see Fig 1). The experimental tests were performed in both conditions using the mouthpiece and ARM (no valves were used in the CE condition) to keep the same dead space volume between the two conditions. Neoprene was used on the inner edge of the mouthpiece to ensure proper sealing, and the mouthpiece was attached to the ARM using adhesive tape.

Table 1. Metabolic variables.

Variables	Conditions	Pre	Post	Δ (%)
pH	CE	7,42 ± 0.01	7,33 ± 0.03*	1.21
	CE-ARM	7,42 ± 0.001	7,29 ± 0.06* †	1.71
$cHCO_3$ (mmol/L)&	CE	22,31 ± 1,88	17,96 ± 2,14	19.43
	CE-ARM	21,76 ± 1,66	17,62 ± 3,25	19.02
HHb (%)&	CE	1,87 ± 1,01	3,14 ± 1,25	67.92
	CE-ARM	1,77 ± 0.81	3,79 ± 1,55	114.1
BE (mmol/L)&	CE	-1,54 ± 1,52	-6,98 ± 2,16	353.2
	CE-ARM	-1,98 ± 1,27	-8,25 ± 3,71	319.61
Lactate (mmol/L)&	CE	1,66 ± 0.51	7,71 ± 3,31	364.4
	CE-ARM	1,53 ± 0.45	7,90± 2,92	416.34
pO_2 (mmHg)&	CE	85,15 ± 7,47	80.62 ± 6,32	5.32
	CE-ARM	82,43 ± 4,04	79,96 ± 5,39	3.03
pCO_2 (mmHg)	CE	35,35 ± 3,19	34,76 ± 3,33	1.66
	CE-ARM	34,61 ± 3,25	37,39 ± 4,06 * †	8.03
Hct (%)	CE	44,23 ± 4,28	46,93 ± 4,46*	6.10
	CE-ARM	45,01 ± 4,37	48,99 ± 4,78* †	8.84
SaO_2 (%)&	CE	98,09 ± 1,03	96,81 ± 1,26	1.30
	CE-ARM	98,19 ± 0.84	96,16 ± 1,58	2.06

Abbreviations: BE, base excess; CE-ARM, continuous exercise with mask; CE, continuous exercise without mask; Hct, hematocrit; HCO3, bicarbonate concentration; HHb, deoxyhemoglobin; SaO2, oxygen saturation; pO2, partial oxygen pressure; pCO2, and partial carbon dioxide pressure.

Data are expressed as Mean ± SD.

* interaction effect between time for the same condition ($p < 0.05$). & difference between time ($p < 0.05$).

† difference between conditions ($p < 0.05$).

Maximum incremental test

The test was performed on a cycle ergometer (CompuTrainer™, RacerMate®, Seattle, USA) with continuous collection of gas exchange ($\dot{V}O_2$, $\dot{V}CO_2$ and VE) using an automatic gas analyzer (CPX Ultima™, Medgraphics®, Minnesota, USA). The calibration of the cycle ergometer and automatic gas analyzer was identical in all tests. The gear ratios and adjustments were identical in all tests and experimental sessions for both conditions. The participants performed progressive exercise to exhaustion. The load was considered maximum when at least two of the following criteria were met: 1. Plateau in oxygen uptake, defined as an increase of less than 2.1 ml/kg/min in oxygen volume ($\dot{V}O_2$) even with increased load; 2. Respiratory exchange ratio ≥ 1.1; and 3. Heart rate (HR) $\geq 90\%$ of the maximum predicted by age (220 –age). These criteria were based on the study by Howley, Bassett, and Welch [10]. The test comprised a preliminary five-min rest and five-min warm-up to 50 W, followed by increments of 25 W every minute. The subjects were instructed to maintain a cadence of 70–75 rpm. When it was impossible to maintain the desired cadence, two reestablishment attempts were given. The test was discontinued when the participant failed to maintain cadence on the third attempt, even under strong verbal encouragement. The third failed attempt was considered physical exhaustion. Heart rate was recorded during the test using a cardio-frequency meter (Polar, Kempele, Finland).

Blood collection and analysis

For analysis of the lactate concentration [Lac], 25μL blood samples were collected from the distal phalanges of the fingers by puncture with disposable lancets after cleaning the fingers with

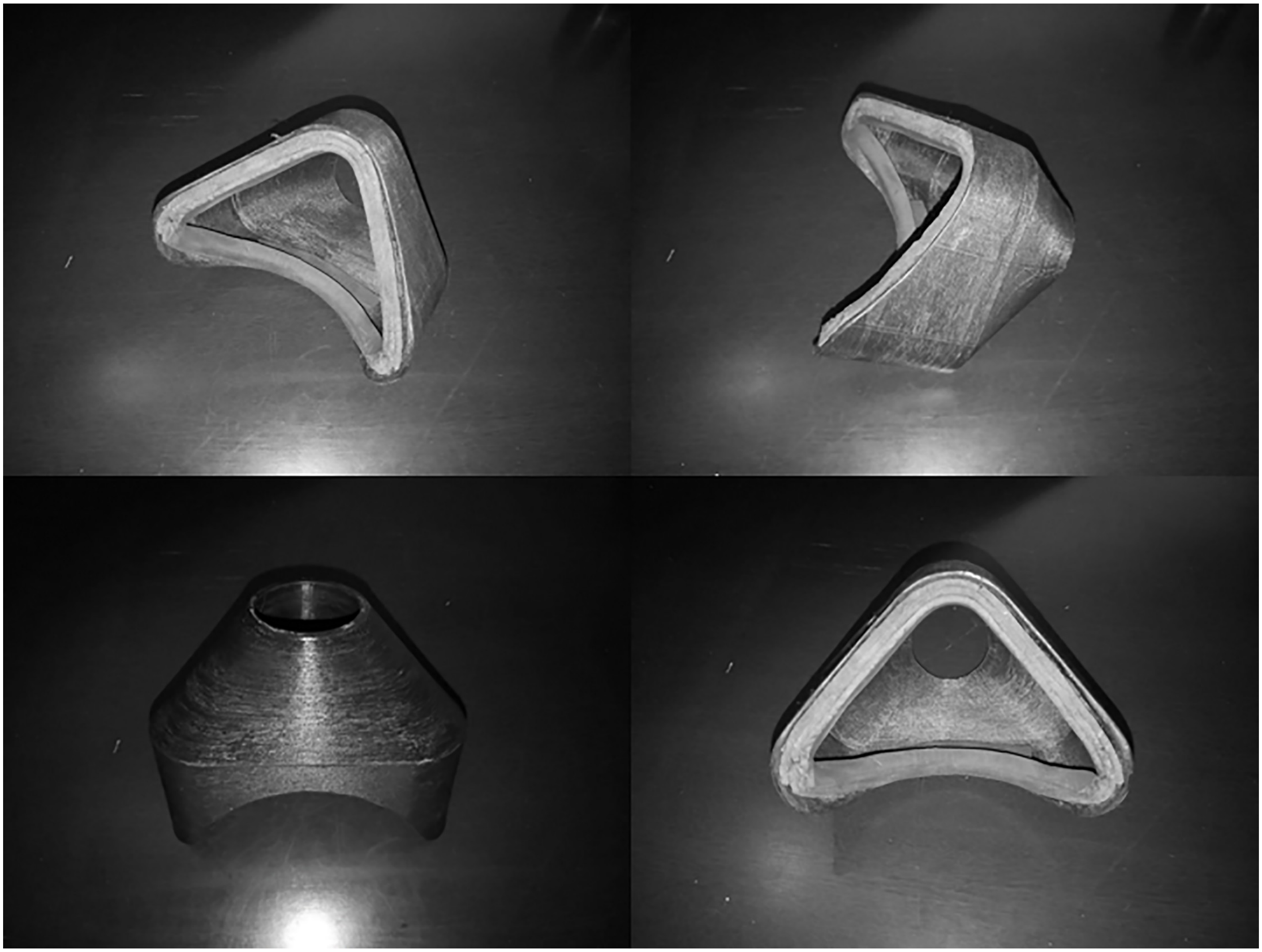

Fig 1. Piece to connect the ARM to the gas analyser. The 3D piece was used to connect the ARM to the gas analyser. The complex ARM + 3D piece had a dead space of 350 ml.

70% alcohol. Blood was collected immediately before, immediately after, and at three, five, and seven min after the end of the exercise. The blood samples were immediately transferred to microtubes containing 25μL of 1% sodium fluoride and centrifuged for 10 min at 5,000 rpm (Centrifuge Excelsa Baby I, Fanem, São Paulo, Brazil). The supernatant plasma was harvested using an automatic pipette and stored at −80˚C for further analysis. Lactate concentration was measured in a spectrophotometer (ELx800, Biotek®, Vermont, USA) using commercial kits (Biotecnica®, São Paulo, Brazil).

Blood (approximately 125 μl) was collected from the earlobe in capillary tubes (Roche, Diagnostics GmbH, Mannheim, Germany) before and immediately after the exercise to analyze the following blood gas variables: partial oxygen pressure (pO_2), partial carbon dioxide pressure (pCO_2), pH, deoxyhemoglobin (HHb), base excess (BE), hematocrit (Hct), bicarbonate concentration (HCO_3), and oxygen saturation (SaO2). Ten minutes before the first blood collection, the earlobe was pre-warmed for 5 min to activate blood flow and facilitate absorption of a vasodilator cream (Finalgon; Laboratorios FHER, SA, Barcelona, Spain) [11]. When necessary, the cream was applied again before the second collect. Blood samples were immediately analyzed using a point-of-care blood gas analyzer (Cobas b 123 POC System; Roche Diagnostics GmbH, Mannheim, Germany).

Electromyographic signal acquisition and processing

A Biopac EMG system model MP150 was used (Biopac System, Inc.; Santa Barbara, CA, USA) for EMG signal acquisition. This model has 16 channels, active bipolar electrodes (model TSD-150), and a common-mode rejection ratio of >95 dB. AcqKnowledge software version 3.8.1 (Biopac System, Inc., CA, USA) was used for signal analysis. The sample rate acquisition was 2000 Hz, and the bandpass filter was 20–500 Hz.

The surface electrodes were attached to the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM) muscles with adhesive tape according to the recommendations of Surface EMG for Non-Invasive Assessment of Muscles [12]. The centers of the electrodes were kept at a fixed distance of two centimeters. A reference electrode was placed on the tibial tuberosity of the right leg of each participant. Hair and dead cells were removed from the skin to decrease impedance. After signal collection, the root mean square (RMS) of EMG signals (100 bursts) was calculated using the software AcqKnowledge version 3.8.1 (Biopac System, Inc., CA, USA). After that, data were extracted, and a specific routine created in the MATLAB (MathWorks) software was used to calculate the mean values per minute. The RMS result per minute was normalized by the RMS of the first minute of each protocol. The normalized signals of the RF, VM, and VL muscles were summed and presented as a single variable (SUM QF).

Statistical analysis

The normality of the data was confirmed using the Shapiro-Wilk test. Two-way analysis of variance (ANOVA) was used for repeated measurements. The Tukey *post-hoc* test was applied to analyze the interaction effect between *condition* and *time* (pre and post) in ANOVA. The significant main effects of *time* and *condition* are indicated in the text. When appropriate, Tukey *post-hoc* test was used to examine the main effect of *time*. The STATISTICA software version 6.0 was used for all statistical analyses (StatSoft, Inc., Tulsa, OK, USA). Data are presented as mean and standard deviation (SD), and the significance level adopted for all comparisons was $p<0.05$.

Results

Metabolic variables

The results of the metabolic variables are shown in Table 1. The pH decreased in the CE and CE-ARM condition after exercise (CE $p<0.01$; CE-ARM $p<0.01$), and the decrease was larger in the CE-ARM condition ($p<0.01$). HCO_3, HHb, BE, and [Lac] showed a main effect of time (HCO_3 $p<0.01$; HHb $p<0.01$; BE $p<0.01$ and [Lac] $p<0.01$).

The pO_2 showed a main effect of time ($p = 0.05$); as the concentration of pO_2 pre-exercise was smaller than post-exercise for both conditions. The pCO_2 remained unaltered in the CE condition and increased in the CE-ARM condition ($p<0.01$), and the difference between the conditions was significant ($p<0.01$). Hct increased in both conditions post-exercise (both at $p = 0.05$) and was higher in the CE-ARM condition ($p<0.01$). SaO_2 did not present an interaction effect between the conditions ($p = 0.08$) but showed a main effect of time ($p<0.01$) and was lower after the test in both conditions.

Gas analysis and ventilatory variables

A summary of ventilatory variables is presented in Fig 2. $\dot{V}O_2$ was higher in the CE condition in the first minute of the test ($p<0.01$) and higher in the CE-ARM condition from the seventh minute until the end of the test (all $p<0.01$) (Fig 2A). VE was lower in CE-ARM at all time points ($p<0.01$) (Fig 2B). The partial pressure of end-tidal CO_2 ($PETCO_2$) was higher in CE-ARM at all time points ($p<0.01$) (Fig 2C). The partial pressure of end-tidal O_2 ($PETO_2$) was lower in the CE-ARM condition before the test ($p = 0.02$) and from the second minute to end of the test (all $p<0.01$) (Fig 2D). The RR showed a main effect of condition ($p<0.01$) and time ($p<0.01$), and the *post-hoc* test indicated that this variable increased throughout the test. However, the *post-hoc* analysis found no interaction effect between the conditions ($p = 0.24$).

Electromyographic variable

The analysis of SUM QF (Fig 3) data revealed that there was no interaction effect ($p = 0.27$) between condition and time ($p<0.01$). However, there was a main effect of time ($p<0.05$).

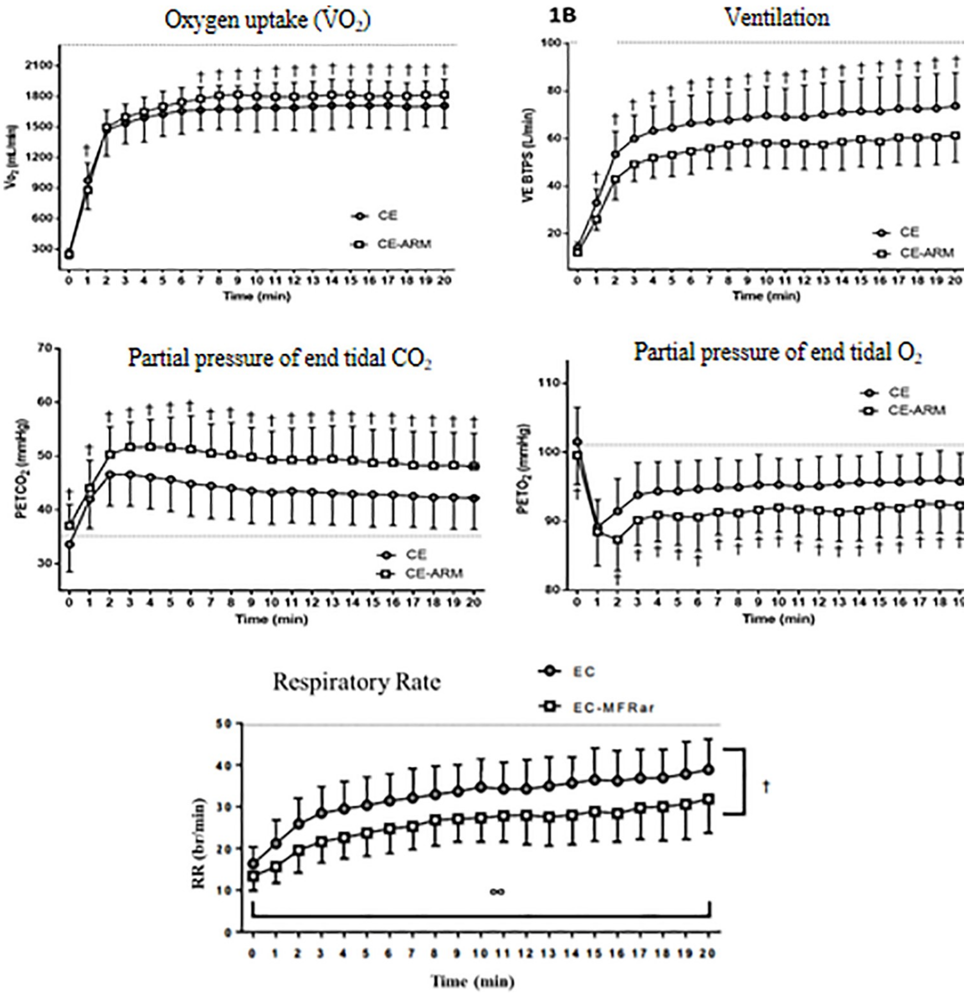

Fig 2. Abbreviations: CE-ARM, continuous exercise with mask; CE, continuous exercise without mask; $PETCO_2$, Partial pressure of end tidal CO_2; $PETO_2$, Partial pressure of end tidal O_2; RR, Respiratory rate; $\dot{\mathbf{V}}\mathbf{O_2}$, Oxygen uptake and VE, Ventilation. Data expressed in Mean (Standard Deviation). † denotes a significant difference between conditions (CE x CE-ARM) identified by the Post Hoc ($p <0.05$). ∞ Denotes main effect of time identified by ANOVA ($p <0.05$).The dashed line indicates the maximum mean value reached in the incremental test.

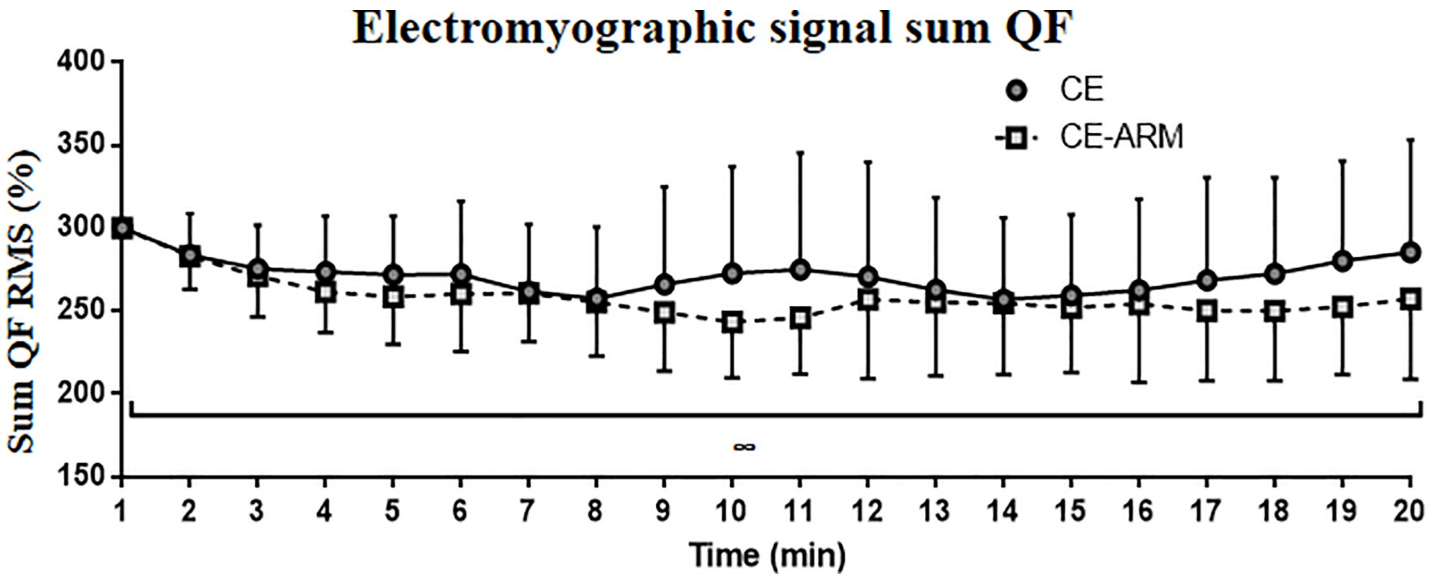

Fig 3. EMG response to the continues test. Abbreviations: CE-ARM, continuous exercise with mask; CE, continuous exercise without mask; Sum QF, the sum of the signals of the already normalized RF, VL and VM muscles. Data are expressed as Mean (Standard Deviation). ∞ Denotes main effect of time identified by ANOVA ($p < 0.05$).

The *post-hoc* analysis indicated that the main effect of time was not significant at any time point.

Discussion

This study evaluated whether the use of ARMs in continuous aerobic exercises could simulate training in hypoxic conditions and cause metabolic and electromyographic changes. ARMs caused significant changes in ventilatory variables, with a decrease in VE and $PETO_2$ and RR and an increase in O_2 consumption and $PETCO_2$. Moreover, there were significant changes in the metabolic profile with the use of ARMs relative to CE, evidenced by lower blood pH, higher blood CO_2, and elevated hematocrit.

Our findings indicated that ARM did not induce hypoxia because the level of decrease in SaO_2 was similar between the two conditions. Furthermore, the absence of hypoxia was evidenced by the lack of significant differences in pO_2 values between the conditions. The other analyzed variables, as Lactate, corroborate that ARMs did not cause hypoxia. Hypoxic training causes pronounced changes in [Lac] when compared with normoxic training [1]. There were no significant differences in [Lac] between the conditions. In addition, it is known that myoelectrical activity is attenuated under hypoxic conditions [1], which did not occur in the present study, whereby there were no significant differences in SUM QF between the conditions.

ARM did not cause hypoxia, but significantly increased Hct. Hematopoietic changes due to exposure to hypoxic environments are considered essential for improving athletic performance [13]. Adaptations such as an increase in baseline Hct are observed in conditions involving chronic exposure to environments with low O_2 saturation as a result of the action of the hormone erythropoietin [14]. However, the increase in Hct at the end of the exercise cannot be attributed to a hypoxic condition, and may be a mechanism to control acid-base balance because the pH was lower in the CE-ARM condition, even with an increase in HHb after exercise in both conditions. The increase in HHb suggests that hemoglobin serves to buffer excess hydrogen protons (H^+) [15]. In addition, it is possible that the decrease in pH and the increase in pCO_2 stimulated a sympathetic response for spleen contraction, releasing reticulocytes into the bloodstream, which could contribute to the increase in Hct [16].

The lower blood pH found in the present study was attributed to respiratory acidosis. The occurrence of metabolic acidosis was discarded because [Lac] was similar in both conditions after exercise, demonstrating that the additional source of H^+ protons did not come from the active leg muscles. This is in agreement with non-differences in quadriceps muscles activation, since there was no significant differences in the electromyographic signal of SUM QF ($p = 0.27$).

Respiratory acidosis is common in some conditions such as chronic obstructive pulmonary disease [17], but this condition can be induced by some techniques such as prolonged expiration, as demonstrated in triathletes [11]. The impaired respiratory mechanics affect the acid-base balance and cause the accumulation of blood CO_2. In this study, ARMs impaired respiratory dynamics, evidenced by the decrease in VE, a main effect of condition on RR, and accumulation of blood CO_2, demonstrating a relationship between changes in ventilatory patterns and changes in the metabolic profile caused by ARMs. In the present study, the magnitude of changes in the ventilatory response was not sufficient to significantly alter SaO_2, and the variables relating to acid-base balance were the most strongly affected.

Studies that use large dead space volumes for RMT found changes in the ventilatory pattern and accumulation of blood CO_2 [18]. These changes may be due to an increase in acidosis and alterations in the composition of the inspired air [18]. The dead volume of the ARM set (adapted mouthpiece and mask) used in the present study was approximately 350 ml, and a small dead space combined with the use of ARM valves may have altered the composition of the air in the mask. There were changes in the expired concentrations of O_2 and CO_2, and the CE-ARM condition presented a decrease in $PETO_2$ and an increase in $PETCO_2$. These results indicate that the air in the dead space had a higher concentration of CO_2 and a lower concentration of O_2, which may have increased blood CO_2 concentration due to rebreathing of the expired air, as postulated previously [7,8].

The ARM may be used as a RMT device, this evidence was confirmed by changes in the ventilatory pattern (VE and RR) via a decrease in air volume, indicating the increased difficulty to perform the respiratory cycle and decreased RR, and demonstrating that time under tension of the respiratory muscles are increased. Additionally, ARM induced respiratory acidosis by increased CO_2 rebreathing; a mechanism used in another RMT devices that use large dead space [18].

A relevant finding of this study was the increase in O_2 consumption in the CE-ARM condition, characterized by high $\dot{V}O_2$ values. Given the lack of increase in electromyographic activity from SUM QF, we hypothesized that the higher consumption of O_2 was due to the higher activity of respiratory muscles, which might decrease ventilatory efficiency because of the lower number of breaths. This phenomenon was been reported in previous studies that have used prolonged expiration as RMT [11].

Limitations

The idea of the project was to evaluate the effect of wearing the mask on a population of physically active individuals. In this sense, adopting a mixed group did not seem to compromise the results. Other studies that aimed to evaluate the effects of using an implement or supplementation also adopted similar methodological approach [19]. A detailed overview of male and female analysis can be found in the supplementary material S1–S4 Tables.

Additionally, the present study has the following limitations. First, blood gas analysis indicated systemic alterations but not metabolic changes in the muscle, so a muscle specific analysis such as from muscle biopsies or even NIR-S could give us a better picture of muscle metabolism. Secondly, ventilatory muscles were not evaluated directly; therefore, the higher activation of these muscles is speculative. Finally, it is possible to speculated a potential additive ergogenic effect due any supplements taken by subjects, however as any supplementation was maintained during the entire study, any effect would occur equally in both conditions.

We recommend that future researchers investigate the relationship between the use of the mask and a heightened activation of the inspiratory musculature (through electromyography or inspiratory force assessments). Future studies should take into account the increased intensity of the exercise protocol, in order to exacerbate the physiological variations with the use of the mask. In conclusion, our results demonstrate that the use of ARMs did not cause hypoxia. Moreover, changes in VE affected metabolic parameters suggesting that the use of ARMs during exercise represents an additional challenge for the control of acid-base balance, therefore the use of ARM for RMT may be appropriate.

Supporting information

S1 Table. Separate man and woman characterization.
(DOCX)

S2 Table. Separate results of lactate for Man (M) and Woman (W).
(DOCX)

S3 Table. Mens gasometric values in CE and ARM.
(DOCX)

S4 Table. Women gasometric values in CE and ARM.
(DOCX)

S1 Dataset. Data used for statistical analysis.
(XLSX)

Acknowledgments

The authors would like to acknowledge all the participants in this project.

Author Contributions

Conceptualization: João Francisco Barbieri, Arthur Fernandes Gáspari, Leonardo Motta.

Data curation: João Francisco Barbieri, Arthur Fernandes Gáspari, Leonardo Motta, Luz Albany Arcila Castaño.

Formal analysis: João Francisco Barbieri, Arthur Fernandes Gáspari, Cassia Lopes Teodoro, Leonardo Motta, Luz Albany Arcila Castaño.

Funding acquisition: João Francisco Barbieri, Arthur Fernandes Gáspari, Cassia Lopes Teodoro, Luz Albany Arcila Castaño, Mara Patrícia Traina Chacon-Mikahil, Antonio Carlos de Moraes.

Investigation: João Francisco Barbieri, Arthur Fernandes Gáspari, Cassia Lopes Teodoro, Leonardo Motta, Luz Albany Arcila Castaño, Celene Fernandes Bernades.

Methodology: João Francisco Barbieri, Arthur Fernandes Gáspari, Cassia Lopes Teodoro, Leonardo Motta, Luz Albany Arcila Castaño, Romulo Bertuzzi, Celene Fernandes Bernades.

Project administration: Cassia Lopes Teodoro, Luz Albany Arcila Castaño, Mara Patrícia Traina Chacon-Mikahil.

Resources: João Francisco Barbieri, Leonardo Motta, Antonio Carlos de Moraes.

Software: João Francisco Barbieri, Antonio Carlos de Moraes.

Supervision: Arthur Fernandes Gáspari, Romulo Bertuzzi, Celene Fernandes Bernades, Mara Patrícia Traina Chacon-Mikahil, Antonio Carlos de Moraes.

Validation: Romulo Bertuzzi, Celene Fernandes Bernades, Mara Patrícia Traina Chacon-Mikahil, Antonio Carlos de Moraes.

Visualization: João Francisco Barbieri, Romulo Bertuzzi, Celene Fernandes Bernades, Mara Patrícia Traina Chacon-Mikahil, Antonio Carlos de Moraes.

Writing – original draft: João Francisco Barbieri, Arthur Fernandes Gáspari, Cassia Lopes Teodoro, Celene Fernandes Bernades, Mara Patrícia Traina Chacon-Mikahil, Antonio Carlos de Moraes.

Writing – review & editing: Arthur Fernandes Gáspari, Cassia Lopes Teodoro, Leonardo Motta, Luz Albany Arcila Castaño, Romulo Bertuzzi, Celene Fernandes Bernades, Mara Patrícia Traina Chacon-Mikahil, Antonio Carlos de Moraes.

References

1. Rusko HK, Tikkanen HO, Peltonen JE. Altitude and endurance training. Journal of Sports Sciences. 2004.
2. Hamlin MJ, Marshall HC, Hellemans J, Ainslie PN, Anglem N. Effect of intermittent hypoxic training on 20 km time trial and 30 s anaerobic performance. Scand J Med Sci Sport. 2010;
3. Kurobe K, Huang Z, Nishiwaki M, Yamamoto M, Kanehisa H, Ogita F. Effects of resistance training under hypoxic conditions on muscle hypertrophy and strength. Clin Physiol Funct Imaging. 2015;
4. Illi SK, Held U, Frank I, Spengler CM. Effect of respiratory muscle training on exercise performance in healthy individuals: A systematic review and meta-analysis. Sports Medicine. 2012.
5. Sellers JH, Monaghan TP, Schnaiter JA, Jacobson BH, Pope ZK. Efficacy of a Ventilatory Training Mask to Improve Anaerobic and Aerobic Capacity in Reserve Officers' Training Corps Cadets. J Strength Cond Res. 2016;
6. Warren B, Spaniol F, Bonnette R. The Effects of an Elevation Training Mask on VO2max of Male Reserve Officers Training Corps Cadets. Int J Exerc Sci. 2017;
7. Porcari JP, Probst L, Forrester K, Doberstein S, Foster C, Cress ML, et al. Effect of wearing the elevation training mask on aerobic capacity, lung function, and hematological variables. J Sport Sci Med. 2016;
8. Romero-Arenas S, López-Pérez E, Colomer-Poveda D, Márquez G. Oxygenation Responses While Wearing the Elevation Training Mask During an Incremental Cycling Test. J Strength Cond Res. 2019;
9. Granados J, Gillum TL, Castillo W, Christmas KM, Kuennen MR. "Functional" respiratory muscle training during endurance exercise causes modest hypoxemia but overall is well tolerated. J Strength Cond Res. 2016;
10. Howley ET, Bassett DR, Welch HG. Criteria for maximal oxygen uptake: review and commentary. Medicine and science in sports and exercise. 1995;
11. Woorons X, Mollard P, Pichon A, Duvallet A, Richalet JP, Lamberto C. Prolonged expiration down to residual volume leads to severe arterial hypoxemia in athletes during submaximal exercise. Respir Physiol Neurobiol. 2007;
12. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol. 2000;
13. Levine BD, Stray-Gundersen J. Dose-response of altitude training: How much altitude is enough? In: Advances in Experimental Medicine and Biology. 2006.
14. Levine BD. Intermittent hypoxic training: Fact and fancy. High Alt Med Biol. 2002;

15. Hall JE, Guyton AC. GUYTON A.C; HALL J. E. Guyton & Hall: Tratado de Fisiologia Médica 11ª ed., Elsevier, Rio de Janeiro, 2006. Journal of Chemical Information and Modeling. 2011.

16. Richardson MX, Lodin A, Reimers J, Schagatay E. Short-term effects of normobaric hypoxia on the human spleen. In: European Journal of Applied Physiology. 2008.

17. Calverley PMA. Respiratory failure in chronic obstructive pulmonary disease. In: European Respiratory Journal, Supplement. 2003.

18. Smolka L, Borkowski J, Zaton M. The effect of additional dead space on respiratory exchange ratio and carbon dioxide production due to training. J Sport Sci Med. 2014

19. Banerjee P, Caulfield B, Crowe L, Clark A. Prolonged electrical muscle stimulation exercise improves strength and aerobic capacity in healthy sedentary adults. Journal of applied physiology. 2005 Dec; 99 (6):2307–11. https://doi.org/10.1152/japplphysiol.00891.2004 PMID: 16081619

9

Preschool environment and preschool teacher's physical activity and their association with children's activity levels at preschool

Chu Chen[1,2]*, **Viktor H. Ahlqvist**[2], **Pontus Henriksson**[3], **Cecilia Magnusson**[1,2], **Daniel Berglind**[1,2]

1 Centre for Epidemiology and Community Medicine, Region Stockholm, Stockholm, Sweden, **2** Department of Global Public Health, Karolinska Institutet, Solna, Sweden, **3** Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden

* chen.chu@ki.se

Editor: David Paul, University of Idaho, UNITED STATES

Abstract

Objective

The aim of this study was to investigate the association between preschool playground size, formalized physical activity (PA) policies, time spent outdoors and preschool teacher's levels of PA and children's objectively assessed levels of PA and sedentary time (ST) during preschool hours.

Methods

In total, 369 children and 84 preschool teachers from 27 preschools in Södermalm municipally, Stockholm Sweden wore an Actigraph GT3X+ accelerometer during 7 consecutive days. Preschool environmental and structural characteristics were measured via the Environment and Policy Evaluation Self-Report (EPAO-SR) instrument and time in- and outdoors was recorded by preschool teachers during the PA measurements. Weight and height of children were measured via validated scales and parents filled out a questionnaire on demographical and descriptive variables. Linear mixed models, nested on preschool level, were used to assess the association between predictors and outcomes.

Results

The mean child age was 4.7 years (SD 0.8) and 45% were girls. We found that children were more active in preschools with a formalized PA policy, compared to preschools without such a policy, but not less sedentary. The association between policy and activity seemed to be more pronounced when accounting for other environmental factors. Similar associations were found in children spent most time outdoors (uppermost quartile) compared with children spent least time outdoors (Lowermost quartile). Preschool teachers' light PA (LPA) ($\beta = 0.25$, $P = 0.004$) and steps ($\beta = 0.52$, $P<0.001$) were associated with children's LPA and steps while the preschool playground size showed no association with PA in children, when accounting for other environmental factors.

Conclusion

The current study showed that preschool structural characteristics such as formalized PA policies and more time spent outdoors were positively associated with children's PA. These findings suggest that formalized PA policies and time outdoors may be of importance for promoting children's PA during preschool hours.

Competing interests: Lindhés Advokatbyrå has no competing interest in terms of its ownership of stocks, employment or consultancy, board membership, patent applications (pending or actual), research grants, travel grants or gifts.

Introduction

Total physical activity (PA), moderate to vigorous PA (MVPA) [1] and steps per day [2] are positively associated with multiple health indicators in young children, while more conflicting findings have been reported for sedentary time (ST) [3]. Furthermore, several studies have shown that physically active children tend to remain more physically active across their lifespan [4]. Despite the many known benefits of PA, children are in general physically inactive [5]. A review on preschoolers' physical activity level based on objective measure has shown highly variable results that preschool children spend 2%–41% of their day in MVPA, 4%–33% in light PA (LPA), and 34%–94% sedentary [6]. Moreover, Swedish data with objectively measured PA and ST, show that preschoolers' levels of PA are low [7, 8]. Hence, effective strategies informed by objective data are urgently required to promote child PA.

A theory base is suggested to be beneficial for the effectiveness of PA promoting strategies [9], therefore the social ecological model was employed as framework to understand systematically what factors may enable effective PA promotion in preschool children [10]. According to the social ecological model, there are different levels of determinants of health behaviors such as physical activity namely individual level, interpersonal level, organizational level and physical environment level [10]. Environmental intervention in preschool which lay emphasize on non-individual level determinants of PA, can potentially be an effective PA promotion strategy also addressing sustainability and equity but the evidence-base is scarce [11]. In Sweden, approximately 92% of all children 1–5 years of age are attending preschools, regardless of their parents' socioeconomic status [12]. Furthermore, approximately 50% of children's daily MVPA is accumulated during preschool hours [8]. Therefore, the preschool environment presents an ideal arena to promote early development of healthy PA and ST behaviors [13].

Potential modifiable characteristics for preschool includes physical environment, policy, time spent outdoors on the organizational level and teachers' PA on the interpersonal level, but the evidence supporting effectiveness is preliminary. In terms of interventions to modify physical environment, studies have shown that structural environmental factors such as playground size, play equipment accessibility and design of the preschool playground may be of importance for children's PA during preschool hours [14, 15]. However, consensus on playground size is hampered by the lack of evidence, application of objective measure on PA, and the difficulty in studying this issue with randomized experimental design [16]. Although physical environment level interventions address all children in the environment with potentially promising sustainability, they are seldom practical due to the requirement on resources especially on large scale [17].

Modifying organizational factors, such as policy and time spent outdoors, is less resource-dependent but may be effective provided adequate evidence base. Having a PA policy in preschool is suggested to be beneficial for preschooler's PA but objective measure on PA is lacking [14, 18, 19]. Conflicting results have been demonstrated by the few existing studies with accelerometer data. While Dowda et al found more MVPA in PA promoting preschools where policy is one of the components [20], Erinosho et al showed a negative association between PA policy and accelerometer measured PA level in preschool children [21]. More research with objective PA data is needed to determine the association between policy and PA levels in preschool children. Similarly, studies investigating associations between time spent outdoors at preschool and children's levels of PA using objective measures on PA are also scarce. Only a few existing studies indicate that the amount of time preschool children spend outdoors is positively associated with their levels of PA and negatively associated with ST [22, 23]. A recent randomized controlled trial showed that scheduling both shorter more frequent and longer outdoor sessions during preschool hours significantly increased preschool children's MVPA [24]. Thus, increasing time spent outdoors during preschool hours may, in addition to policies and environmental factors, be an effective strategy to promote healthy PA among preschool children. However, more studies are warranted to further explore the potential of organizational level factors.

Interpersonal level factor such as preschool teacher's attitude, initiative, and participation in physical activities along with children, may play an important role in promoting preschool children's PA [25]. However, there is a lack of studies among the preschool population. Only one Norwegian study has used objectively measured PA in both preschool teachers' and children and found a small, but statistically significant association, between preschool teachers' and preschool children's levels of PA during preschool hours [26]. More studies with objective measure on both preschool teachers and children are imperative to confirm the potential association between preschooler's PA and preschool teachers' PA.

To address these knowledge gaps, the aim of the current study is to assess to what extent the physical preschool environment, formalized PA policies, time spent outdoors and preschool teachers' levels of PA were associated with children's objectively assessed PA, steps and ST during preschool hours to deepen knowledge in informing strategy development for child PA promotion.

Materials and methods

Study design, setting and study population

In this cross-sectional observational study, 30 out of the total 51 municipal preschools within the Södermalm district of Stockholm Sweden, were invited to participate. Preschools were chosen to reflect a representative sample of the different environmental characteristics (outdoor operation and different size of the playground) within the Södermalm district. In Sweden, all children from the age of 1 to 5 are eligible to go to preschool. However, children aged 1–2 years are often separated in physical activity daily routines from children aged 3–5 years because of the difference in their development stage [27]. Further, WHO have formulated different physical activity guidelines due to this variation in growth between 1–2 years old toddlers and 3–5 years old preschool children [28]. As such, preschool children of 3–5 years old were chosen as the study population of this research and children between 3–5 years of age, at the participating preschools, were invited to participate. Written informed consent was obtained from all participating children's parents and preschool teachers and the study has been approved by the Stockholm Ethical Review Board (EPN), Dnr: 2018/890-31/2. The fieldwork measurements, comprising questionnaires for preschool teachers and parents, body measures of children and 7 days of accelerometer measures of PA in children and preschool teachers, were carried out at the participating preschools from September to November 2018.

Preschool environmental characteristics, policies and time outdoors

The Environment and Policy Evaluation Self-Report (EPAO-SR) Instrument, showing good to excellent validity and reliability [29], was administered to preschool teachers. The EPAO-SR instrument includes both questions about nutrition and physical activity and only questions regarding physical activity were distributed in this study. Subscales of the EPAO-SR were used to measure environmental characteristics and formalized PA policies in the participating preschools. The specific questions asked were “How large is your preschool playground?” and “Does your preschool has written policy or any other written document about physical activity? Answers about playground size were categorized and modified to include the Swedish outdoor activity practice; (1) $\leq$200m^2, (2) around 900m^2, (3) $>$2700m^2 and (4) outdoors activity (all time at the preschool is spent outdoors). Formalized PA policy was analyzed as a dichotomous variable (Yes/No), depending on if the preschool had any written policy concerning PA or not. Time in-and outdoors was aggregated from in-out report, in which preschool teachers recorded time spent “indoors” or “outdoors” in 30-minute periods for every child on all weekdays during the PA measurements. Time outdoors was thereafter converted into quartiles (Q1 $<$138min, Q2 138min$\leq$ to $<$187.5min, Q3 187.5min$\leq$ to $<$234min and Q4 $\geq$234min), where Q1 comprises those 25% of preschool children who spent the least time outdoors and Q4 comprises those 25% who spent the most time outdoors.

Body measures

Weight and height of participating children were measured via validated scales and stadiometers, respectively (calibrated scale: VB2-200-EC, Vetek AB, Väddö, Sweden; portable stadiometer: Seca 213, Seca, Chino, CA, USA). Body mass index (BMI) was classified as normal, overweight or obese according to an international classification by Cole et al., correcting for age and sex [30].

Physical activity and sedentary time

PA and ST were measured via the triaxial Actigraph GT3X+ accelerometer, which has been tested extensively for reliability and validity and is widely used in epidemiological pediatric research [31]. Wear protocol and analyzing techniques followed best practices and used the latest recommendations to increase accuracy [31]: children and preschool teachers were instructed to wear the accelerometer, at the right hip, all waking hours for 7 consecutive days. A sampling rate of 60 Hz was used and vector magnitude (V_m) activity counts ($V_m = \sqrt{}(X^2 + Y^2 + Z^2)$) was analyzed. Accelerometer data were considered valid if the child wore the accelerometer for at least 3 days, 10 hours/day. Non-wear time was defined as 60 or more consecutive minutes with zero counts, allowing up to 2 min of interruptions with non-zero counts [31]. Steps were determined using the manufacturer’s step algorithm, using the normal filter. MVPA, LPA and ST were calculated based on cut-offs and epochs developed specifically for the GT3X+ accelerometer, using V_m activity counts, in 4-year-old children [32]. A 60-s epoch length was used in analysis according to the epoch setting in the validation study that developed these cut-offs [32]. ST was calculated as any minute of less than 820 counts per minute (cpm), LPA as 820–3907 cpm and MVPA as $\geq$3908 cpm. For preschool teachers, MVPA, LPA and ST were calculated based on cut-offs and epochs developed by Santos-Lozano et al. for the GT3X+ accelerometer, using V_m activity counts [33]. ST was calculated as any minute of less than 150 cpm, LPA as 150–3207 cpm and MVPA as $\geq$3208 cpm [33]. After the validation and classification of PA level for whole day PA accelerometer measure, the time-stamped accelerometer data was further matched with preschool time information to extract PA during preschool time due to the focus of the preschool factors in this study. In Sweden, preschool hours vary to fit parents' working schedule, but most preschools are open from 7:00 to 19:00. The preschool arrival and departure time information for each child was documented by preschool teachers daily in the same in/out report that recorded children's activity indoor or outdoor in 30 minutes periods from 7:00 to 19:00.

Family characteristics

At baseline, parents filled out a questionnaire on demographical and descriptive variables on anthropometry (height and weight) and highest education level, categorized into elementary school, upper secondary school and university education.

Teacher PA

Teachers' PA outcomes were aggregated at preschool level by calculating the means of the respective PA outcomes of all teachers in each preschool. Every outcome was then categorized into high and low by the median. Cut-offs for teacher PA outcomes aggregated at preschool level were defined as: $MVPA_{low}<24.5$ min, $LPA_{low}<310.4$ min, $Steps_{low}<6656$ steps, $ST_{low}\leq 184.5$ min.

Statistical analyses

Descriptive analyses included the distribution (mean and standard deviation (SD)) of various background characteristics and PA outcomes by preschool policy, playground size, time outdoors and teacher PA.

Next, we used Linear Mixed Models (LMM), nested on preschool level, to examine associations between existence of formalized PA policy, playground size, time spent outdoors and preschool teachers aggregated levels of MVPA, LPA, steps and ST with child levels of MVPA, LPA, steps and ST. We analyzed each association between the exposures and outcomes independently and all predictors jointly in both unadjusted and adjusted models. Adjustments, in all models presented, were made for age of the child, sex and BMI [34] that has been selected based on causal diagram [35]. There are no defined classrooms in Swedish preschools where all teachers take care and interact will all children in principle [36]. Although children can be divided into groups, these groups are not fixed, and most children participate in different group constellations [27]. Therefore, a 2-level nesting, children/teachers nested in preschools, was adopted in the LMM. In addition, we estimated the intra class correlation for each mixed model to determine the preschool-level cluster effect.

All statistical analysis was performed in software STATA version 16.0.

Results

Fig 1 demonstrates the derivation of the analytical dataset. In total, 404 children and 92 preschool teachers from 27 preschools participated in the current study. First, 10 children and 6 teachers were excluded because they had less than 3 days or 10 hours/day of accelerometer data. Second, 25 children and 2 teachers were excluded due to missing recorded preschool hours, as such information were required to determine PA during preschool time. Thus, the final analytical sample comprised 369 children and 84 preschool teachers. The mean child age was 4.7 years (SD 0.1) and 45% were girls. On average, a child spent 475 minutes (7.9h) in preschool per day, of which 269 (SD 97.5) and 206 (SD 107.7) minutes were spent in- and outdoors, respectively.

Table 1 provides an overview of the preschool characteristics by preschool policy, playground size and time spent outdoors (exposures) and child's daily average levels of PA, steps and ST

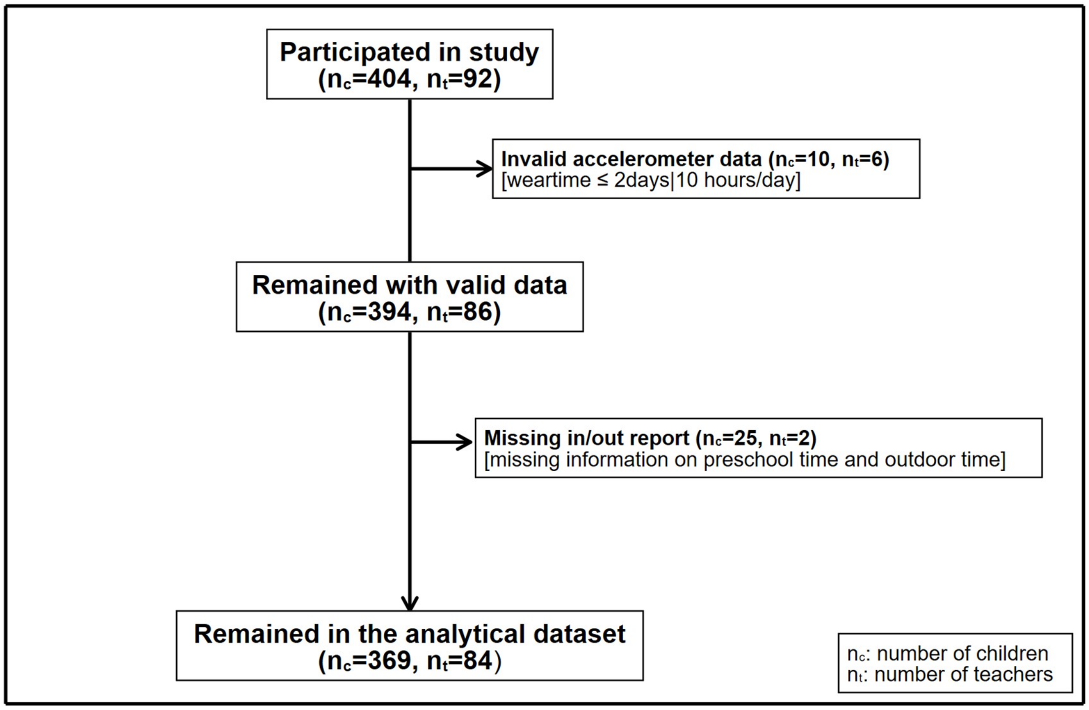

Fig 1. Flowchart of participants.

(outcomes) during preschool hours. The overview of teacher's PA, steps and ST aggregated on preschool level and child's daily average of PA during preschool time, steps and ST respectively is presented in S1 Table. In total, girls spent 19% less minutes in MVPA, 5% less minutes in LPA and spend 14% more minutes in ST compared with boys during preschool hours.

Associations between formalized preschool policy and children's PA, steps and ST

When formalized preschool policy was analyzed as the only predictor in the independent models, the effect of formalized preschool PA policy was hardly manifested (Figs 2–5, independent model). However, when all predictors were analyzed simultaneously (Figs 2–5, joint model), i.e. the direct association between each predictor and outcome, assuming all other predictors constant, children spent 10.2 (95% CI: 2.8, 17.6) minutes more in MVPA (Fig 2, joint model), 15.6 (95% CI: 1, 30.2) minutes more in LPA (Fig 3, joint model) and acquired 997 (95% CI: 181, 1813) more steps (Fig 4, joint model) in preschools with formalized PA policies compared with preschools with no such policies.

Associations between preschool playground area and children's PA, steps and ST

As is shown in Figs 2–4, the independent model shows dose-response association between preschool playground area and children's PA and steps. However, this dose-response association is not displayed when the preschool playground area is analyzed with other predictors simultaneously.

Table 1. Descriptive characteristics in relation to preschool level features.

		Formalized PA Policy		Playground area (m^2)				Time spent outdoors			
	Total	**No**	**Yes**	**≤200**	**Around 900**	**>2700**	**Out group**	**Q1**	**Q2**	**Q3**	**Q4**
	N = 369	**N = 290**	**N = 79**	**N = 98**	**N = 69**	**N = 151**	**N = 51**	**N = 94**	**N = 93**	**N = 90**	**N = 92**
Individual characteristics											
Boys, n (%)	204 (55.3)	125 (43.1)	40 (50.6)	55 (56.1)	34 (49.3)	88 (58.3)	27 (52.9)	40 (43)	50 (54)	38 (42)	37 (40)
Age, mean (SD)	4.7 (0.1)	4.6 (0.8)	4.9 (0.7)	4.7 (0.7)	4.6 (0.9)	4.7 (0.7)	4.4 (0.9)	4.8 (0.8)	4.6 (0.8)	4.7 (0.7)	4.5 (0.8)
BMI, mean (SD)	15.7 (0.1)	15.7 (2.8)	15.5 (2.1)	15.2 (3.8)	16.0 (1.2)	15.6 (2.5)	16.2 (1.7)	14.6 (4.3)	15.7 (2.0)	16.2 (1.1)	16.2 (1.6)
Overweight, n (%)	25 (6.8)	20 (6.9)	5 (6.3)	7 (7.1)	6 (8.7)	8 (5.3)	4 (7.8)	5 (5)	6 (6)	8 (9)	6 (7)
Obesity, n (%)	8 (2.2)	7 (2.4)	1 (1.3)	3 (3.1)	1 (1.4)	2 (1.3)	2 (3.9)	1 (1)	0 (0)	3 (3)	4 (4)
Preschool children's physical activity level during preschool time, mean (SD)											
MVPA (min)	39.2 (1.2)	37.2 (20.8)	46.4 (28.6)	33.5 (17.0)	36.1 (24.5)	43.3 (24.4)	42.0 (24.6)	37.4 (22.0)	33.5 (18.2)	42.3 (27.1)	43.7 (22.9)
LPA (min)	258.8 (45.5)	258.5 (45.4)	259.8 (45.9)	240.3 (42.3)	247.6 (42.0)	266.9 (45.5)	285.4 (37.3)	226.7 (38.7)	248.6 (38.4)	273.2 (41.8)	287.7 (37.7)
Steps (counts)	7343 (116)	7253 (2219)	7674 (2252)	6447 (1734)	6469 (1793)	7425 (1808)	10007 (2589)	6058 (1916)	6630 (1540)	7675 (1725)	9053 (2400)
ST (min)	177.5 (2.4)	181.9 (45.2)	161.1 (44.0)	175.3 (45.7)	177.5 (36.4)	180.9 (53.5)	171.4 (29.4)	164.7 (42.0)	178.3 (42.5)	182.7 (50.7)	184.6 (45.3)
Wear time (min)	446.1 (61.8)	448.6 (63.8)	437.1 (53.3)	419.9 (54.7)	431.2 (44.1)	462.2 (69.7)	469.0 (46.6)	401.5 (55.5)	431.2 (39.6)	467.6 (58.9)	485.8 (54.9)
Preschool time (min)	475.6 (62.1)	477.8 (64.2)	467.4 (53.6)	449.3 (54.5)	461.3 (45.2)	491.2 (70.1)	499.0 (46.6)	429.0 (53.7)	460.5 (40.1)	498.3 (58.8)	516.2 (54.8)
Parental characteristics											
Education, n (%)	N = 337	N = 265	N = 72	N = 87	N = 64	N = 140	N = 46	N = 81	N = 84	N = 88	N = 84
University	271 (80.4)	214 (80.8)	57 (79.2)	73 (83.9)	52 (81.3)	106 (75.7)	40 (87.0)	70 (86)	66 (79)	66 (75)	69 (82)

Abbreviations: BMI = body mass index, MVPA = moderate to vigorous physical activity, LPA = light physical activity, ST = sedentary time, Q1-4 = quartile 1–4.

Associations between time spent outdoors and children's PA, steps and ST

In the independent model where time spent outdoors was analyzed as the only predictor for the association with children's PA outcomes, children in the uppermost quartile of time spent outdoors (Q4) spend 11.1 (95% CI: 3.5, 18.7) minutes more in MVPA (Fig 2, independent model), 59.8 (95% CI: 45.5, 74.1) minutes more in LPA (Fig 3, independent model) and acquired 2685 (95% CI: 2037, 3333) more steps compared with children in the lowermost quartile of time spent outdoors (Q1) (Fig 4, independent model). Moreover, when analyzed with all predictors and confounders simultaneously, children in the uppermost quartile of time spent outdoors (Q4) spend 11.5 (95% CI: 3.0, 20.0) minutes more in MVPA (Fig 2, joint model), 59.1 (95% CI: 43.1, 75.2) minutes more in LPA (Fig 3, joint model) and acquired 2092 (95% CI: 1399, 2785) more steps (Fig 4, joint model) compared with children in the lowermost quartile of time spent outdoors (Q1).

Association between preschool teachers' PA and children's PA

Fig 6 illustrates the association between preschool teacher's aggregated levels of MVPA, LPA, steps and ST with children's levels of MVPA, LPA, steps and ST on an individual level. Both preschool teacher's aggregated levels of LPA and steps were statistically significant associated with children's individual levels of LPA (*ß* = 0.25, *P* = 0.004) and steps (*ß* = 0.52, $P<0.001$). However, there was no statistically significant association between preschool teachers

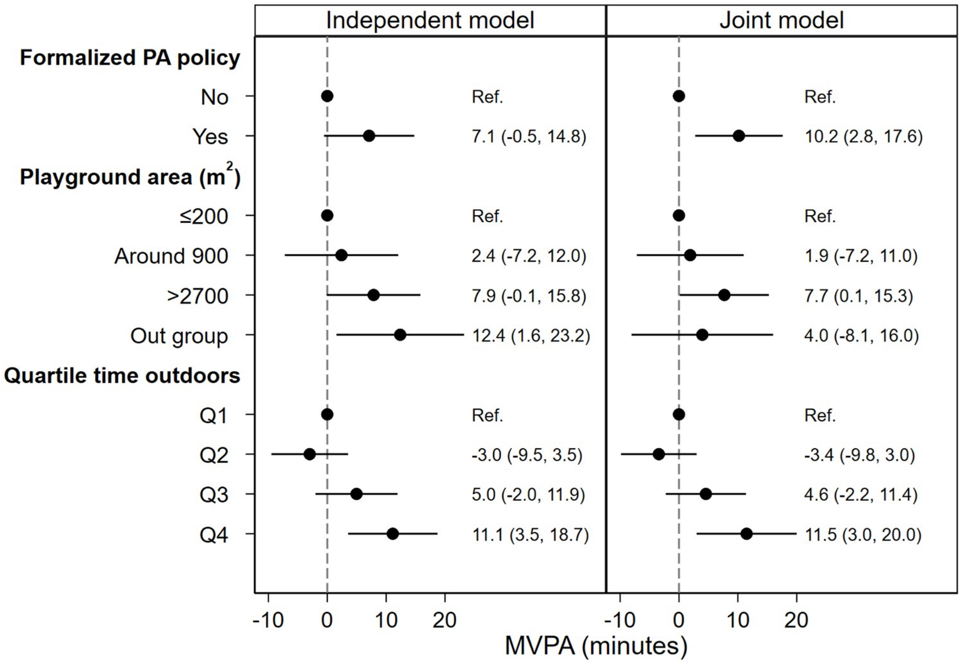

Fig 2. Association between predictors and children's MVPA during preschool time. Both models are adjusted for age, sex and BMI category. Abbreviations: PA = physical activity, MVPA = moderate to vigorous physical activity, Q1-4 = quartile 1–4, BMI = body mass index.

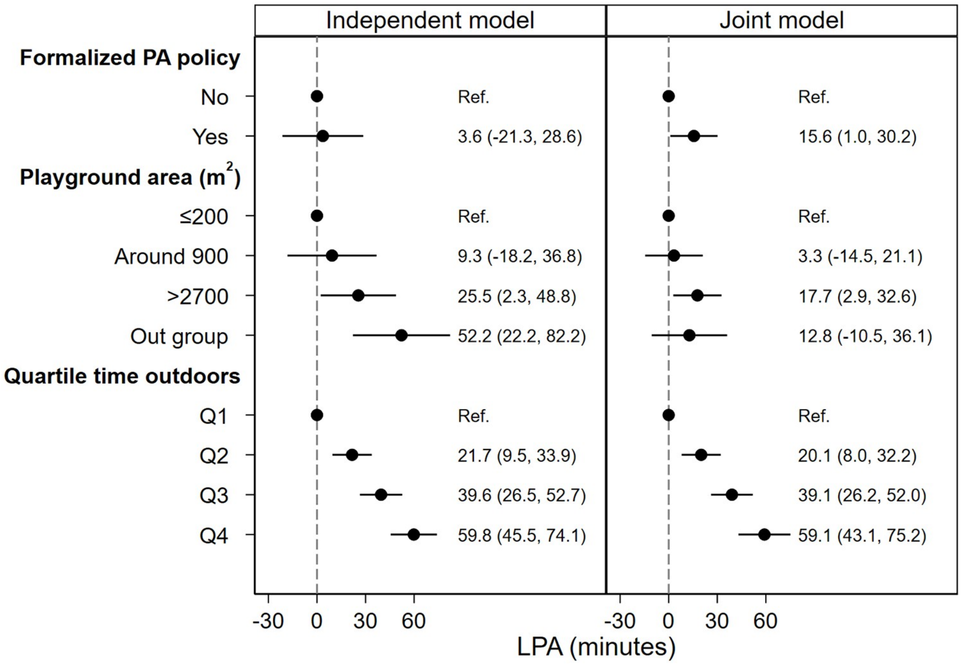

Fig 3. Association between predictors and children's LPA during preschool time. Both models are adjusted for age, sex and BMI category. Abbreviations: PA = physical activity, LPA = light physical activity, Q1-4 = quartile 1–4, BMI = body mass index.

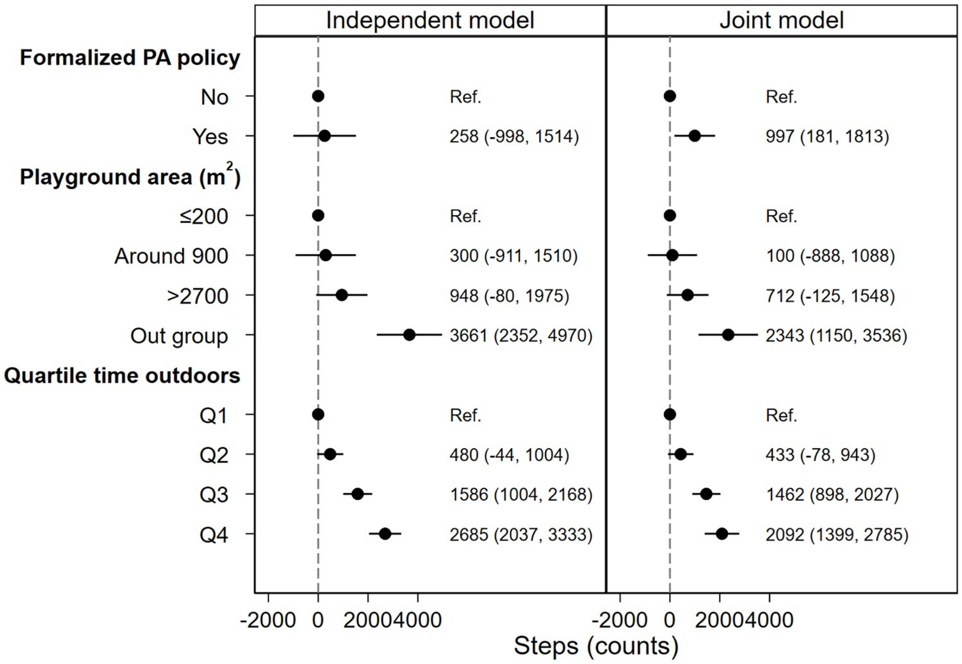

Fig 4. Association between predictors and children's steps during preschool time. Both models are adjusted for age, sex and BMI category. Abbreviations: Q1-4 = quartile 1–4, BMI = body mass index.

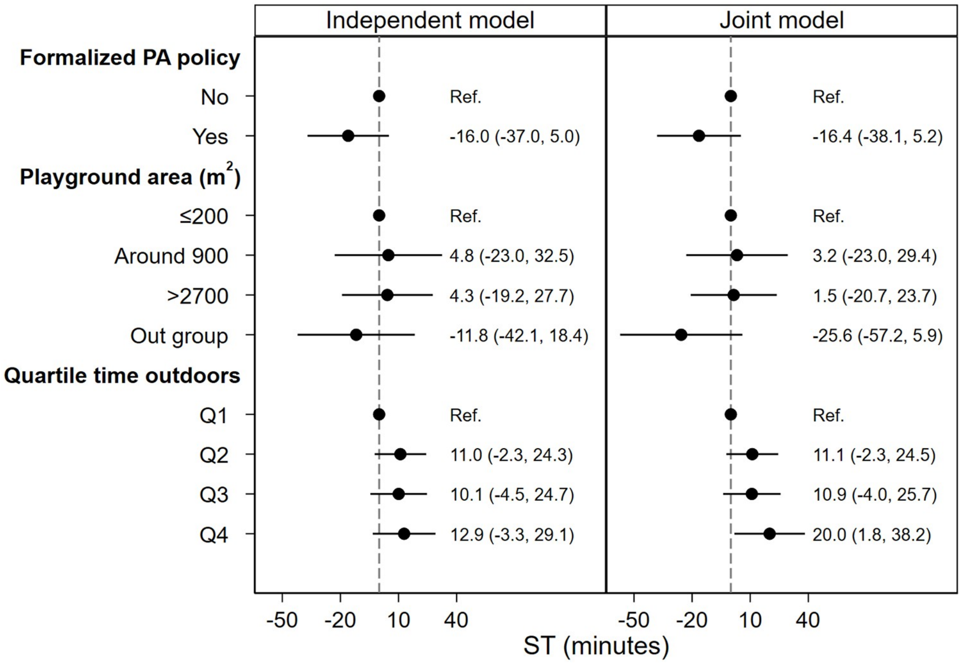

Fig 5. Association between predictors and children's ST during preschool time. Both models are adjusted for age, sex and BMI category. Abbreviations: ST = sedentary time, Q1-4 = quartile 1–4, BMI = body mass index.

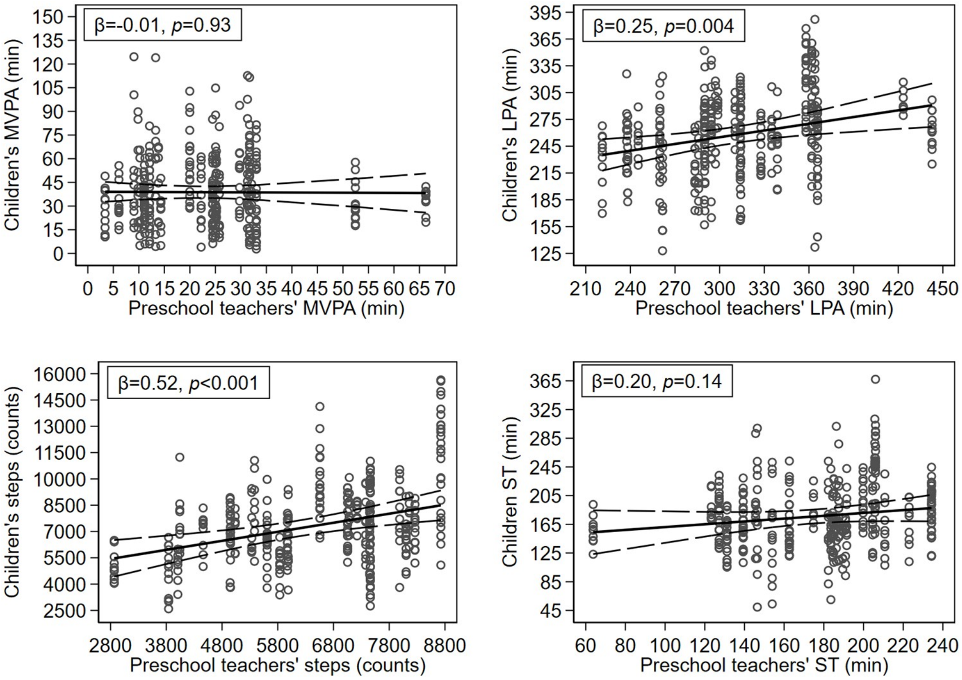

Fig 6. Association between preschool teachers' and children's PA and ST during preschool time. Abbreviations: PA = physical activity, MVPA = moderate to vigorous physical activity, LPA = light physical activity, ST = sedentary time.

aggregated levels of MVPA and ST and children's levels of MVPA (*ß* = -0.01, *P* = 0.93) and ST (*ß* = 0.20, *P* = 0.14).

Sensitivity analyses

In sensitivity analyses, we estimated the intra class correlation of each model shown in S2 Table. We observed small to modest ICC for all models (and all outcomes); there was a small ICC for MVPA, and somewhat higher for Steps, LPA and ST (S3 Table). In addition, we ran all models included in S2 Table with additional adjustment made for parental education in a subset population of children (n = 337) (S4 Table). S4 Table shows that none of the estimates are affected, to any substantial extent, by the introduction of parental education as a covariate. Finally, we ran descriptive analyses comparing children in the analytical sample (n = 369) with children with incomplete accelerometer data or missing data on anthropometrics and or missing data on time spent in- and outdoors (n = 35) (S5 Table). As shown in S5 Table, children excluded from analyses, due to missing data, had a higher prevalence of obesity (33% vs. 2%) and lower PA level. The excluded sample were generally distributed across the preschools except for that one preschool contributed to 11 (31.4%) exclusions due to loss of sub-report document for in/out report with information on preschool time and time spent outdoors. This preschool had the smallest preschool playground and did not have a formalized policy.

Discussion

The current study examined associations between preschool playground size, formalized PA policies, time spent outdoors and preschool teacher's levels of PA with children's levels of PA and ST at preschool. Our findings showed that preschool characteristics such as formalized PA policies and more time spent outdoors were positively associated with children's levels of PA. Moreover, preschool teachers aggregated levels of LPA and steps were statistically significant associated with children's individual levels of LPA and steps. However, preschool playground size showed no significant association with children's levels of PA. These findings may be of importance for promoting children's PA during preschool hours and intervention development.

Comparison with previous research

Our finding that time spent outdoors was positively associated with children's levels of PA is supported by previous systematic reviews on positive associations between time outdoors with PA [37] and negative associations with ST [38]. However, most previous studies have relied on potentially biased self-reported levels of PA or retrospective information on time spent in- and outdoors during preschool hours. One small (n = 46) observational study with objectively measured PA and GPS-assessed time spent outdoors showed that children were approximately twice as active and less sedentary when comparing outdoor verses indoor time in a preschool setting [39]. The association in the aforementioned study is somewhat stronger than the 68% more MVPA and 37% less ST accumulated during time spent outdoors compared to indoors we observed in the current study. However, the study by Tandon et al. had a more precise measure of the exposure, i.e. time spend in- and outdoors (GPS vs. preschool teacher reports), which to some extent may explain the observed differences between the two studies.

There is limited evidence that PA policies alone positively stimulate PA and reduce ST in preschool children [40]. In general, PA interventions in preschool settings generate small to moderate effect on children's MVPA, where multicomponent interventions including structured outdoor activity are most effective [13]. However, a review on the promotion of PA in preschool children [41] highlights the importance of implementing policies concerning PA in preschools to promote children's levels of PA during preschool hours. Findings in the current study that children spent 10.2 minutes more in MVPA during preschool hours in preschools having formalized PA policies compared with preschools with no such policies, further supports the importance of implementing formalized PA policies.

Systematic review data indicate that preschool playground size and playground software characteristics e.g. play equipment are associated with levels of PA in preschool children [42]. Hence, having enough space to play and having favorable playground conditions may be sufficient for preschool children to be physically active. However, these observational associations have not been reinforced in the few existing interventions studies. In the current study, we found a dose-response association between playground size and children's levels of PA during preschool hours. However, this association was deteriorated when the association between playground size and PA was evaluated together with PA policy and time outdoors. Thus, the association between playground size and children's PA may partly be explained by PA policy and time spent outdoors. Nevertheless, more complex relationships may exist. For example, it is possible that these correlating factors interact, and such interactions may be of relevance for PA. However, the potential complex interplay was not explored due to the limited sample size and the cross-sectional design of this study, future studies that perform such detailed investigations are warranted.

Preschool teachers' individual attitudes and behaviors may play an important role in promoting preschool children's PA [43]. However, most previous research on the topic are based on qualitative approaches. Thus far, only one Norwegian study has explored accelerometer assessed associations between preschool teachers' and children's levels of MVPA during preschool hours [44]. This study demonstrated that there was a statistically significant association between preschool teacher's aggregated levels of MVPA and preschool children's individual levels of MVPA. In contrast, we were unable to detect any such association in the current study. However, we observed a statistically significant association between preschool teachers aggregated levels of LPA and steps and children's individual levels of LPA and steps. The discrepancy between these studies may to some extent be explained by differences in approaches used to classify MVPA intensity in both preschool children and preschool teachers.

Strengths and limitations

The current study possesses several strengths. First, PA in both preschool children and teachers were assessed objectively with accelerometers during all preschool hours. Thus, limiting certain biases associated with self-reported measures, e.g. social desirability and recall difficulties. Second, the detailed in- and outdoor reports enabled us to match accelerometer data with children's in- and outdoor time with high resolution (in 30-minute intervals). Third, the study included a large number of participants in preschools with different environmental characteristics (e.g. playground size). Finally, we used a validated instrument (EPAO-SR) to assess preschool characteristics, e.g. PA policies.

Nevertheless, the current study has several limitations that need consideration. First, both preschool characteristics, assessed via the EPAO-SR, and the in- and outdoor reporting relied on preschool teacher's self-reporting, which have several inherited biases. In addition, formalized PA policy was dichotomized into yes/no which may have disregarded the influence of policy's specific content on PA outcomes [19, 21]. Investigation further into content of formalized policy was hindered by the limited number of preschools that had formalized policy and unavailability of implementation information. Second, the geographical distribution of preschools was limited to a small area in Stockholm with a homogenous socioeconomic distribution. Furthermore, our sensitivity analysis showed that children with incomplete data had a higher prevalence of obesity and lower PA level compared with those included in the main analyses. However, it is important to note that few observations (25 observations for obesity status and 8 observations for PA level) in the excluded sample due to missing value may limit the representativeness of this result in the excluded sample. Further, one preschool, with no formalized policy and a playground $\leq 200\text{m}^2$, contributed to 31.4% of the excluded participants due to loss of sub-document of in/out report with information on preschool time and time spent outdoors. This is of importance in relation to the center-level influence on missing data. Nevertheless, the constricted socioeconomic and body size distribution limit the generalizability of findings in the current study. Third, although accelerometry is considered as a preferable measurement of PA among preschool children in free-living conditions, it is unable to detect all PA when attached to the hip, e.g. cycling or upper-body movements [45]. Thus, some of preschoolers PA during preschool hours may not be captured, which may impact or estimates of PA and ST. Forth, information regarding child and preschool teacher associations in PA may have been diminished due to aggregating preschool teacher levels of PA within the preschools. Moreover, with the cross-sectional nature of data, it is not possible to conclude if preschool teachers PA affect children's PA or vice versa. Finally, by using the normal filter to process accelerometer data we may have underestimated the number of steps taken during preschool hours [46]. In addition, a 60s epoch was adopted in accelerometer data analysis while a shorter epoch length has been suggested to suit the young children's sporadic moving nature [31]. However, studies also suggest that scaling the cut-offs to suit a different epoch may be problematic [47]. The accuracy of cut-offs to classify PA level may be optimal when they are applied under the same epoch setting as the calibration setting that developed these cut-offs [47, 48]. Therefore, accelerometer data was analyzed in 60 s epoch strictly following the epoch length used in the validation study [32].

Conclusions

The current study showed that modifiable preschool characteristics such as formalized PA policies and more time spent outdoors were positively associated with children's objectively measured levels of PA during preschool hours. For promoting children's PA during preschool hours, preschools should consider incorporating formalized PA policies and aim to increase the daily amount of time spent outdoors. However, given the cross-sectional nature of the current study, these findings need further examination, preferably using experimental research designs.

Supporting information

S1 Table. Cross tabulation of teachers' PA and children' s PA. High and low are classified by the median of the respective teacher PA variable Abbreviations: PA = physical activity, MVPA = moderate to vigorous physical activity, LPA = light physical activity, ST = sedentary time.
(DOCX)

S2 Table. Associations between predictors and physical activity indicators during preschool time (n = 369). Model 1 = crude model each predictor independently, Model 2 = Model 1 adjusted for age, sex, BMI category Model 3 = all predictors jointly, Model4 = Model 3 adjusted for age, sex, BMI category Abbreviations: PA = physical activity, BMI = body mass index, MVPA = moderate to vigorous physical activity, LPA = light physical activity, ST = sedentary time, Q1-4 = quartile 1–4 Reference level: Formalized PA policy = No, Playground area = ≤200 m^2, Time spend outdoors = Q1.
(DOCX)

S3 Table. Preschool-level cluster effect (intra class correlation) in each linear mixed model. Model 1 = crude model each predictor independently, Model 2 = Model 1 adjusted for age, sex, BMI category Model 3 = all predictors jointly, Model 4 = Model 3 adjusted for age, sex, age, BMI category Abbreviations: MVPA = moderate to vigorous physical activity, LPA = light physical activity, ST = sedentary time Reference level: Formalized PA policy = No, Playground area = ≤200 m^2, Time spend outdoors = Q1.
(DOCX)

S4 Table. Associations between predictors and physical activity indicators during preschool time (n = 337). Model 1 = crude model each predictor independently, Model 2 = Model 1 adjusted for age, sex, BMI category and parental education Model 3 = all predictors jointly, Model4 = Model 3 adjusted for age, sex, age, BMI category and parental education Abbreviations: PA = physical activity, BMI = body mass index, MVPA = moderate to vigorous physical activity, LPA = light physical activity, ST = sedentary time, Q1-4 = quartile 1–4 Reference level: Formalized PA policy = No, Playground area = ≤200 m2, Time spend outdoors = Q1.
(DOCX)

S5 Table. Comparison of descriptive characteristics between analytical dataset and excluded observations. PA = physical activity, BMI = body mass index, MVPA = moderate to vigorous physical activity, LPA = light physical activity, ST = sedentary time, Q1-4 = quartile 1–4.
(DOCX)

S6 Table. Policy content in seven preschool reported formalized policy.
(DOCX)

Acknowledgments

The authors would like to thank participating children, their families, preschool teachers and personnel at Södermalm municipally.

Author Contributions

Conceptualization: Daniel Berglind.

Data curation: Chu Chen.

Formal analysis: Chu Chen, Viktor H. Ahlqvist.

Funding acquisition: Daniel Berglind.

Investigation: Chu Chen, Daniel Berglind.

Methodology: Chu Chen, Viktor H. Ahlqvist, Daniel Berglind.

Project administration: Chu Chen.

Resources: Daniel Berglind.

Software: Chu Chen, Viktor H. Ahlqvist.

Supervision: Daniel Berglind.

Visualization: Chu Chen, Viktor H. Ahlqvist.

Writing – original draft: Daniel Berglind.

Writing – review & editing: Chu Chen, Viktor H. Ahlqvist, Pontus Henriksson, Cecilia Magnusson.

References

1. Carson V, Lee EY, Hewitt L, Jennings C, Hunter S, Kuzik N, et al. Systematic review of the relationships between physical activity and health indicators in the early years (0–4 years). BMC public health. 2017; 17(Suppl 5):854. https://doi.org/10.1186/s12889-017-4860-0 PMID: 29219090
2. Tudor-Locke C, Craig CL, Beets MW, Belton S, Cardon GM, Duncan S, et al. How Many Steps/Day are Enough? for Children and Adolescents. Int J Behav Nutr Phy. 2011; 8.
3. Cliff DP, Hesketh KD, Vella SA, Hinkley T, Tsiros MD, Ridgers ND, et al. Objectively measured sedentary behaviour and health and development in children and adolescents: systematic review and meta-analysis. Obes Rev. 2016; 17(4):330–44. https://doi.org/10.1111/obr.12371 PMID: 26914664
4. Jones RA, Hinkley T, Okely AD, Salmon J. Tracking Physical Activity and Sedentary Behavior in Childhood: A Systematic Review. American Journal of Preventive Medicine. 2013; 44(6):651–8. https://doi.org/10.1016/j.amepre.2013.03.001 PMID: 23683983
5. Bornstein DB, Beets MW, Byun W, McIver K. Accelerometer-derived physical activity levels of preschoolers: a meta-analysis. Journal of Science and Medicine in Sport. 2011; 14(6):504–11. https://doi.org/10.1016/j.jsams.2011.05.007 PMID: 21684809
6. Hnatiuk JA, Salmon J, Hinkley T, Okely AD, Trost S. A review of preschool children's physical activity and sedentary time using objective measures. Am J Prev Med. 2014; 47(4):487–97. https://doi.org/10.1016/j.amepre.2014.05.042 PMID: 25084681
7. Berglind D, Hansson L, Tynelius P, Rasmussen F. Levels and Patterns of Objectively Measured Physical Activity and Sedentary Time in Four-Year-Old Swedish Children. Journal of physical activity & health. 2016:1–23.
8. Berglind D, Tynelius P. Objectively measured physical activity patterns, sedentary time and parent-reported screen-time across the day in four-year-old Swedish children. BMC public health. 2017; 18(1):69. https://doi.org/10.1186/s12889-017-4600-5 PMID: 28764730
9. Finch M, Jones J, Yoong S, Wiggers J, Wolfenden L. Effectiveness of centre-based childcare interventions in increasing child physical activity: a systematic review and meta-analysis for policymakers and practitioners. Obes Rev. 2016; 17(5):412–28. https://doi.org/10.1111/obr.12392 PMID: 27005942

10. Bronfenbrenner U. The ecology of human development: Harvard university press; 1979.

11. Mehtälä MAK, Sääkslahti AK, Inkinen ME, Poskiparta MEH. A socio-ecological approach to physical activity interventions in childcare: a systematic review. Int J Behav Nutr Phy. 2014; 11(1):22.

12. Pearce M, Page AS, Griffin TP, Cooper AR. Who children spend time with after school: associations with objectively recorded indoor and outdoor physical activity. Int J Behav Nutr Phys Act. 2014; 11 (1):45. https://doi.org/10.1186/1479-5868-11-45 PMID: 24679149

13. Gordon ES, Tucker P, Burke SM, Carron AV. Effectiveness of physical activity interventions for preschoolers: a meta-analysis. Res Q Exerc Sport. 2013; 84(3):287–94. https://doi.org/10.1080/02701367.2013.813894 PMID: 24261007

14. Hinkley T, Carson V, Hesketh KD. Physical environments, policies and practices for physical activity and screen-based sedentary behaviour among preschoolers within child care centres in Melbourne, Australia and Kingston, Canada. Child: Care, Health and Development. 2015; 41(1):132–8.

15. Villarreal-Calderon A, Acuna H, Villarreal-Calderon J, Garduno M, Henriquez-Roldan CF, Calderon-Garciduenas L, et al. Assessment of physical education time and after-school outdoor time in elementary and middle school students in south Mexico City: the dilemma between physical fitness and the adverse health effects of outdoor pollutant exposure. Archives of environmental health. 2002; 57 (5):450–60. https://doi.org/10.1080/00039890209601437 PMID: 12641189

16. Broekhuizen K, Scholten A-M, de Vries SI. The value of (pre)school playgrounds for children's physical activity level: a systematic review. Int J Behav Nutr Phy. 2014; 11(1):59.

17. Swerissen H, Crisp BR. The sustainability of health promotion interventions for different levels of social organization. Health promotion international. 2004; 19(1):123–30. https://doi.org/10.1093/heapro/dah113 PMID: 14976180

18. Byrd-Williams CE, Dooley EE, Thi CA, Browning C, Hoelscher DM. Physical activity, screen time, and outdoor learning environment practices and policy implementation: a cross sectional study of Texas child care centers. BMC public health. 2019; 19(1):274. https://doi.org/10.1186/s12889-019-6588-5 PMID: 30845946

19. Dooley EE, Thi CA, Browning C, Hoelscher DM, Byrd-Williams CE. Examining physical activity policies to practice implementation: Results from the Texas Early Childhood Physical Activity Survey in non-Head Start childcare centers. Preventive medicine reports. 2020; 17:101019. https://doi.org/10.1016/j.pmedr.2019.101019 PMID: 32021757

20. Dowda M, Brown WH, McIver KL, Pfeiffer KA, O'Neill JR, Addy CL, et al. Policies and characteristics of the preschool environment and physical activity of young children. Pediatrics. 2009; 123(2):e261–6. https://doi.org/10.1542/peds.2008-2498 PMID: 19171578

21. Erinosho T, Hales D, Vaughn A, Mazzucca S, Ward DS. Impact of Policies on Physical Activity and Screen Time Practices in 50 Child-Care Centers in North Carolina. Journal of physical activity & health. 2016; 13(1):59–66.

22. Boldemann C, Blennow M, Dal H, Martensson F, Raustorp A, Yuen K, et al. Impact of preschool environment upon children's physical activity and sun exposure. Prev Med. 2006; 42(4):301–8. https://doi.org/10.1016/j.ypmed.2005.12.006 PMID: 16448688

23. Tandon PS, Saelens BE, Zhou C, Christakis DA. A Comparison of Preschoolers' Physical Activity Indoors versus Outdoors at Child Care. International journal of environmental research and public health. 2018; 15(11).

24. Razak LA, Yoong SL, Wiggers J, Morgan PJ, Jones J, Finch M, et al. Impact of scheduling multiple outdoor free-play periods in childcare on child moderate-to-vigorous physical activity: a cluster randomised trial. Int J Behav Nutr Phy. 2018; 15.

25. Mikkelsen BE. Associations between pedagogues attitudes, praxis and policy in relation to physical activity of children in kindergarten—results from a cross sectional study of health behaviour amongst Danish pre-school children. Int J Pediatr Obes. 2011; 6:12–5.

26. Fossdal TS, Kippe K, Handegard BH, Lagestad P. "Oh oobe doo, I wanna be like you" associations between physical activity of preschool staff and preschool children. PloS one. 2018; 13(11).

27. Sheridan S, Williams P, Pramling Samuelsson I. Group size and organisational conditions for children's learning in preschool: a teacher perspective. Educational Research. 2014; 56(4):379–97.

28. WHO. WHO guidelines on physical activity, sedentary behaviour and sleep for children under 5 years of age. In: WHO, editor. https://apps.who.int/iris/bitstream/handle/10665/311664/9789241550536-eng.pdf?sequence=1&isAllowed=y: WHO; 2019.

29. Ward D, Mazzucca S, McWilliams C, Hales D. Use of the Environment and Policy Evaluation and Observation as a Self-Report Instrument (EPAO-SR) to measure nutrition and physical activity environments in child care settings: validity and reliability evidence. Int J Behav Nutr Phy. 2015; 12 (1):124.

30. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000; 320(7244):1240–3. https://doi.org/10.1136/bmj.320.7244.1240 PMID: 10797032

31. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nystrom C, Mora-Gonzalez J, Lof M, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. Sports medicine. 2017.

32. Butte NF, Wong WW, Lee JS, Adolph AL, Puyau MR, Zakeri IF. Prediction of energy expenditure and physical activity in preschoolers. Med Sci Sports Exerc. 2014; 46(6):1216–26. https://doi.org/10.1249/MSS.0000000000000209 PMID: 24195866

33. Santos-Lozano A, Santin-Medeiros F, Cardon G, Torres-Luque G, Bailon R, Bergmeir C, et al. Actigraph GT3X: Validation and Determination of Physical Activity Intensity Cut Points. Int J Sports Med. 2013.

34. Dolinsky DH, Brouwer RJN, Evenson KR, Siega-Riz AM, Østbye T. Correlates of sedentary time and physical activity among preschool-aged children. Prev Chronic Dis. 2011; 8(6):A131–A. PMID: 22005624

35. Williamson EJ, Aitken Z, Lawrie J, Dharmage SC, Burgess JA, Forbes AB. Introduction to causal diagrams for confounder selection. Respirology. 2014; 19(3):303–11. https://doi.org/10.1111/resp.12238 PMID: 24447391

36. Skolverket. Curriculum for the Preschool Lpfö 18 https://www.skolverket.se/download/18.6bfaca41169863e6a65d897/1553968298535/pdf4049.pdf2019

37. Ferreira I, van der Horst K, Wendel-Vos W, Kremers S, van Lenthe FJ, Brug J. Environmental correlates of physical activity in youth—a review and update. Obes Rev. 2007; 8(2):129–54. https://doi.org/10.1111/j.1467-789X.2006.00264.x PMID: 17300279

38. Hinkley T, Salmon J, Okely AD, Trost SG. Correlates of sedentary behaviours in preschool children: a review. Int J Behav Nutr Phys Act. 2010; 7:66. https://doi.org/10.1186/1479-5868-7-66 PMID: 20825682

39. Tandon PS, Saelens BE, Zhou C, Christakis DA. A Comparison of Preschoolers' Physical Activity Indoors versus Outdoors at Child Care. Int J Environ Res Public Health. 2018; 15(11).

40. Bower JK, Hales DP, Tate DF, Rubin DA, Benjamin SE, Ward DS. The childcare environment and children's physical activity. Am J Prev Med. 2008; 34(1):23–9. https://doi.org/10.1016/j.amepre.2007.09.022 PMID: 18083447

41. Tremblay L, Boudreau-Lariviere C, Cimon-Lambert K. Promoting Physical Activity in Preschoolers: A Review of the Guidelines, Barriers, and Facilitators for Implementation of Policies and Practices. Can Psychol. 2012; 53(4):280–90.

42. Broekhuizen K, Scholten AM, de Vries SI. The value of (pre)school playgrounds for children's physical activity level: a systematic review. Int J Behav Nutr Phys Act. 2014; 11:59. https://doi.org/10.1186/1479-5868-11-59 PMID: 24885611

43. Mikkelsen BE. Associations between pedagogues attitudes, praxis and policy in relation to physical activity of children in kindergarten—results from a cross sectional study of health behaviour amongst Danish pre-school children. Int J Pediatr Obes. 2011; 6 Suppl 2:12–5.

44. Fossdal TS, Kippe K, Handegard BH, Lagestad P. "Oh oobe doo, I wanna be like you" associations between physical activity of preschool staff and preschool children. PLoS One. 2018; 13(11):e0208001. https://doi.org/10.1371/journal.pone.0208001 PMID: 30496229

45. Cliff DP, Reilly JJ, Okely AD. Methodological considerations in using accelerometers to assess habitual physical activity in children aged 0–5 years. J Sci Med Sport. 2009; 12(5):557–67. https://doi.org/10.1016/j.jsams.2008.10.008 PMID: 19147404

46. Tudor-Locke C, Barreira TV, Schuna JM. Comparison of Step Outputs for Waist and Wrist Accelerometer Attachment Sites. Med Sci Sport Exer. 2015; 47(4):839–42.

47. Hibbing PR, Bassett DR, Crouter SE. Modifying Accelerometer Cut-Points Affects Criterion Validity in Simulated Free-Living for Adolescents and Adults. Res Q Exerc Sport. 2020:1–11.

48. Marissa Hope M, Ruth C, Shannon M, Laura AF-L. Accelerometry Analysis Options Produce Large Differences in Lifestyle Physical Activity Measurement. Physiological Measurement. 2020.

10

Motor control integrated into muscle strengthening exercises has more effects on scapular muscle activities and joint range of motion before initiation of radiotherapy in oral cancer survivors with neck dissection: A randomized controlled trial

Yueh-Hsia Chen[1,2], **Chi-Rung Lin**[2], **Wei-An Liang**[2], **Cheng-Ya Huang**[1]*

1 School and Graduate Institute of Physical Therapy, College of Medicine, National Taiwan University, Taipei, Taiwan, **2** Rehabilitation Center, Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital Linkou Branch, Taoyuan, Taiwan

* rcyhuang@ntu.edu.tw

Editor: Leonardo A. Peyré-Tartaruga, Universidade Federal do Rio Grande do Sul, BRAZIL

Abstract

Background

Accessory nerve shoulder dysfunction is common after neck dissection in oral cancer survivors. This study aimed to investigate the short-term effects of scapular muscle strengthening exercises with motor-control techniques on neck dissection-related shoulder dysfunction in oral cancer survivors before the initiation of radiotherapy.

Methods

Thirty-eight participants were randomly allocated into the motor-control and regular-exercise groups. Each group received conventional physical therapy and specific scapular muscle strengthening exercises for 1 month immediately after neck dissection. Motor control techniques were integrated with scapular strengthening exercises for the motor-control group. Shoulder pain, active range of motion (AROM) of shoulder abduction, and scapular muscle activities including upper trapezius (UT), middle trapezius (MT), lower trapezius (LT), and serratus anterior (SA) when performing maximal voluntary isometric contraction (MVIC) and scapular muscle exercises were evaluated at baseline and after 1 month of training.

Results

Both groups reduced shoulder pain and increased muscle activity of maximum voluntary isometric contraction (MVIC) of each muscle after the intervention. Increased AROM of shoulder abduction was only observed in the motor-control group (95% CI 3.80 to 20.51, $p = 0.004$). Relative to baseline evaluation, muscle activities of UT decreased in the motor-control group when performing shoulder shrug with 1-kg weight (95% CI -33.06 to -1.29, $p = 0.034$). Moreover, the SA activity decreased in the motor-control group (95% CI -29.73 to

-27.68, $p<0.001$) but increased in the regular-exercise group (95% CI 28.16 to 30.05, $p<0.001$) when performing shoulder horizontal adduction and flexion.

Competing interests: The authors have declared that no competing interests exist.

Conclusion

Early strengthening exercise with motor control techniques has greater benefits for improving AROM of shoulder abduction, muscle economy, and reducing compensatory scapular muscle activities in patients with neck dissection-related shoulder dysfunction before the initiation of radiotherapy.

Introduction

Accessory nerve shoulder dysfunction is one of the most frequent complications after neck dissection. Ewing and Martin first described the clinical signs of shoulder disability after radical neck dissection, such as shoulder dropping, and limited range of motion of shoulder joint [1]. Even with selective neck dissection, the prevalence of spinal accessory nerve dysfunction is still around 9% to 25% [2–5]. In addition, electromyogram (EMG) studies showed significant spinal accessory nerve impairment [2] and decreased trapezius muscle activity after neck dissection [6, 7]. The decreased amplitudes of trapezius muscle persisted at least 9 months after neck dissection [8].

Behavior phenomena of accessory nerve shoulder dysfunction (e.g., pain and limited active range of motion (AROM) of shoulder joint) are often observed in head and neck cancer (HNC) survivors with neck dissection [9–11]. McGarvey et al. identified that the EMG activities of the affected upper trapezius (UT) and middle trapezius (MT) were lower than those of the unaffected side when performing scapular exercises such as shoulder shrug, overhead press, shoulder adduction and flexion, and one-arm row [6, 12]. In contrast, higher activities of rhomboid and the serratus anterior (SA) were observed in the affected side than the unaffected side [12]. The symptoms of scapular muscle imbalance highlight the need for muscle training and reeducation for HNC survivors with neck dissection. "Scapular dyskinesis" is associated with abnormal scapular muscle activation and muscle balance during static and dynamic movement [13–16], and has been linked to shoulder dysfunction and impairments in many previous studies [13, 15, 16]. Few articles have addressed the effects of scapular muscle training in HNC patients with neck dissection, which showed an improvement in behavior phenomena (e.g., shoulder pain and AROM) after scapular muscle training [11, 17, 18]. However, the training effect regarding scapular muscle activation is lacking.

In addition to neck dissection, radiation therapy is one of the contributing factors for shoulder dysfunction. Based on a large population-based study using the national cancer registry database, most cases (81%) start to receive adjuvant radiotherapy 4 weeks post-operation [19]. Many studies reported that patients suffer from shoulder impairment [20–22] and brachial plexus-related neuropathic symptoms [23] after adjuvant radiotherapy. For the problem of shoulder impairment, systematic reviews have shown early exercise implementation was more effective in improvement of shoulder joint range of motion following breast cancer surgery [24] and early intervention could prevent long-standing limitation of shoulder joint range of motion and function for frozen shoulder [25]. Although early physical therapy intervention for shoulder function has been conducted in some studies [11, 17], the training effect was confounded with radiation therapy.

It has been proposed that strengthening exercise has positive effect on regaining scapular muscle balance for scapular dyskinesis [26–29]. Furthermore, recent studies suggested motor control intervention is beneficial to restore scapular muscle balance by improving muscle recruitment pattern and scapular alignment with altering neurophysiological and biomechanical effects [30, 31]. The motor control techniques, such as palpation, manual contact, verbal cues or visual feedback, have been used to restore neuromuscular control [30, 32]. In addition, motor control intervention is also used to educate, correct, and facilitate alignment and coordination of movements [33].

The purpose of this study was to explore the effects of early motor control intervention with specific scapular strengthening exercises on behavior phenomena and scapular muscle activities in oral cancer survivors before initiation of adjuvant radiotherapy. We hypothesized that scapular strengthening exercises with motor control techniques would be more effective than regular exercise in releasing shoulder pain, improving AROM of shoulder joint and muscle activities.

Materials & methods

Participants

This study is a design of randomized controlled trial. The participants were enrolled from a Memorial Hospital from June 2018 to December 2018. The inclusion criteria were as follows: (1) newly diagnosed oral cancer subjects with neck dissection; (2) age between 20 and 65 years; and (3) having all of the clinical signs of accessory nerve shoulder dysfunction, which were shoulder droop, limited AROM of shoulder abduction, and insufficient muscle strength of shoulder abduction to against gravity. Participants were excluded if they (1) were pregnant or breastfeeding; (2) had distant metastasis or recurrence; (3) were unable to communicate or comprehend the questionnaires; (4) had a history of shoulder dysfunction before neck dissection (e.g., shoulder pain, tendinitis, tendon rupture, shoulder capsulitis, or neuropathy); or (5) had any disorder that could influence movement performance.

This study was approved by the Chang Gung Medical Foundation Institutional Review Board (Approval No: 201800026A3 and 201800026A3C502) and Clinical Trials (Approval No: NCT03545100). Written informed consent was obtained from all participants. Participants were randomly allocated into the motor-control group or regular-exercise group with block randomization by a researcher who did not involve in intervention and evaluations. The method of 4 participants in one block was used, and 2 participants were assigned into each group in every 4 participants. Each participant was blinded to the intervention allocation and accepted a 1-month intervention by a physical therapist. All the interventions were conducted by a different group of two certified physical therapists with an average of 6.5 years of clinical experience. Before the study, these two physical therapists accepted one-month training for intervention procedures and motor control techniques. Besides, all evaluations, including baseline (pre-test) and 1-month after the intervention (post-test), were conducted by another physical therapist with 24 years of clinical experience who was blinded to subject allocation.

A priori sample size calculation was performed using G*power software based on a pilot study of 10 patients. The test family and statistical test which we used were F tests and 'MANOVA: Repeated measures, within-between interaction', respectively. We used the absolute values of serratus anterior muscle activities when performing a scapular muscle exercise (e.g., horizontal adduction and flexion) to estimate sufficient sample size (motor-control group: pre-test: 178.42±107.68; post-test: 123.99±20.37; regular-exercise group: pre-test: 211.36 ±217.69; post-test: 325.13±263.77). The significance level was set at $\alpha = 0.05$, and the power was set at 0.8. Considering a 10% drop-out rate, the estimation indicated that a sample size of 38 participants was required (effect size = 0.5).

Interventions

Both regular-exercise and motor-control groups received conventional physical therapy, including pain management, scar massage, stretching, active and passive range of motion exercise of shoulder joint, and specific scapular strengthening exercises. Specific scapular strengthening exercises for the UT, MT, LT and SA muscles were based on previous studies and were administered respectively [12, 26–29]. The details of the strengthening exercises are shown in S1 Table. For the specific scapular strengthening exercises, participants in the regular-exercise group were instructed to perform the exercises without any information about the muscle involved or alignment of scapula. In contrast, participants in the motor-control group received anatomy education about the scapular muscles, including their function and proper alignment before performing specific scapular strengthening exercises. A physical therapist instructed and facilitated the participants in controlling the scapula with arm movement by manual contact and verbal cues during exercises for the motor-control group. The intervention sessions were performed 5 days a week during hospitalization and 1 day a week after discharged from the hospital, with 60 minutes for each session. All participants were instructed to perform individual home-programs for 60 minutes per day, and they were requested to record the performed exercise in exercise diaries.

Outcomes

To assess shoulder behavior, we measured the AROM of shoulder abduction since it is the most affected movement after neck dissection [3, 34] and shoulder pain at rest by a 10 cm visual analog scale (VAS) [4, 35]. AROM was taken by a senior physical therapist with a two-arm goniometer under standard procedures, and the means of three measurements were recorded. The internal reliability of the two-arm goniometer is 0.58 to 0.99, and the concurrent validity was good compared with a digital inclinometer (ICC = 0.85) for shoulder abduction [36].

To measure the muscle activities during exercises, muscle activities of the UT, MT, LT, and SA were recorded using surface EMG electrodes (Ambu® BlueSensor NF-50-K, Malaysia) and an AC amplifier (gain: 5000, cut-off frequency: 10–450 Hz; Model: QP511, GRASS, USA). Surface EMG is a non-invasive and high reliable methodology to measure muscle activity [37, 38]. The investigator conducted surface EMG recording with a standardization procedure, especially for the electrode position. The placement of the EMG electrodes was in accordance with the recommendations for surface EMG sensor placement [39] and previous studies [26, 40]. For the UT, the EMG electrodes were placed in the middle between the 7th cervical vertebra and the posterior tip of the acromion process. For the MT, the EMG electrodes were placed between the 3rd thoracic vertebra and the root of the spine of the scapula. For the LT, the EMG electrodes were placed at the 2/3 position of the line from the trigonum spinea to the 8th thoracic vertebra. For the SA, the EMG electrodes were placed at the intersection of mid axillar line and the inferior angle of the scapula. Reference electrodes were placed over the 7th cervical vertebra, 3rd and the 8th thoracic vertebra, and acromion process for the UT, MT, LT, and SA, respectively. The sampling rate of the EMG signal was 1000 Hz.

Before electrode application, the skin was cleaned with alcohol and shaved if needed. Every participant was requested to perform 7 testing tasks, including 4 maximum voluntary isometric contraction (MVIC) tasks for the UT, MT, LT and SA muscles, and 3 tasks of scapular muscle exercise. Because some participants were unable to maintain prone position due to tracheostomy at pre-test, the 3 scapular muscle exercises were performed in an upright posture, including shoulder shrug with 1-kg weight, shoulder horizontal adduction and flexion, and one-arm row with 1-kg weight for each participant [11, 12, 28, 29]. Details of the MVIC tasks and 3 tasks of scapular muscle exercise were illustrated in S2 Table.

When performing the MVIC tasks, the participants placed their limbs to the standard testing position, and then kept the limbs in the standard testing position with bearing the force resistance which was provided by the physical therapist for 5 seconds. Each MVIC task was repeated 3 times with a 30-second rest between each repetition. There was a 60-second rest between different MVIC tasks. The root mean square (RMS) of the EMG data from the 2nd to the 5th second of the MVIC task was analyzed. When performing the tasks of scapular muscle exercise, the participants were asked to remain at the target position for 10 seconds and the task was repeated 3 times. The RMS of the EMG data from the 3rd to 6th seconds for each scapular muscle was analyzed. The RMS of the EMG data was normalized by the MVIC and presented as %MVIC. All raw EMG data were visually inspected for artifacts. If there was an artifact, artifacts were excluded and the task was repeated.

Statistical analysis

The Generalized Estimating Equations (GEE) procedure was conducted to analyze repeated measures outcome variables over time [41]. GEE has the benefit to provide higher power with small sample size for repeated measurements with complete or missing data [42–44]. We used the GEE model with an exchangeable working correlation matrix. Separate models were run for each muscle and each task. The level of significance was set at $p<0.05$. Statistical analyses were completed using SPSS version 21 (SPSS Inc., USA).

Results

A total of 38 participants were analyzed in the present study. Thirty-five participants received single-side neck dissection; whereas 3 participants received bilateral neck dissection (2 in the motor-control group; 1 in the regular-exercise group), and the data of the worse side were analyzed. The CONSORT flow diagram is shown in Fig 1. Table 1 presents the participants' demographic and clinical characteristics. There was no significant difference at baseline measurements and in the number of intervention sessions during hospitalization between the two groups (regular-exercise group: 4.6±2.9 sessions; motor-control group: 5.8±3.6 sessions, $p = 0.251$). Each participant accepted intervention for 3 consecutive weeks after discharged from the hospital. The exercise diaries containing home-programs were checked by the physical therapist that provided the treatment. All participants followed the instructions and didn't present any side effects or complain about the treatment. Only 2 participants discontinued in the motor control group due to a busy schedule (Fig 1).

Shoulder behavior outcomes

The GEE results showed a significant time effect (95% CI 0.01 to 2.42, $p = 0.049$) on the VAS score of shoulder pain without group (95% CI: -0.90 to 2.01, $p = 0.456$) and interaction (95% CI: -1.51 to 1.88, $p = 0.830$) effects. The shoulder pain score (VAS) decreased by 1.40 (95% CI: -0.21 to -2.59, $p = 0.021$) in the regular-exercise group and by 1.21 (95% CI: -0.01 to -2.42, $p = 0.049$) in the motor-control group. Also, the AROM of shoulder abduction had a significant time effect (95% CI: -20.51 to -3.80, $p = 0.004$) without group (95% CI: -22.91 to 1.97, $p = 0.099$) and interaction (95% CI: -6.28 to 16.61, $p = 0.376$) effects. The post-hoc showed the improvement of AROM was only found in the motor-control group which was from 124.75

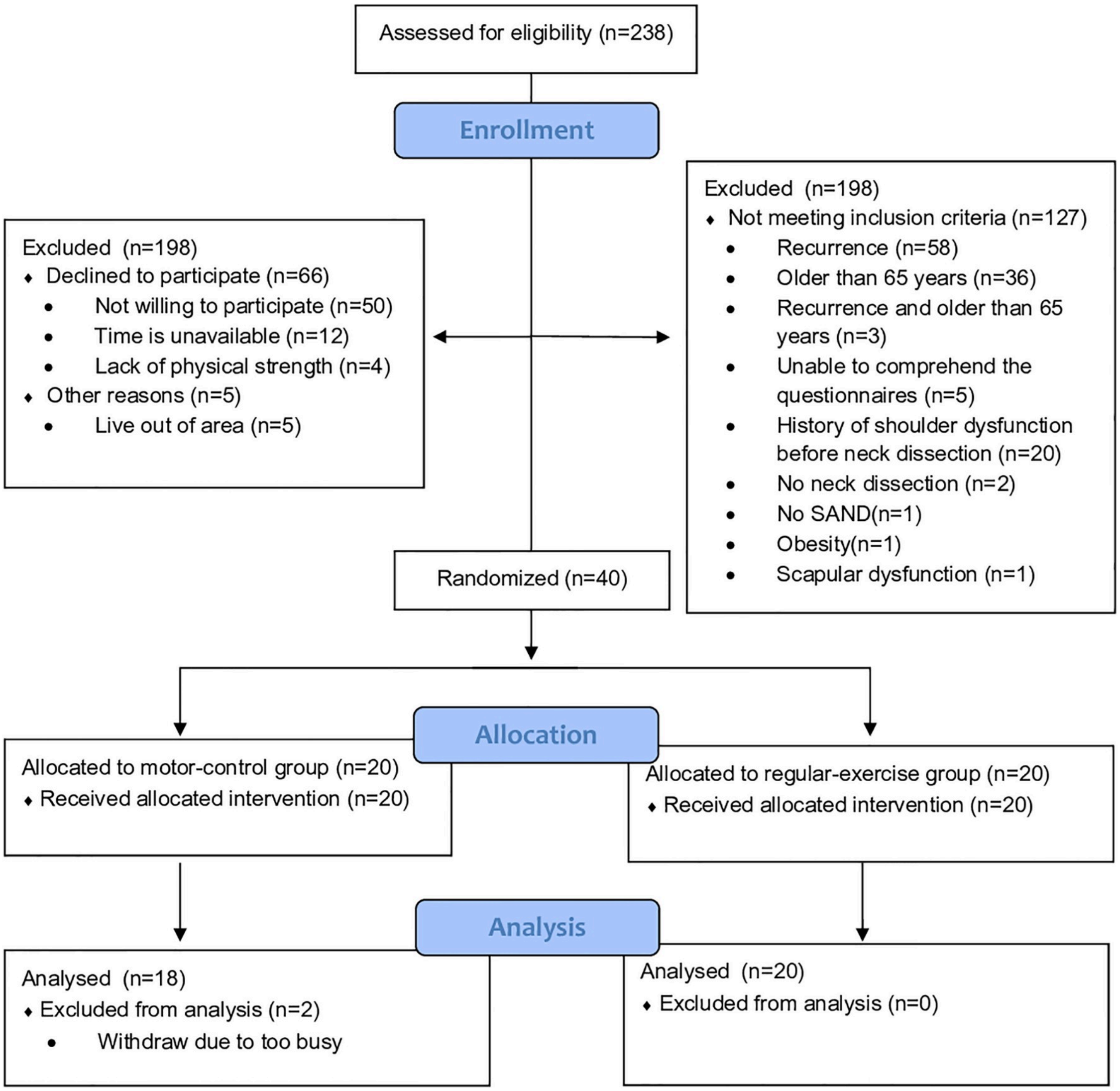

Fig 1. CONSORT flow diagram.

degrees to 136.91 degrees (95% CI: 3.80 to 20.51, $p = 0.004$), but not in the regular-exercise group which was 119.44 degrees at pre-test and 126.44 degrees at post-test.

Scapular muscle activations

Fig 2 illustrates the results of the EMG activities under the 4 MVIC conditions. Although there were no group and interaction effects in each muscle under any MVIC condition, there were significant time effects of EMG RMS in the UT, MT, LT, and SA muscles ($p<0.001$) with greater RMS value after 1-month intervention.

For the task of scapular muscle exercise of shoulder shrug with 1-kg weight (Fig 3), the model analyzed by GEE revealed a time effect (95% CI: 1.29 to 33.07, $p = 0.034$) on the UT activation (%MVIC) without group (95% CI: -20.73 to 20.33, $p = 0.985$) and interaction (95% CI: -29.80 to 13.97, $p = 0.478$) effects. The UT activation decreased after a 1-month training only

Table 1. Demographic and clinical characteristics of the study participants.

Characteristic	Randomized (n = 38)	
	Motor-control group (n = 18)	**Regular-exercise group (n = 20)**
Age (yr), mean (SD)	52.7 (9)	49.1 (9)
Male, n (%)	17 (94)	20 (100)
Days after surgery (dy), mean (SD)	11.4 (5)	12.3 (5)
Area of cancer, n (%)		
Buccal	11 (61)	9 (45)
Lower gingiva	1 (6)	0 (0)
Lower gum	3 (17)	3 (15)
Lower lip	1 (6)	1 (5)
Mouth floor	0 (0)	2 (10)
Tongue	2 (11)	5 (25)
Disease stage, n (%)		
I	0 (0)	2 (10)
II	6 (33)	2 (10)
III	2 (11)	3 (15)
IV	10 (56)	13 (65)
Neck dissection, n (%)		
Selective neck dissection	13 (72)	17 (85)
Modified neck dissection	5 (28)	3 (15)
Affected side, n (%)		
Left	9 (50)	12 (60)
Right	9 (50)	8 (40)
Affected side is dominant side, n (%)	10 (56)	8 (40)
Donor site, n (%)		
ALT flap	8 (44)	13 (65)
ALT and VL flap	1 (6)	0 (0)
Fubular OSC flap	5 (28)	4 (20)
Fubular OSC flap and ALT flap	2 (11)	0 (0)
Medial sural artery perforator flap	1 (6)	0 (0)
Profunda artery perforator flap	1 (6)	3 (15)

ALT, Anterolateral thigh flap; VL, vastus lateralis; Fibular OSC flap, Fubular osteoseptocutaneous flap.

in the motor-control group (95% CI: -33.06 to -1.29, $p = 0.034$). However, muscle activation of the MT, LT, and SA did not change after a 1-month training in both groups.

For the task of shoulder horizontal adduction and flexion (Fig 4), the model analyzed by GEE revealed a time effect (95% CI: 1.32 to 29.68, $p = 0.032$) on the MT activation without group (95% CI: -21.58 to 20.01, $p = 0.941$) and interaction (95% CI: -16.92 to 21.97, $p = 0.799$) effects. The MT activity decreased after a 1-month training in both motor-control (95% CI: -29.68 to -1.32, $p = 0.032$) and regular-exercise (95% CI: -31.32 to -4.73, $p = 0.008$) groups. Surprisingly, there were group (95% CI: 0.58 to 156.79, $p = 0.048$), time (95% CI: 27.68 to 29.73, $p<0.001$), and interaction (95% CI: -59.20 to -56.42, $p<0.001$) effects on the muscle activity of the SA. The post-hoc showed that after a 1-month training, the SA activity increased in the regular-exercise group (95% CI: 28.16 to 30.05, $p<0.001$) but decreased in the motor-control group (95% CI: -29.73 to -27.68, $p<0.001$). Both the UT and LT muscle activities were not affected during the task of shoulder horizontal adduction and flexion in both groups.

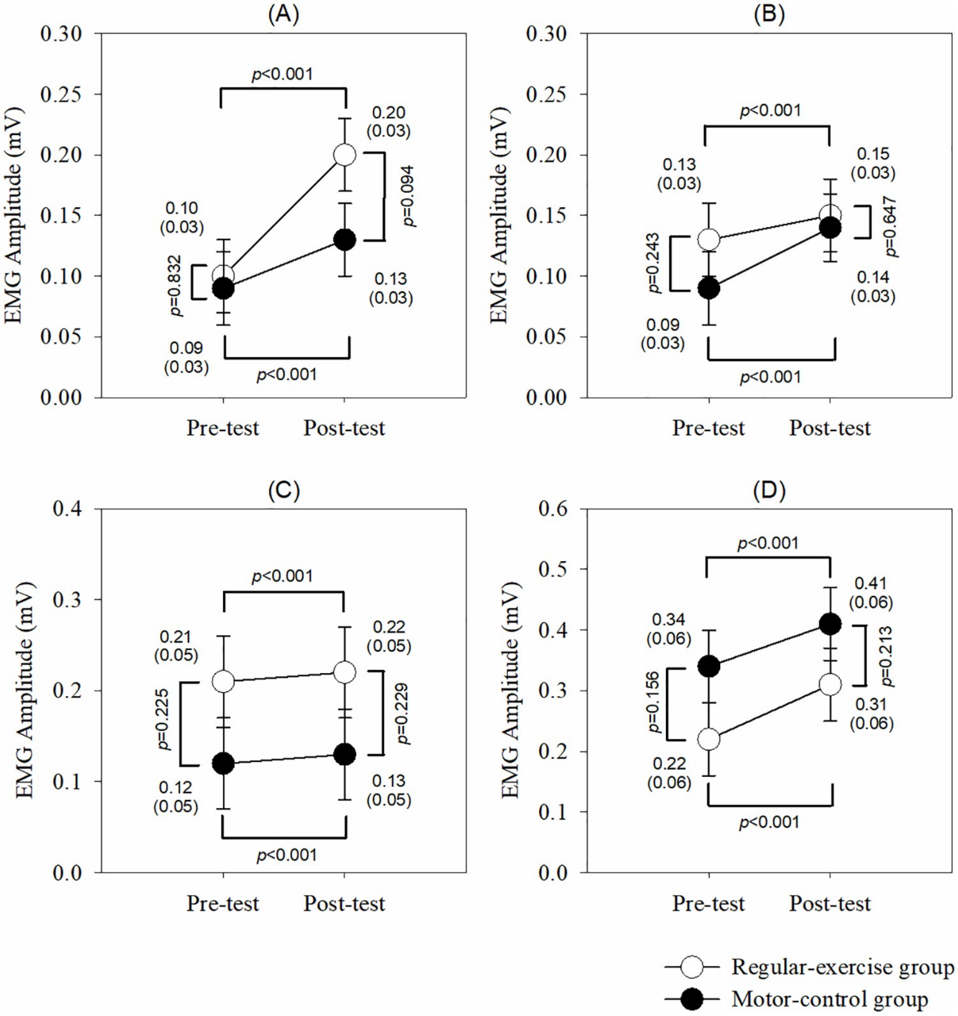

Fig 2. The EMG activities under the 4 MVIC conditions. (A) Upper trapezius. (B) Middle trapezius. (C) Lower trapezius. (D) Serratus anterior. The *p* values are shown if any significant difference ($p<0.05$) between the groups or pre-post tests in that muscle.

For the task of one-arm row (Fig 5), there were no time, group or interaction effects on each muscle activity.

Discussion

To the best of our knowledge, this is the first study to investigate the effects of early intervention of specific scapular strengthening exercises with motor control techniques on shoulder behavior and scapular muscle activation during the interval between surgery and initiation of radiotherapy in oral cancer survivors with shoulder dysfunction. Some previous studies reported that progressive resistance exercise reduced shoulder pain and improved muscle strength of upper extremities for HNC survivors with neck dissection [9, 10]. However, these studies have attracted some criticism because the intervention started up to 18 months after neck dissection and the training was not specific to accessory nerve-related muscles [18, 45]. It has been reported that the MVIC of the trapezius decreased by 70% at 21 to 30 days after neck dissection compared to the pre-operative value [7], and lasted at least for 9 months [8]. Also,

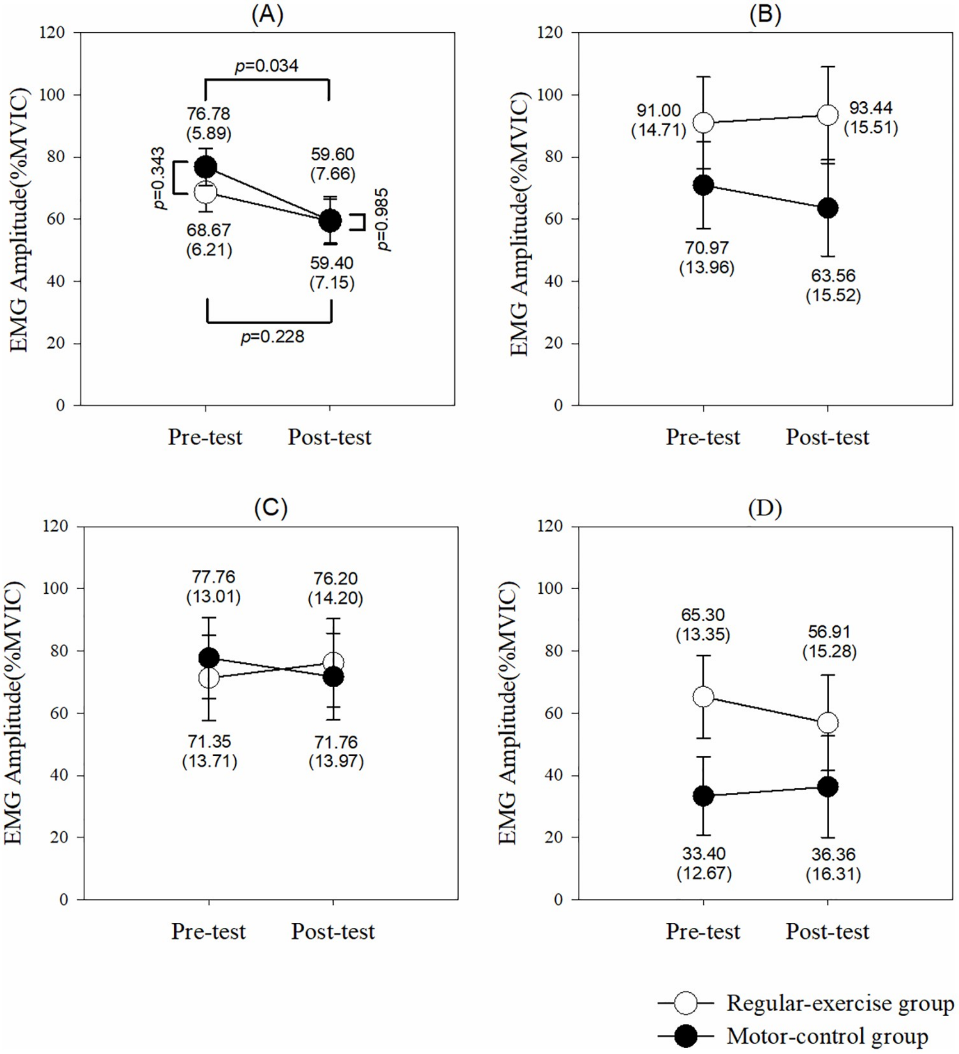

Fig 3. Muscle activites in each muscle to perform shoulder shrug. (A) Upper trapezius. (B) Middle trapezius. (C) Lower trapezius. (D) Serratus anterior. The *p* values are shown if any significant difference ($p<0.05$) between the groups or pre-post tests in that muscle.

neck dissection led to lower EMG activities of affected UT and MT compared with the unaffected side [6, 12]. Therefore, the effect of early intervention specific to accessory nerve-related muscles for the restoration of muscle activation after neck dissection is worth further study. In the model analyzed by the GEE procedure, early intervention of specific scapular strengthening exercises with or without motor control techniques all decreased shoulder pain and increased muscle activity of MVIC of each muscle after a 1-month intervention. Furthermore, motor control has more benefits for shoulder joint ROM of abduction and scapular muscle activities when performing scapular muscle exercises.

Shoulder behavior outcomes

One of our key findings is that early intervention released shoulder pain and improved AROM of shoulder abduction. Regarding shoulder pain measured by VAS, both groups showed a significant decrease in shoulder pain intensity. The evidence of shoulder pain reduction by

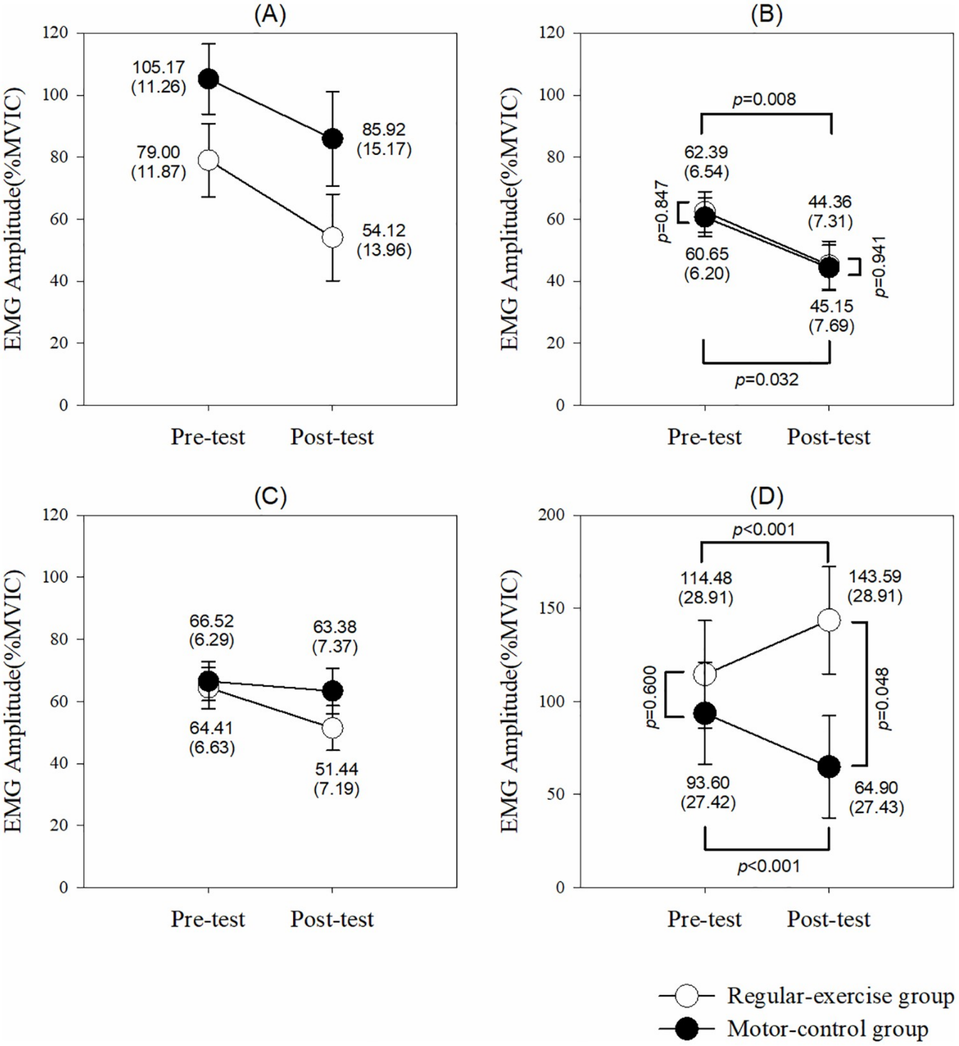

Fig 4. Muscle activites in each muscle to perform horrizontal adduction and flexion. (A) Upper trapezius. (B) Middle trapezius. (C) Lower trapezius. (D) Serratus anterior. The *p* values are shown if any significant difference ($p<0.05$) between the groups or pre-post tests in that muscle.

specific scapular strengthening exercises was provided in patients with shoulder impingement syndrome [46, 47]. In addition, it has been proposed that pain reduction after scapular strengthening exercises was related to regained scapular muscle balance [26–29]. Although McGarvey et al.'s study showed that early physical therapy intervention with scapular strengthening exercises did not have benefits to shoulder pain for patients with neck dissection, they suggested the non-improvement phenomenon might because many participants underwent radiation therapy during the intervention, and radiation therapy would impede the effects of the intervention [11]. In the present study, none participants underwent radiation therapy during the intervention, and the participants of both the motor-control group and the regular-exercise group showed reduced shoulder pain after a 1-month intervention. Our results proved that early physical therapy intervention with specific scapular strengthening exercises indeed had a positive effect on pain reduction in oral cancer survivors with neck dissection.

Furthermore, improvement in AROM of shoulder abduction was observed only in the motor-control group, the mean change achieved a minimal clinically important difference

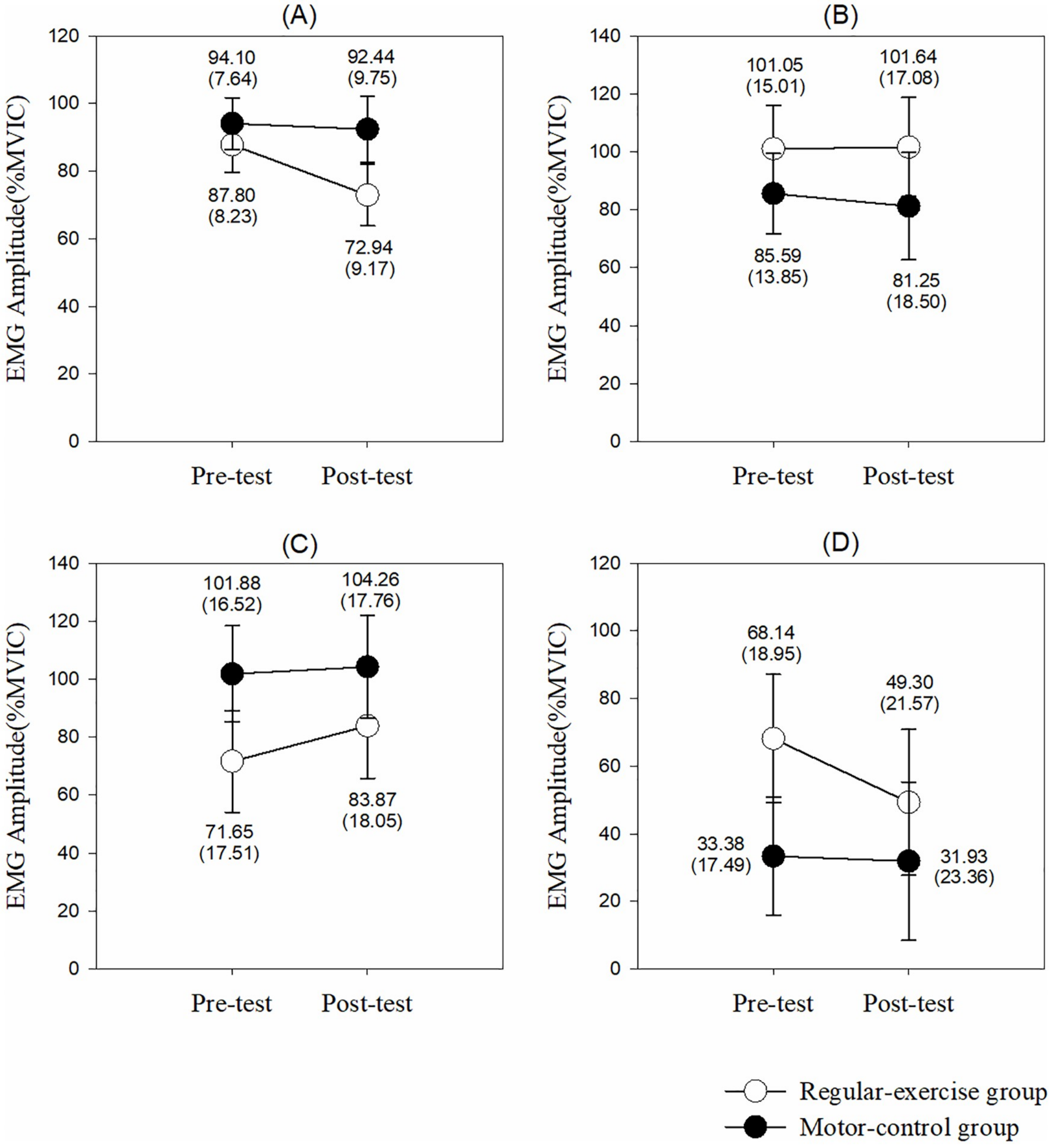

Fig 5. Muscle activites in each muscle to perform one-arm row. (A) Upper trapezius. (B) Middle trapezius. (C) Lower trapezius. (D) Serratus anterior. The *p* values are shown if any significant difference ($p<0.05$) between the groups or pre-post tests in that muscle.

(11–16 degrees) [48]. In contrast, there was no significant improvement in the regular-exercise group after the intervention. It has been reported that motor control training with consciously correct scapular orientation could change scapular kinematics and increase AROM of shoulder joint for patients with shoulder impingement [30, 32], but the training effect for patients with HNC was not proven. In the present study, the physical therapist used manual contact and verbal cues to instruct and correct scapular movement for the motor-control group. Combined verbal and hepatic feedbacks allow participants to enhance motor learning by improving temporal muscle activation of motor tasks [49]. Because the control of scapular alignment (e.g., scapular upward rotation and posterior tilt) is critical for the movement of arm elevation such as shoulder abduction [50, 51], motor control training with manual contact and verbal cues from a physical therapist could help the patients to learn how to control the alignment of the scapula during arm movement. The present study confirmed that motor control intervention has a greater benefit to improve AROM of shoulder abduction for HNC survivors with neck dissection.

Scapular muscle activations

Specific scapular strengthening exercises could increase trapezius muscle activation in patients with shoulder pathology [27, 29] and in HNC patients with shoulder dysfunction [12]. The present study investigates the effects of scapular muscle exercises on patients with oral cancers based on a 1-month intervention duration. According to our results, increased absolute muscle activation values under MVIC conditions for each target muscle were observed in both groups after intervention. EMG activities increased after short-term training was associated with neural adaption by increased motor unit firing rate leading to increased muscle activation [52, 53]. Even though shoulder girdle stretching and manual therapy (e.g., glenohumeral and scapulothoracic joints mobilizations) were proposed to be effective in reducing shoulder pain [54, 55], it is less clear if either stretching or manual therapy could lead benefits to muscle activities. Based on the results of the present study, we suggested that specific scapular strengthening exercises are not only effective in pain reduction but also in the restoration of scapular muscle activities in oral cancer survivors with neck dissection.

Besides increased muscle activities under MVIC conditions, a decreased muscle activation of UT when performing shoulder shrug with 1-kg weight was identified in the motor-control group after the 1-month intervention. The decreased muscle activation could be a phenomenon of neural adaptation or muscle economy after resistance training that less motor units are required for producing a given force [56]. Since the UT is the primary mover of shoulder shrug, achieving a motor task with smaller muscle activation indicates the participants consumed less muscle effort for the task. The results indicated that motor control intervention prompted participants to be aware of controlling the alignment of scapula during movement and to increase the efficacy to perform the movement.

Surprisingly, relative to pre-test, the present study identified the muscle activation of SA decreased after a 1-month intervention in the motor-control group when performing the task of shoulder horizontal adduction and flexion. In contrast, the activity of SA increased after intervention in the regular-exercise group. In McGarvey et al.'s studies, HNC survivors with neck dissection showed greater muscle activity of SA in the affected side than the unaffected side when performing scapular exercise [6, 12]. The function of SA muscle is to stabilize the medial border of the scapula on the chest wall when elevating the arm overhead and protracting scapula [57] (i.e. shoulder horizontal adduction and flexion). Greater SA activity was suggested to be a compensatory effect for insufficient strength of trapezius muscle [6]. The present study provides the evidence that strengthening exercises with motor control techniques for scapular muscles is effective in proprioception training for scapula orientation and inhibiting muscle compensation in oral cancer patients with shoulder dysfunction.

Similar to our findings, Huang and his colleagues have reported that motor control intervention by progressive conscious control scapular orientation with arm movement could immediately restore intramuscular ratio in patients with clinical shoulder impingement [31]. In addition, enhanced muscle recruitments and duration of activation of SA muscle were identified in patients with clinical shoulder impingement after a 12-week motor control intervention [30]. Compared to pre-intervention, the recruitment pattern of SA muscle was similar to healthy controls without delayed onset and early termination of activity during arm movement. Different from impingement syndrome, neck dissection-related shoulder dysfunction originates from neuromuscular dysfunction. As the nerve function recovers after neck dissection, motor control intervention is also recommended for regaining neuromuscular interaction to stabilize the scapula and to coordinate with arm movement.

Although the LT is the primary muscle for the one-arm row [28], our results did not show a muscle activity change of the LT in the task of one-arm row. Since the LT is a large muscle and is innervated by the spinal accessory nerve with the longest distance from the posterior cervical triangle [57]. We suggested it may require longer training duration for nerve reinnervation to recruit motor units to achieve the significant change of LT muscle activation. Besides, the main function of the LT is to depress and posterior tilt the scapula, which is not easy to be trained specifically. Further study to explore the long-term effects of specific scapular muscle strengthening exercises on the LT with motor control is needed.

Study limitations

There were some limitations in this study. First, we did not measure the absolute values of force output in the MVIC conditions. Although the muscle activity of MVIC increased after strengthening exercise, the muscle activity is uncertain to be equivalent to the real muscle force. Second, the scapular kinematics was not measured in this study. It can provide information on scapular movement and the biomechanical effects of motor control intervention. Third, we did not measure the EMG activities before the operation of neck dissection. The value of the EMG activities before the neck dissection may provide the object reference. Further study is needed to evaluate the long-term functional or biomechanical effects of motor control intervention with specific scapular muscle strengthening programs.

Conclusions

Shoulder dysfunction is common in patients with oral cancer after neck dissection. This is the first study to investigate the effects of early intervention of specific scapular strengthening exercises with motor control techniques on shoulder behavior and scapular muscle activation in oral cancer survivors with shoulder dysfunction. Based on our results, we suggested the intervention of specific scapular strengthening exercises with motor control techniques immediately after neck dissection is necessary for relieving shoulder pain, improving shoulder AROM, and reducing compensatory scapular muscle activities in oral cancer survivors with shoulder dysfunction. In the future, exploration of the long-term effects of motor control with scapular strengthening exercise is suggested to understand the progression of shoulder behavior and scapular muscle activations even under the effects of radiation therapy.

Supporting information

S1 Table. Specific scapular strengthening exercises.
(PDF)

S2 Table. Test tasks of scapular muscles.
(PDF)

S1 Checklist.
(DOC)

S1 Protocol.
(PDF)

S2 Protocol.
(PDF)

Author Contributions

Conceptualization: Yueh-Hsia Chen.

Data curation: Yueh-Hsia Chen.

Formal analysis: Yueh-Hsia Chen.

Investigation: Yueh-Hsia Chen, Chi-Rung Lin, Wei-An Liang.

Methodology: Yueh-Hsia Chen.

Project administration: Yueh-Hsia Chen.

Resources: Yueh-Hsia Chen.

Supervision: Cheng-Ya Huang.

Validation: Cheng-Ya Huang.

Writing – original draft: Yueh-Hsia Chen.

Writing – review & editing: Cheng-Ya Huang.

References

1. Ewing MR, Martin H. Disability following radical neck dissection; an assessment based on the postoperative evaluation of 100 patients. Cancer. 1952; 5(5):873–83.
2. Erisen L, Basel B, Irdesel J, Zarifoglu M, Coskun H, Basut O, et al. Shoulder function after accessory nerve-sparing neck dissections. Head & neck. 2004; 26(11):967–71.
3. Gane EM, Michaleff ZA, Cottrell MA, McPhail SM, Hatton AL, Panizza BJ, et al. Prevalence, incidence, and risk factors for shoulder and neck dysfunction after neck dissection: A systematic review. Eur J Surg Oncol. 2017; 43(7):1199–218. https://doi.org/10.1016/j.ejso.2016.10.026 PMID: 27956321
4. Carr SD, Bowyer D, Cox G. Upper limb dysfunction following selective neck dissection: a retrospective questionnaire study. Head & neck. 2009; 31(6):789–92.
5. Dijkstra PU, van Wilgen PC, Buijs RP, Brendeke W, de Goede CJ, Kerst A, et al. Incidence of shoulder pain after neck dissection: a clinical explorative study for risk factors. Head & neck. 2001; 23(11):947–53.
6. McGarvey AC, Osmotherly PG, Hoffman GR, Chiarelli PE. Impact of neck dissection on scapular muscle function: a case-controlled electromyographic study. Archives of physical medicine and rehabilitation. 2013; 94(1):113–9. https://doi.org/10.1016/j.apmr.2012.07.017 PMID: 22864015
7. Lima LP, Amar A, Lehn CN. Spinal accessory nerve neuropathy following neck dissection. Braz J Otorhinolaryngol. 2011; 77(2):259–62. https://doi.org/10.1590/s1808-86942011000200017 PMID: 21537629
8. Orhan KS, Demirel T, Baslo B, Orhan EK, Yucel EA, Guldiken Y, et al. Spinal accessory nerve function after neck dissections. J Laryngol Otol. 2007; 121(1):44–8. https://doi.org/10.1017/S0022215106002052 PMID: 17040583
9. McNeely ML, Parliament M, Courneya KS, Seikaly H, Jha N, Scrimger R, et al. A pilot study of a randomized controlled trial to evaluate the effects of progressive resistance exercise training on shoulder dysfunction caused by spinal accessory neurapraxia/neurectomy in head and neck cancer survivors. Head & neck. 2004; 26(6):518–30.
10. McNeely ML, Parliament MB, Seikaly H, Jha N, Magee DJ, Haykowsky MJ, et al. Effect of exercise on upper extremity pain and dysfunction in head and neck cancer survivors: a randomized controlled trial. Cancer. 2008; 113(1):214–22. https://doi.org/10.1002/cncr.23536 PMID: 18457329
11. McGarvey AC, Hoffman GR, Osmotherly PG, Chiarelli PE. Maximizing shoulder function after accessory nerve injury and neck dissection surgery: A multicenter randomized controlled trial. Head & neck. 2015; 37(7):1022–31.

12. McGarvey AC, Osmotherly PG, Hoffman GR, Chiarelli PE. Scapular muscle exercises following neck dissection surgery for head and neck cancer: a comparative electromyographic study. Phys Ther. 2013; 93(6):786–97. https://doi.org/10.2522/ptj.20120385 PMID: 23431215

13. Kibler WB, Sciascia A. Current concepts: scapular dyskinesis. Br J Sports Med. 2010; 44(5):300–5. https://doi.org/10.1136/bjsm.2009.058834 PMID: 19996329

14. Kibler WB, Ludewig PM, McClure PW, Michener LA, Bak K, Sciascia AD. Clinical implications of scapular dyskinesis in shoulder injury: the 2013 consensus statement from the 'Scapular Summit'. Br J Sports Med. 2013; 47(14):877–85. https://doi.org/10.1136/bjsports-2013-092425 PMID: 23580420

15. Cools AM, Declercq GA, Cambier DC, Mahieu NN, Witvrouw EE. Trapezius activity and intramuscular balance during isokinetic exercise in overhead athletes with impingement symptoms. Scand J Med Sci Sports. 2007; 17(1):25–33. https://doi.org/10.1111/j.1600-0838.2006.00570.x PMID: 16774650

16. Struyf F, Cagnie B, Cools A, Baert I, Brempt JV, Struyf P, et al. Scapulothoracic muscle activity and recruitment timing in patients with shoulder impingement symptoms and glenohumeral instability. J Electromyogr Kinesiol. 2014; 24(2):277–84. https://doi.org/10.1016/j.jelekin.2013.12.002 PMID: 24389333

17. Chen YH, Liang WA, Hsu CY, Guo SL, Lien SH, Tseng HJ, et al. Functional outcomes and quality of life after a 6-month early intervention program for oral cancer survivors: a single-arm clinical trial. PeerJ. 2018; 6:e4419. https://doi.org/10.7717/peerj.4419 PMID: 29492348

18. Carvalho AP, Vital FM, Soares BG. Exercise interventions for shoulder dysfunction in patients treated for head and neck cancer. Cochrane Database Syst Rev. 2012; 4:CD008693.

19. Cheng YJ, Tsai MH, Chiang CJ, Tsai ST, Liu TW, Lou PJ, et al. Adjuvant radiotherapy after curative surgery for oral cavity squamous cell carcinoma and treatment effect of timing and duration on outcome-A Taiwan Cancer Registry national database analysis. Cancer Med. 2018.

20. Gallagher KK, Sacco AG, Lee JS, Taylor R, Chanowski EJ, Bradford CR, et al. Association Between Multimodality Neck Treatment and Work and Leisure Impairment: A Disease-Specific Measure to Assess Both Impairment and Rehabilitation After Neck Dissection. JAMA Otolaryngol Head Neck Surg. 2015; 141(10):888–93. https://doi.org/10.1001/jamaoto.2015.2049 PMID: 26426565

21. Sun Q, Guo S, Wang D, Xu N, Fang QG. Shoulder Dysfunction After Radiotherapy in Surgically and Nonsurgically Treated Necks: A Prospective Study. Medicine (Baltimore). 2015; 94(30):e1229.

22. Chepeha DB, Taylor RJ, Chepeha JC, Teknos TN, Bradford CR, Sharma PK, et al. Functional assessment using Constant's Shoulder Scale after modified radical and selective neck dissection. Head & neck. 2002; 24(5):432–6.

23. Chen AM, Wang PC, Daly ME, Cui J, Hall WH, Vijayakumar S, et al. Dose—volume modeling of brachial plexus-associated neuropathy after radiation therapy for head-and-neck cancer: findings from a prospective screening protocol. Int J Radiat Oncol Biol Phys. 2014; 88(4):771–7. https://doi.org/10.1016/j.ijrobp.2013.11.244 PMID: 24606846

24. McNeely ML, Campbell K, Ospina M, Rowe BH, Dabbs K, Klassen TP, et al. Exercise interventions for upper-limb dysfunction due to breast cancer treatment. Cochrane Database Syst Rev. 2010(6): CD005211. https://doi.org/10.1002/14651858.CD005211.pub2 PMID: 20556760

25. Wong CK, Levine WN, Deo K, Kesting RS, Mercer EA, Schram GA, et al. Natural history of frozen shoulder: fact or fiction? A systematic review. Physiotherapy. 2017; 103(1):40–7. https://doi.org/10.1016/j.physio.2016.05.009 PMID: 27641499

26. Cools AM, Dewitte V, Lanszweert F, Notebaert D, Roets A, Soetens B, et al. Rehabilitation of scapular muscle balance: which exercises to prescribe? Am J Sports Med. 2007; 35(10):1744–51. https://doi.org/10.1177/0363546507303560 PMID: 17606671

27. Cricchio M, Frazer C. Scapulothoracic and scapulohumeral exercises: a narrative review of electromyographic studies. J Hand Ther. 2011; 24(4):322–33; quiz 34. https://doi.org/10.1016/j.jht.2011.06.001 PMID: 21820276

28. Andersen CH, Zebis MK, Saervoll C, Sundstrup E, Jakobsen MD, Sjogaard G, et al. Scapular muscle activity from selected strengthening exercises performed at low and high intensities. J Strength Cond Res. 2012; 26(9):2408–16. https://doi.org/10.1519/JSC.0b013e31823f8d24 PMID: 22076101

29. Schory A, Bidinger E, Wolf J, Murray L. A Systematic Review of the Exercises That Produce Optimal Muscle Ratios of the Scapular Stabilizers in Normal Shoulders. Int J Sports Phys Ther. 2016; 11 (3):321–36. PMID: 27274418

30. Worsley P, Warner M, Mottram S, Gadola S, Veeger HE, Hermens H, et al. Motor control retraining exercises for shoulder impingement: effects on function, muscle activation, and biomechanics in young adults. J Shoulder Elbow Surg. 2013; 22(4):e11–9. https://doi.org/10.1016/j.jse.2012.06.010 PMID: 22947240

31. Huang TS, Du WY, Wang TG, Tsai YS, Yang JL, Huang CY, et al. Progressive conscious control of scapular orientation with video feedback has improvement in muscle balance ratio in patients with scapular dyskinesis: a randomized controlled trial. J Shoulder Elbow Surg. 2018; 27(8):1407–14. https://doi.org/10.1016/j.jse.2018.04.006 PMID: 29886062

32. Roy JS, Moffet H, Hebert LJ, Lirette R. Effect of motor control and strengthening exercises on shoulder function in persons with impingement syndrome: a single-subject study design. Man Ther. 2009; 14 (2):180–8. https://doi.org/10.1016/j.math.2008.01.010 PMID: 18358760

33. Shumway-Cook A. Motor control: theory and practical applications. 2nd ed. Woollacott MH, editor. Philadelphia: Lippincott Williams & Wilkins; 2001.

34. Goldstein DP, Ringash J, Bissada E, Jaquet Y, Irish J, Chepeha D, et al. Scoping review of the literature on shoulder impairments and disability after neck dissection. Head & neck. 2014; 36(2):299–308.

35. Chan JY, Wong ST, Chan RC, Wei WI. Shoulder Dysfunction after Selective Neck Dissection in Recurrent Nasopharyngeal Carcinoma. Otolaryngol Head Neck Surg. 2015; 153(3):379–84. https://doi.org/10.1177/0194599815590589 PMID: 26138607

36. Norkin CC, White DJ. Measurement Of Joint Motion: A Guide To Goniometry: F.A. Davis Company; 2016.

37. Beretta-Piccoli M, Cescon C, Barbero M, D'Antona G. Reliability of surface electromyography in estimating muscle fiber conduction velocity: A systematic review. J Electromyogr Kinesiol. 2019; 48:53–68. https://doi.org/10.1016/j.jelekin.2019.06.005 PMID: 31229925

38. McIntosh KC, Gabriel DA. Reliability of a simple method for determining muscle fiber conduction velocity. Muscle Nerve. 2012; 45(2):257–65. https://doi.org/10.1002/mus.22268 PMID: 22246883

39. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol. 2000; 10(5):361–74. https://doi.org/10.1016/s1050-6411(00)00027-4 PMID: 11018445

40. Ekstrom RA, Soderberg GL, Donatelli RA. Normalization procedures using maximum voluntary isometric contractions for the serratus anterior and trapezius muscles during surface EMG analysis. J Electromyogr Kinesiol. 2005; 15(4):418–28. https://doi.org/10.1016/j.jelekin.2004.09.006 PMID: 15811612

41. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics. 1986; 42(1):121–30. PMID: 3719049

42. Paik MC. The generalized estimating equation approach when data are not missing completely at random. Journal of the American Statistical Association. 1997; 92(440):1320–9.

43. Birhanu T, Molenberghs G, Sotto C, Kenward MG. Doubly robust and multiple-imputation-based generalized estimating equations. J Biopharm Stat. 2011; 21(2):202–25. https://doi.org/10.1080/10543406.2011.550096 PMID: 21390997

44. Ma Y, Mazumdar M, Memtsoudis SG. Beyond repeated-measures analysis of variance: advanced statistical methods for the analysis of longitudinal data in anesthesia research. Reg Anesth Pain Med. 2012; 37(1):99–105. https://doi.org/10.1097/AAP.0b013e31823ebc74 PMID: 22189576

45. McGarvey AC, Chiarelli PE, Osmotherly PG, Hoffman GR. Physiotherapy for accessory nerve shoulder dysfunction following neck dissection surgery: a literature review. Head & neck. 2011; 33(2):274–80.

46. Struyf F, Nijs J, Mollekens S, Jeurissen I, Truijen S, Mottram S, et al. Scapular-focused treatment in patients with shoulder impingement syndrome: a randomized clinical trial. Clinical rheumatology. 2013; 32(1):73–85. https://doi.org/10.1007/s10067-012-2093-2 PMID: 23053685

47. Park SI, Choi YK, Lee JH, Kim YM. Effects of shoulder stabilization exercise on pain and functional recovery of shoulder impingement syndrome patients. J Phys Ther Sci. 2013; 25(11):1359–62. https://doi.org/10.1589/jpts.25.1359 PMID: 24396188

48. Muir SW, Corea CL, Beaupre L. Evaluating change in clinical status: reliability and measures of agreement for the assessment of glenohumeral range of motion. N Am J Sports Phys Ther. 2010; 5(3):98–110. PMID: 21589666

49. Frikha M, Chaari N, Elghoul Y, Mohamed-Ali HH, Zinkovsky AV. Effects of Combined Versus Singular Verbal or Haptic Feedback on Acquisition, Retention, Difficulty, and Competence Perceptions in Motor Learning. Percept Mot Skills. 2019; 126(4):713–32. https://doi.org/10.1177/0031512519842759 PMID: 31033405

50. Johnson G, Bogduk N, Nowitzke A, House D. Anatomy and actions of the trapezius muscle. Clin Biomech (Bristol, Avon). 1994; 9(1):44–50.

51. Ludewig PM, Cook TM, Nawoczenski DA. Three-dimensional scapular orientation and muscle activity at selected positions of humeral elevation. The Journal of orthopaedic and sports physical therapy. 1996; 24(2):57–65. https://doi.org/10.2519/jospt.1996.24.2.57 PMID: 8832468

52. Del Balso C, Cafarelli E. Adaptations in the activation of human skeletal muscle induced by short-term isometric resistance training. J Appl Physiol (1985). 2007; 103(1):402–11.

53. Gabriel DA, Kamen G, Frost G. Neural adaptations to resistive exercise: mechanisms and recommendations for training practices. Sports Med. 2006; 36(2):133–49. https://doi.org/10.2165/00007256-200636020-00004 PMID: 16464122

54. Camargo PR, Alburquerque-Sendin F, Avila MA, Haik MN, Vieira A, Salvini TF. Effects of Stretching and Strengthening Exercises, With and Without Manual Therapy, on Scapular Kinematics, Function, and Pain in Individuals With Shoulder Impingement: A Randomized Controlled Trial. The Journal of orthopaedic and sports physical therapy. 2015; 45(12):984–97. https://doi.org/10.2519/jospt.2015.5939 PMID: 26471852

55. Turgut E, Duzgun I, Baltaci G. Effects of Scapular Stabilization Exercise Training on Scapular Kinematics, Disability, and Pain in Subacromial Impingement: A Randomized Controlled Trial. Archives of physical medicine and rehabilitation. 2017; 98(10):1915–23 e3. https://doi.org/10.1016/j.apmr.2017.05.023 PMID: 28652066

56. Sale DG. Neural adaptation to resistance training. Med Sci Sports Exerc. 1988; 20(5 Suppl):S135–45. https://doi.org/10.1249/00005768-198810001-00009 PMID: 3057313

57. Didesch JT, Tang P. Anatomy, Etiology, and Management of Scapular Winging. The Journal of hand surgery. 2019; 44(4):321–30. https://doi.org/10.1016/j.jhsa.2018.08.008 PMID: 30292717

11

Evidence for the effects of prehabilitation before ACL-reconstruction on return to sport-related and self-reported knee function: A systematic review

Florian Giesche[1]*, **Daniel Niederer**[2], **Winfried Banzer**[1], **Lutz Vogt**[2]

1 Division of Preventive and Sports Medicine, Institute of Occupational, Social and Environmental Medicine, Goethe University Frankfurt, Frankfurt/Main, Germany, **2** Department of Sports Medicine & Exercise Physiology, Institute of Sports Sciences, Goethe University Frankfurt, Frankfurt/Main, Germany

* giesche@sport.uni-frankfurt.de

Abstract

Study design

Systematic review.

Background and objectives

Preoperative neuromuscular function is predictive for knee function and return to sports (RTS) after reconstruction of the anterior cruciate ligament (ACL). The aim of this review was to examine the potential benefits of prehabilitation on pre-/postoperative objective, self-reported and RTS-specific outcomes.

Methods

A systematic search was conducted within three databases. From the 1.071 studies screened, two randomized control trials (RCTs), two control trials (CTs) and two cohort studies (CS) met the inclusion criteria. Methodological quality rating adopted the PEDro- (RCT, CT) or Newcastle-Ottawa-Scale (CS).

Results and conclusions

Methodological quality of the included studies was moderate (PEDro score: 6.5 ± 1.7; range 4 to 9). Two studies reported higher increases of the maximal quadriceps torque from baseline to pre-reconstruction: one study in the limb symmetry index (LSI), and one in both legs of the prehabilitation group compared to the controls. At 12-weeks post-reconstruction, one study (from two) indicated that the prehabilitation group had a lesser post-operative decline in the single-leg-hop for distance LSI (clinically meaningful). Similar findings were found in terms of quadriceps strength LSI (one study). At both pre-reconstruction (three studies) and two-year post-surgery (two studies), the prehabilitation groups reached significantly higher self-reported knee function (clinically meaningful) than the controls. RTS tended to be faster (one study). At two years post-surgery, RTS rates (one study) were higher in the

Editor: Dragan Mirkov, University of Belgrade, SERBIA

Competing interests: All authors declare no support from any organisation for the submitted work, no financial rela-tionships with any organisations that might have an interest in the submitted work in the pre-vious three years and no other relationships or activities that could appear to

have influenced the submitted work. We declare that we have no competing interests.

prehabilitation groups. The results provide evidence for the relevance of prehabilitation prior to ACL-reconstruction to improve neuromuscular and self-reported knee function as well as RTS. More high quality confirmatory RCTs are warranted.

Registration number

PROSPERO 2017: CRD42017065491.

Introduction

Anterior cruciate ligament (ACL) reconstruction is the usual treatment for athletes after ACL tears, followed by evidence-based physical rehabilitation therapy to restore function [1, 2]. The final goal of the rehabilitation process after ACL reconstruction (ACLR), is to return to sport (RTS) to pre-injury level as quickly as possible without exposing the athlete at undue risk for re-injury [3, 4].

The RTS-decision should be based on the systematic and stepwise assessment of potential risk factors [5, 6]. Particularly, the use of clinical tests to assess an athlete's neuromuscular function of the affected limb compared to the non-affected limb expressed by limb symmetry indices (LSI) appear to be a crucial criterion for RTS decision [7–9]. More symmetrical limb LSI are demonstrated to reduce the risk of reinjury [3, 10, 11]. Compared to healthy controls, individuals and athletes who returned to sport after primary ACLR show a up to six times higher incidence rate for re-injury within two years after surgery [12]. Furthermore, athletes who successfully returned to sports nevertheless often display a shorter career duration and an impaired game performance compared to controls after ACL reconstruction and RTS [13]. These issues highlight the importance of strategies for the improvement of the RTS-process.

One of these strategies is pre-operative rehabilitation (prehabilitation). Numerous studies indicate the relevance of preoperative neuromuscular performance factors, such as knee extension and flexion strength as well as single-leg-hop performance in ACL-injured individuals for postoperative knee function [14–19]. Accordingly, evidence-based guidelines for rehabilitation after ACLR also recommend pre-operative rehabilitation (prehabilitation) programmes with the aim to increase pre- and postoperative function [1].

Low-level evidence supports the relevance of prehabilitation to improve return to sports (RTS)-rates and two years self-reported knee function [20]. Prehabilitation appears to be effective for improving postoperative LSI of neuromuscular performance [16, 21]. Recently, a systematic Review conducted by Alshewaier et al. [22] indicates the positive value of preoperative training and post injury rehabilitation particularly in terms of increased knee-related function and improved muscle strength. As the authors of the review mainly included studies using a non-operative approach (no ACLR) and/or non-controlled trials (no usual care), the systematic assessment of potential benefits of prehabilitation on objective and self-reported outcomes before and after ACLR compared to usual care and its effects on RTS, is still lacking. Therefore, the aim of this systematic review was to examine the evidence for the effects of prehabilitation prior to ACLR and postoperative rehabilitation on pre- and postoperative RTS-specific neuromuscular outcomes, long-term self-reported knee functions and RTS-rates compared to ACLR and postoperative rehabilitation without prehabilitation.

Materials and methods

The protocol for this systematic review was registered in the PROSPERO international prospective register of systematic reviews https://www.crd.york.ac.uk/PROSPERO/ (registration number: blinded for review). As studies on prehabilitation before ACLR often include participants below 18 years of age, the inclusion criteria on age was changed accordingly after the PROSPERO registration. This review is reported in accordance to the PRISMA statement.

Study inclusion and exclusion criteria

We included randomized controlled trials, controlled trials (i.e. no randomized allocation into control and intervention groups) and prospective cohort studies published in English and German. We considered only studies including participants with primarily unilateral ACL rupture scheduled for reconstruction regardless of the surgical technique (i.e. single or double bundle technique), graft type (i.e. patellar or semitendinosus tendon) and concomitant injuries. Included studies had to assess, at least, one objective or subjective functional outcome measure and, at least, two of the following measuring points: Baseline (pre-prehabilitation), post-intervention (post-prehabilitation/ pre-surgery), post-surgery/after rehabilitation or follow-up.

Non-controlled studies (i.e. no usual care group) case reports, protocols, oral presentations and studies not based on original data were not included. Furthermore, trials including patients who were not scheduled for reconstructive surgery (non-operative rehabilitation), undergoing other orthopaedic operations than the reconstruction of the isolated, unilateral injured ACL, and scheduled for secondary ACLR were not included.

Intervention groups consisted of patients who received a preoperative exercise programme prior to ACLR and standard care following surgery (i.e. rehabilitation). Patients scheduled for ACLR who received standard treatment (usual care, i.e. no prehabilitation) before and standard care following reconstruction (i.e. rehabilitation) were considered controls.

Database research

The databases PubMed/MEDLINE, Web of Knowledge and the Cochrane Library were searched using the following search terms:

PubMed/Medline: *(("acl"[All Fields]) OR "anterior cruciate ligament"[All Fields]) AND (Prehabilitation [All Fields] OR prehab [All Fields] OR pre-rehabilitation [All Fields] OR preoperative [All Fields] OR pre-operative [All Fields]) AND (exercise OR physiotherapy OR training OR intervention OR rehabilitation)*

Web of Knowledge and the Cochrane Library: *TOPIC: ((("acl" OR "anterior cruciate ligament") AND (Prehabilitation OR prehab OR pre-rehabilitation OR preoperative OR pre-operative)) AND (exercise OR physiotherapy OR training OR intervention OR rehabilitation))*

The initial search was performed for studies published until October 31th, 2017; an update search was performed on June 12th, 2019.

Reference lists of the studies of interest were screened to identify extra articles. Two authors (FG, DN) independently selected trials for inclusion based on titles, keywords, and abstracts to determine eligibility. Any disagreements in terms of the study selection were discussed. If a conclusion could not be reached after discussion, a third reviewer (LV) was asked to resolve any conflicts. Full-texts of all trials considered eligible were retrieved. Then, one author (FG) performed first data extraction. Another author (DN) reviewed all data blindly. Again, any disagreements were discussed bilaterally. If a conclusion could not be reached, the third reviewer delivered the decisive vote. The following data from included papers were extracted: sample size, participant characteristics (diagnosis, number, age, sex and time since injury, if indicated), study methods (statistics), study specifics (setting, intervention, time points of assessment, and length of follow-up). Outcomes of interest were quadriceps strength and single-leg hop test (limb symmetry indices) as well as self-reported knee function. If not retrievable from the original publications, the authors were asked to send us the required data by e-mail.

Study quality and risk of bias assessment

The methodological quality of (randomized) controlled trials was assessed using the PEDro scale (11 criteria). The PEDro scale is a valid and reliable tool to assess the methodological quality of clinical studies [23, 24]. The methodological quality of cohort studies was assessed by using the Newcastle Ottawa scale (NOS, 9 criteria) [25]. Two authors (FG, DN) independently assessed the risk of bias of the included (randomized) controlled trials using the Risk of bias tool described in the Cochrane Handbook version 5.1.0. We rated the risk of bias of the individual studies on an outcome-based level (self-reported and objective outcomes separately) according to the handbook. Outcomes were graded for risk of bias in each of the following domains: sequence generation, allocation concealment, blinding (participants, personnel, and outcome assessment), incomplete outcome data, selective outcome reporting, and other sources of bias. Risk of bias of the included cohort studies was rated using an assessment tool contributed by the CLARITY Group at McMaster University. Any disagreements were discussed. If a conclusion could not be reached after discussion, a third reviewer (LV) made the final decision.

Results

Search and synthesis of included studies

Fig 1 displays the research history and flow of the studies. From PubMed/ MEDLINE, we retrieved n = 674, from Web of Knowledge n = 305 and from The Cochrane Library n = 92 potentially eligible studies. Seven studies were excluded because they were 1) no controlled trials as both groups participated in a prehabilitation programme, or 2) used a non-operative rehabilitation approach (no ACLR) [16, 26–31]. One study was excluded, because it was a systematic review [22]. From the six trials included in qualitative analysis are two cohort studies [20, 32]; two controlled trials [33, 34] and two randomized controlled trials [35, 36].

Methodological quality

According to the PEDro-Rating (11 points maximum), the controlled and randomized controlled trials were rated from four (one study [34]) over seven (two studies [33, 36]) to eight (one study [35]). The mean score of the studies was 6.5, which indicates an overall moderate methodological quality. The two cohort studies [20, 32] assessed with the NOS-scale displayed a fair to good quality (five [20] to seven [32] out of nine points).

Risk of bias. Risk of bias assessed for the controlled and randomized controlled trials was rated low for one study's [35] (five out of seven), moderate for two studies' [33, 36] (four out of seven points) and high for one studies' outcomes [34] (two out of seven points) (Table 1). Risk of bias were mainly not rated different between the self-reported and objective studies' outcomes (Table 1). In terms of the included cohort studies [20, 32], risk of bias was rated rather low for most items (Table 2).

Participants characteristics. A total of 5.131 participants (thereof n = 4.961 from cohort studies) were included. Two studies recruited men only [35, 36]. The remaining four studies

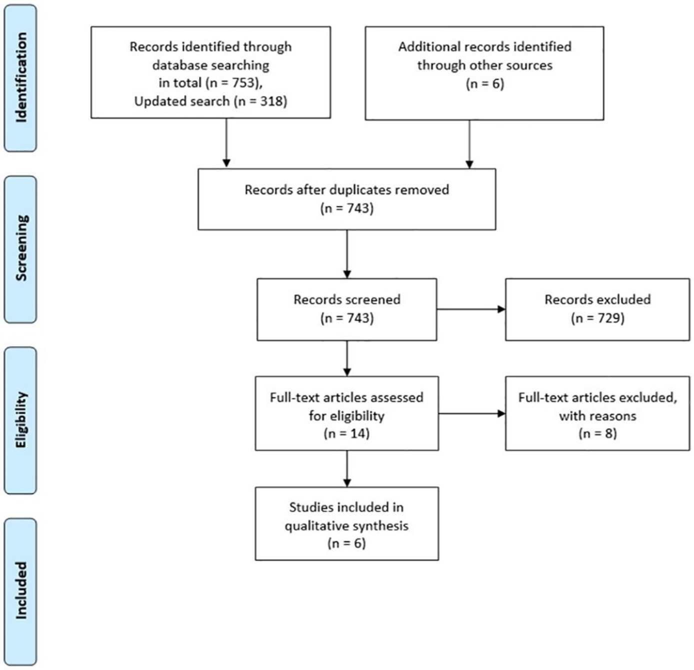

Fig 1. PRISMA flow diagram.

recruited both men and women. The mean age of the participants in the included trials ranged from 24.5 to 41 years (Table 1).

Participants'inclusion and exclusion criteria. Five studies [20, 26, 32, 42, 51] reported the inclusion of participants with primarily unilateral ACL rupture and one study included only individuals with chronic unilateral ACL ruptures [33] awaiting reconstruction. The clinical assessment of knee instability (i.e. pivot shift test, anterior drawer or Lachman test) was performed in two studies [33, 35], both with positive results. The remaining studies have not assessed knee stability. In one study recreational sports persons were included [35]. Two studies included only individuals who participated, at minimum, twice a week in jumping, cutting and pivoting sports such as football, basketball, American football, skiing or tennis, respectively performed these activities more than 50 hours per year before injury [20].

Table 1. Risk of bias assessment of CT and RCT.

	Shaarani et al. [35]		Keays et al. [33]		Kim et al. [36]		Zdunski et al. [34]	
	self-reported	**objective**	**self-reported**	**objective**	**self-reported**	**objective**	**self-reported**	**objective**
Sequence generation	Low risk	Low risk	High risk	High risk	Low risk	Low risk	High risk	-
Allocation sequence concealment	Low risk	Low risk	High risk	High risk	unclear	unclear	High risk	-
Blinding of participants and personnel	unclear	unclear	unclear	unclear	unclear	unclear	unclear	-
Blinding of outcome assessment	Low risk	High risk	Low risk	Low risk	unclear	unclear	High risk	-
Incomplete outcome data	High risk	High risk	Low risk	Low risk	Low risk	Low risk	unclear	-
Selective outcome reporting	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	-
Other potential threats to validity	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	-
Score	**5**	**4**	**4**	**4**	**4**	**4**	**2**	-

Table 2. Risk of bias assessment of cohort studies.

	Grindem et al. [32]		**Failla et al. [20]**	
	self-reported	**objective**	**self-reported**	**objective**
Was selection of exposed and non-exposed cohorts drawn from the same population?	**Definitely yes (low risk of bias)**	-	**Definitely yes (low risk of bias)**	-
	Probably yes		Probably yes	
	Probably no		Probably no	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	
Can we be confident in the assessment of exposure?	**Definitely yes (low risk of bias)**	-	**Definitely yes (low risk of bias)**	-
	Probably yes		Probably yes	
	Probably no		Probably no	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	
Can we be confident that the outcome of interest was not present at start of study?	Definitely yes (low risk of bias)	-	Definitely yes (low risk of bias)	-
	Probably yes		Probably yes	
	Probably no		**Probably no**	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	
Did the study match exposed and unexposed for all variables that are associated with the outcome of interest or did the statistical analysis adjust for these prognostic variables?	Definitely yes (low risk of bias)	-	Definitely yes (low risk of bias)	-
	Probably yes		Probably yes	
	Probably no		**Probably no**	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	
Can we be confident in the assessment of the presence or absence of prognostic factors?	Definitely yes (low risk of bias)	-	Definitely yes (low risk of bias)	-
	Probably yes		**Probably yes**	
	Probably no		Probably no	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	
Can we be confident in the assessment of outcome?	Definitely yes (low risk of bias)	-	Definitely yes (low risk of bias)	-
	Probably yes		Probably yes	
	Probably no		**Probably no**	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	
Was the follow up of cohorts adequate?	**Definitely yes (low risk of bias)**	-	Definitely yes (low risk of bias)	-
	Probably yes		Probably yes	
	Probably no		**Probably no**	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	
Were co-interventions similar between groups?	Definitely yes (low risk of bias)		Definitely yes (low risk of bias)	
	Probably yes		**Probably yes**	
	Probably no		Probably no	
	Definitely no (high risk of bias)		Definitely no (high risk of bias)	

Exclusion criteria. Participants with any injury induced associated fractures, injuries to other ligaments in the same knee, collateral ligament injuries requiring repair/reconstruction and contralateral injuries [20, 32, 35, 36], full-thickness articular cartilage lesions [20, 32, 34], symptomatic meniscal injuries [20, 32], or previous injury or surgery of the involved or uninvolved knee [20] were excluded. Two studies specifically reported that only participants with isolated ACL rupture were included [35, 36]. One study has not reported any exclusion criterion [33].

ACL grafts. Bone-patellar tendon-bone (BPTB) grafts, Hamstring autografts and soft tissue allografts [20, 32] or BPTB grafts only [35] were used for reconstruction. The remaining three studies did not report the graft types used for reconstruction surgery [33, 34, 36].

Time before surgery/enrollment. In one study, the time from injury to surgery was 6.3 ± 4.1 months in the prehabilitation, and 6.8 ± 4.2 months in the rehabilitation cohort (control group) [32]. In another study, the mean time from ACL injury to reconstruction was approximately 5 months in the prehabilitation and 9 months in the control group [34]. One study stated that the time from injury to enrollment, which was 1.9 ± 1 months in the prehabilitation, and lower than 6 months in the rehabilitation cohort (control group) [20]. In another study, the average time from injury to baseline assessment was 6.7 months, ranging from 5 to 15 months [35], followed by a waiting time for surgery after baseline assessment of approximately 6 weeks. The remaining two studies have not reported any timeframes between injury and baseline assessment before reconstructive surgery [33, 36]. Further details on the study characteristics are displayed in Table 3.

Intervention

Intervention vs. usual care (no prehabilitation). All six studies compared preoperative exercise interventions with no treatment or usual care in participants scheduled for unilateral ACLR [20, 32, 33, 35, 36, 51]. In one study, usual care consisted of the maintenance of a normal daily physical activity level without any specific preoperative physical interventions [35]. In another trial [34], the participants of the control group received general recommendations and instructions on particular exercises for people with ACL tears. The remaining studies did not specifically define usual care [20, 32, 33, 36].

Postoperatively, all participants received a standardised, criterion-based rehabilitation programme aiming to increase knee RoM and weight bearing to restore gait pattern and limb symmetry of neuromuscular performance factors. In two studies, only preoperative measuring points were considered [33, 34].

Preoperative training interventions. Both prospective cohort studies [20, 32] did not describe any details about the training protocols used. Therefore, the authors referred to a study of Eitzen et al. [31].

Type/content. The preoperative interventions included strengthening exercises mainly of the lower limb in open and closed chain, [12, 16, 20, 23, 24, 32, 41, 50] neuromuscular (perturbation, balance, stability, proprioceptive exercises) training, [20, 32–36] muscle control and co-contraction exercises of the knee muscles with particular attention of the quadriceps [33, 34, 36], as well as stretching [33, 34] and RoM exercises [36] of the lower limb. One study included mobilization of the patella and used kinesiology taping of the patellofemoral joint [34]. Two studies included plyometric exercises, such as single leg hops with soft landing [20, 32].

Intensity. The same two studies used progressively increased strength training, with maximal effort for 3 or 4 sets of 6 to 8 repetitions. Progress was made, as the patients were told to perform as many repetitions as they could manage in the last three or four sets. If they were

Table 3. Characteristics for which data were extracted for each study included into qualitative and quantitative synthesis.

Study (year)	Participants (diagnosis, N analyzed, age (mean, SD), gender)	Measuring points /follow-up period	Treatment (n)	Control (n)	Outcomes	Statistics
Failla et al. (2016) [20]	Primarily unilateral ACL-R awaiting reconstruction; n = 2.187; 24,5 ± 9,5 years; 54,5% male	• Baseline before (MOON) and after impairment resolution (DOC; before training) • 2 years post reconstruction	*DOC-patients*: Prehabilitation, Rehabilitation (n = 192)	*MOON-Cohort*: Usual care, Rehabilitation (n = 1.995)	*Self-reported knee function* (IKDC, KOOS–subscales: Pain, symptoms, ADLs, sports/ recreation, QoL), RTS rates	*ANCOVA*: 2-year IKDC/ KOOS scores between groups (covariate: baseline IKDC/ KOOS scores) *ANOVA*: interaction of group and meniscal surgery/ graft types on 2-year IKDC scores
Grindem et al. (2015) [32]	Primarily unilateral ACL-R awaiting reconstruction; n = 2.774; 25,1 ± 7,5 years; 48,5% male	• no baseline • pre reconstruction • 2 years post reconstruction	*NAR-patients*: Prehabilitation, Rehabilitation (n = 84)	*NKLR-Cohort*: Usual care, Rehabilitation (n = 2.690)	*Self-reported knee function* (KOOS–subscales: Pain, symptoms, ADLs, sports/ recreation, QoL)	ANCOVA: • Comparison of KOOS in the two cohorts preoperatively and 2 years postoperatively (covariates: sex, age, time from injury to surgery, presence of cartilage and meniscus injury, Baseline KOOS) • Stratification of preoperative KOOS subscale scores (Low/high scores were defined as scores below/above the median preoperative scores) • Calculation of sex-specific KOOS cutoff points for each subscale to quantify the percentage of patients with KOOS within the normative range (18–34 yrs. age group)
Do Kyung Kim et al. (2015) [36]	Isolated ACL Rupture awaiting reconstruction; n = 80; 27.8 ± 5.7 years, 100% male	• Baseline • 3 month post reconstruction	Prehabilitation, Rehabilitation (n = 40)	Usual care, Rehabilitation (n = 40)	• *Isokinetic knee strength (*range of 0 to 90° at an angular speed of 60°/ sec, with four repetitions) • *sokinetic knee strength endurance* (angular speed of 180°/sec, with 20 repetitions) → therefrom derived *strength deficit* for each angular speed • SI of single-leg hop distance	Independent sample T-Tests: • The highest peak torque value for each velocity was compared with the uninjured side (percent of strength deficit). • For the single-leg hop test, the mean average distance was quantified by limb symmetry index (LSI) • Repeated measures analysis to investigate the change in knee extensor strength and single-leg hop distance between groups
Keays et al. (2006) [33]	Chronic, unilateral ACL rupture awaiting reconstruction; n = 36; 29 ± 8 years; 69,4% male	• Baseline • Post training (pre reconstruction)	*ACLD-group 1*: Prehabilitation (n = 12)	*ACLD-group 2*: Usual care (n = 12) *Healthy controls*: nothing (n = 12)	• *Knee joint stability* (KT1000 arthrometer which measured anterior translation on application of 67 Newton (N) and 89 N forces, and a manual maximum displacement test (MMDT); clinical stability assessed via anterior drawer, Lachman and pivot shift tests) • *Quadriceps/ hamstring strength* at 60° and 120°/s via dynamometer • single leg standing balance • *Objective Functional performance* (agility: shuttle run, side-step and carioca tests) • *Self-reported function* (via modified Noyes and Trust questionnaire)	ANOVA: • 2 x 3, side-to-side differences for strength, knee stability and balance were calculated (injured vs. uninjured side) • Raw values were compared for the subjective questionnaires and the three agility tests. • (Group matching for age, gender and activity level)

(*Continued*)

Table 3. (Continued)

Study (year)	Participants (diagnosis, N analyzed, age (mean, SD), gender)	Measuring points /follow-up period	Treatment (n)	Control (n)	Outcomes	Statistics
Shaarani et al. (2013) [35]	Isolated ACL tear awaiting reconstruction; n = 23; 18–45 years (inclusion criteria); 100% male	• Baseline • Post-training (pre reconstruction) • 3 month post reconstruction	Prehabilitation, Rehabilitation (n = 14)	Usual care, Rehabilitation (n = 9)	• *Quadriceps and hamstrings strength* (average peak torque, average work per repetition, and deficits) assessed by use of isokinetic dynamometry (90°/s.) • *Jump performance* (single legged hop test) • *Pain and function* assessed via the Modified Cincinnati Knee Rating System score and Tegner activity level • *RTS duration* (defined as return to preinjury levels of sport activities) • *Quadriceps CSA* (via fMRI) • CSA, MHC, mRNA, IGF-1, MuRF-1, MAFbx	ANOVA: • One-way ANOVA was used to evaluate potential group differences in the baseline characteristics • Mixed-design for repeated measures was used to analyze potential differences between groups over time
Zduński et al. 2015 [34]	Isolated ACL Rupture awaiting reconstruction; n = 30; 40 ± 8 years, 56.7% male	• Baseline • Post training (pre reconstruction)	Prehabilitation, Rehabilitation (n = 15)	Usual care, Rehabilitation (n = 15)	Self-reported knee function assessed by the Lysholm-Gillquist scale	Students t-Test and Mann-Whitney U tests were applied to detect differences from baseline to pre-surgery assessment within and between groups

Moon = Multicentre Orthopaedic Outcomes Network; DOC = Delaware-Oslo ACL Cohort; NAR = Norwegian Research Centre for Active Rehabilitation; NKLR = Norwegian Knee Ligament Registry; IKDC = International Knee Documentation Committee; KOOS = Knee injury and Osteoarthritis Outcome Score; ADLs = Activities of daily living; ACL = Anterior cruciate ligament; ACLD = anterior cruciate ligament-deficient; QoL = Quality of life; LSI = Limb symmetry index; CSA = Cross-sectional area; MHC = Myosin heavy chain; mRNA = messenger RNA; IFG-1 = Insulin-like growth factor 1; MuRF-1 = Muscle RING-finger protein-1; MAFbx = Muscle atrophy f-box.

able to add two additional repetitions, weight was increased in the next session. Shaarani et al. [35] also used a progressive strengthening training approach including three sets of 12 repetitions with 10–15% weekly increase in the load. In the home-based exercise program of an ongoing study, strengthening and activation exercises of the lower limb muscles were conducted with 10 to 30 repetitions up to 30 to 50 repetitions, using materials such as elastic bands. Progression was made by increasing the number of sets. Stretching exercises of the lower limbs were performed about three sets with 30 seconds each [33], respectively RoM exercises such as knee extensions and flexions in sitting position for 10 minutes [36]. In one study, balance training, such as single leg standing on a balance board was performed about three sets with 30 secs each [36], 30 secs to 3 min with open eyes and 5–10 sec with closed eyes in another study. [33] Seperated in three consecutive phases, the 10 weeks perturbation protocol [31] performed by two studies [20, 32] contained a total of 10–12 sets of unilateral stance exercises on a rocker or roller board per session. To increase the level of difficulty, the participants executed additional movements of the arms or self-initiated perturbations [33].

Duration/frequency. The preoperative intervention lasted 4.8 weeks on average (range 4–6 weeks; [32, 34–36]. In one study, individuals participated in ten neuromuscular training sessions before ACL [20]. One study did not report information about the length of the intervention period [33]. The training frequency ranged from two to four times per week [20, 32, 34, 35] with a duration of a maximum of 75 to 120 minutes [20, 32, 34], including 10- to

20-minute warm-up exercises on a stationary ergometer cycle [20, 32, 36]. One study reported daily home-based training of 30 minutes' duration [33].

Supervision/setting. In three studies, the prehabilitation group was supervised twice a week with two additional home-based training sessions a week [20, 32, 35]. The participants were supervised, at least, three times a week in one study [36], but the authors of this study did not provide information whether additional home-based training sessions were required. The supervised training took place in a hospital [36] or gym [35]. A completely home-based training approach was used in one study [33].

Training compliance and dropouts. According to the study of Shaarani et al. [35], three participants in the prehabilitation group did not complete the program because of time constraints. All patients in the exercise group completed more than 90% of the exercise protocol. In another study, participants who participated in a home-based training programme, reported high levels of compliance with a minimal self-score rating of 8 out of 10 [33]. None of the remaining four studies have reported dropout rates, nor reported any information about training compliance.

Pre-prehabilitation / baseline to pre-surgery effects

Objective outcomes. Two studies investigated the effects of prehabilitation on the limb symmetry index of quadriceps strength from baseline to pre-reconstruction. Keays et al. [33] observed a significant increase of the limb symmetry index from 85% to 102% (60˚/s, $p < .05$, $d = 1.7$) and from 86% to 103% (120˚/s, $p < .05$, $d = 1.6$) in the prehabilitation group. No significant changes occurred in the usual care and the healthy control group ($p > .05$). Shaarani et al. [35] observed significant increases of the quadriceps peak torque (90˚/s) in both the injured and uninjured limbs compared to baseline in the prehabilitation group only ($p < 0.05$). Nevertheless, the peak torques did not differ systematically between groups neither at baseline nor at pre-surgery ($p > .05$) (Table 4). Contrary to Keays et al. [33], Shaarani et al. [35] found no significantly higher increases of the limb symmetry index in the prehabilitation (+3.2%) relative to the control group (+6.6%) (based on original data sent by the authors).

One study [35] measured the single-leg hop for distance performance: Compared to the control group, the authors found higher increases of the single-leg hop scores of the injured limb in the prehabilitation (13.5%) compared to the control group (9%), resulting in an overall preoperative score of 183.6 ± 16 in the prehabilitation and 156 ± 43 in the control group. These improvements were significant ($p < .05$) in the prehabilitation group only. However, both groups did not differ significantly neither at baseline nor at pre-surgery ($p > .05$).

One study [33] assessed knee joint stability, balance and agility. The authors found significantly higher improvements in each of these outcomes for the prehabilitation compared to the control groups (Table 4). In terms of hamstring peak torque assessed by two studies [33, 35], no significant advantage was found for the prehabilitation group at pre-surgery (Table 4).

Self-reported outcomes. Two studies [33, 34] examined the effects of prehabilitation on pre-operative self-reported knee function (Noyes, Trust [33] and Lysholm score [34]): Significant higher improvements were found for the prehabilitation compared to the control groups for both the Noyes ($d = 1.1$) and Trust ($d = 1.2$) scores (Table 4). In terms of the Lysholm score, Zdunski et al. [34] reported a mean pre- to post change from 46 to 66 points in the prehabilitation and from 59 to 64 points in the control group. Similar findings were gained by another study [35]: The authors observed a significant increase of the mean Cincinnati score (62.6 to 76.5 vs. 66 to 70 points) in the prehabilitation group only. At pre-surgery, both groups did not differ significantly.

Table 4. Descriptive results of the individual studies included into qualitative and quantitative synthesis.

Study (year)	Results
Failla et al. (2016) [20]	*Baseline*:
	•No differences between groups in age, sex, or body mass index
	•Significantly higher proportion of concomitant meniscal surgery performed (p = .029) in the MOON cohort
	•DOC patients had significantly higher baseline IKDC compared to MOON cohort (70 ± 13 vs. 50 ± 17; $p < .001$), which exceeded the MCID
	•The preoperative training group had significantly higher baseline KOOS values across all subscales than MOON cohort patients (Pain: 84 vs. 73, Symptoms: 75 vs. 67, ADL: 93 vs. 82, Sports/Recreation: 66 vs. 48, Quality of Life: 51 vs. 37).
	2-years IKDC scores:
	•After controlling for baseline IKDC scores, DOC patients continued to have significantly higher IKDC scores than MOON cohort (84 ± 25 vs. 71 ± 32; $p < .001$)
	•Post hoc power analysis revealed the ability to detect a difference of 2 points on the IKDC between groups
	•No significant group x meniscal procedure (p = .345) or group x graft type (p = .073) interactions on 2-year IKDC scores
	2-years KOOS-subscale scores:
	•After controlling for baseline KOOS values, DOC patients continued to have higher and clinically meaningful differences across all KOOS subscale scores compared with MOON cohort (Pain: 94 vs. 78, Symptoms: 89 vs. 72, ADL: 98 vs. 82, Sports/Recreation: 85 vs. 70, Quality of Life: 76 vs. 64).
	2-years RTS-rates:
	RTS rates were significantly higher in the DOC compared with MOON cohort ($p < .001$)
Grindem et al. (2015) [32]	*Preoperative KOOS-subscale scores*:
	•No significant differences between the two cohorts in age, sex, time to surgery, presence or severity of cartilage or meniscus injuries
	•NAR-patients had significantly better preoperative KOOS in all subscales (differences in all subscales except Symptoms were clinically relevant): Pain: 87 vs. 75.9, Symptoms: 82.6 vs. 73.6, ADL: 94.7 vs. 85.1, Sports/Recreation: 69.1 vs. 45.2, Quality of Life: 49.6 vs. 36).
	2 years KOOS-subscale scores:
	•NAR cohort still showed significantly better KOOS in all subscales, and clinically relevant differences were found in KOOS Symptoms, Sports and QoL (largest group differences again for KOOS—Sports; 17.7 points)
	•After controlling for the preoperative KOOS, the NAR cohort had significantly better KOOS scores (Pain: 93.5 vs. 86, Symptoms: 89.2 vs. 77.4, ADL: 98 vs. 92.5, Sports/Recreation: 85.1 vs. 67.6, Quality of Life: 78.6 vs. 67.7).
	•In patients who had preoperative scores below the median score, the NAR cohort showed 20.6 higher KOOS—Sports scores (p = .003), and 12.3 points higher KOOS—QoL scores (p = .006)
	•A higher percentage rate of patients in the NAR cohort scored within the normative range in the different KOOS subscales compared to NKLR-cohort
Do Kyung Kim et al. (2015) [36]	•Patients of IG showed a significantly lower post-operative loss of knee extensor strength deficits both at an angular velocity of 60˚/s (Prehab: 22.8±13.7 to 28.5 ±9.0, p = .018), and 180˚/s (16.6±10.6 to 23.3±9.0, p = .033) compared to CG (60˚/s: 23.5±15.8 to 36.5±10.7, $p > .05$; 180˚/s: 17.5±11.9 to 27.9±12.6, $p > .05$).
	•The IG also showed significant improvements in the single leg hop for distance test (higher limb symmetries; p = .029) in comparison to CG.
Keays et al. (2006) [33]	*Comparison between groups (Baseline)*:
	•No significant differences in any measure existed between the two injured groups (Exception: hamstring strength measured at 60˚/s).
	•Significant differences in all measures between each injured group and the control group (Exception: hamstring strength measured at 120˚/s, eyes-open and foam balance tests).
	Comparison between groups (post-training):
	•Significant differences existed between the treated (Group T) and untreated (Group NT) injured groups for quadriceps strength ($p < .001$), standing balance measure, for the three agility measures (p = .002; p = .003; p = .001) and for the Noyes and Trust questionnaires ($p < .001$ for both).
	•No differences existed between the treated and healthy, control group (Group C) for quadriceps and hamstring strength, balance measures or agility measures ($p > .05$). However, differences still existed for objective knee joint stability testing and for subjective testing.
	•Differences between the untreated group (Group NT) and control group (Group C) remained unchanged.
	Time effects (Baseline to post-training):
	•Significant improvements in quadriceps strength for Group T ($p < .01$) from strength indices (60˚/s: 0.85 to 1.02; 120˚/s: 0.86 to 1.03) compared to NT (60˚/s: 0.74 to 0.75; 120˚/s: 0.85 to 0.81) and C (60˚/s: 1.01 to 0.99; 120˚/s: 1.04 to 1.05).
	•No significant improvements of Group T, NT and C in terms of hamstring strength.
	•Significant decrease in side-to-side translation measured at the 89 N testing force in Group T ($p < .003$)
	•Balance improved significantly in Group T for eyes-closed ($p < .001$) as well as for eyes-open (p = .036)
	•Group T improved significantly in all agility measures ($p < .05$)
	•Group T only demonstrated significant improvements in scores for both the Noyes (57±14 to 70±6, $p < .05$, d = 1.1) and Trust (4.7±3.1 to 8.3±2.9, $p < .05$, d = 1.2) assessments ($p < .001$)
	•No significant changes in the other both groups (exception Group NT balance had worsened; p = .002)
	Group x Time interactions:
	•Were found for quadriceps strength (improved limb symmetries) at 60˚/s ($p < .001$) and 120˚/s ($p < .001$), for knee joint stability (p = .041), for standing balance with eyes-open (p = .002) and eyes closed (p = .006; F = 6.13) and foam balance (p = .042), for functional performance such as the shuttle-run (p = .001), the side-step (p = .021), the carioca test (p = .004) and subjective function such as the Noyes score ($p < .001$) and the Trust score ($p < .001$)

(*Continued*)

Table 4. (Continued)

Study (year)	Results
Shaarani et al. (2013) [35]	*Single leg hop performance (before surgery and at 12 weeks' post-reconstruction)*:
	•The single-legged hop test results improved significantly in the injured limb compared with baseline (p = .001). Mean single leg-hop test scores were higher preoperatively in the exercise group than the control group (p = .001).
	•At 12 weeks postoperatively, the rate of decline in the single-legged hop test was reduced in the exercise group compared with control (p = .001).
	Quadriceps peak torque (before surgery and at 12 weeks' post-reconstruction):
	•Quadriceps peak torque increased significantly with similar gains in CSA in both the injured (p = .001) and uninjured limbs (p = .009) after prehabilitation compared with baseline.
	•However, there was a significant decrease in quadriceps peak torque of the injured limb in the exercise group at 12 weeks postoperatively compared with baseline (p = .042) and preoperative time points (p < .001). No statistically significant differences between both groups for the injured limbs at any time point.
	Hamstrings peak torque (before surgery and at 12 weeks' post-reconstruction):
	•Compared with baseline, preoperative hamstring peak torque increased significantly in the injured limb in both the exercise (p = .034) and control group (p < .001). No significant differences were seen between the exercise and control groups at both pre- and postoperative time points.
	Cincinnati scores (before surgery and at 12 weeks' post-reconstruction):
	•The mean modified Cincinnati scores were increased significantly from baseline to pre-operative and to 12 weeks postoperative time points in the exercise group only (p = .004; p = .001). There was a significantly higher mean score (p = .004) in the exercise group compared with the control group only at 12 weeks postoperatively.
	RTS-duration:
	•The mean time to return to sport was shorter for the control and exercise group. The difference almost reached statistical significance (p = .055).
Zduński et al. 2015 [34]	The self-reported knee function (Lysholm score) improved in both groups. At pre-prehabilitation, patients from the prehabilitation group reported poor knee function. At the pre-surgery measurement time point, the mean score had increased significantly. The difference was statistically significant (p < .001). At pre-prehabilitation, the control group reported significant higher self-reported knee function than the prehabilitation group. At the second measurement, directly before the ACL-reconstruction, the mean score improved. However, a greater pre-post improvement of the injured knee joint was found in patients from the prehabilitation group.

Moon = Multicentre Orthopaedic Outcomes Network; DOC = Delaware-Oslo ACL Cohort; NAR = Norwegian Research Centre for Active Rehabilitation; NKLR = Norwegian Knee Ligament Registry; IKDC = International Knee Documentation Committee; KOOS = Knee injury and Osteoarthritis Outcome Score; Group T = Injured group receiving preoperative physiotherapy treatment; Group NT = Injured group receiving no preoperative physiotherapy treatment; Group C = Uninjured control group; IG = Intervention group; CG = Control group; MCID = Minimal clinically important differences; ADL = Activities of daily living; QoL = Quality of life; CSA = Cross-sectional area.

Pre-prehabilitation or baseline to post-rehabilitation (12-week after surgery)

Objective outcomes. Two studies [35, 36] examined the effects of prehabilitation on quadriceps strength at 12-weeks post-surgery. Kim et al. [36] observed a significant lower post-operative loss of the limb symmetry of the knee extensor strength relative to baseline in the prehabilitation compared to the control group at both an angular velocity of 60°/s (-5.7% vs. -13%) and 180°/s (-6.7% vs. -10.4%) (Table 4). This resulted in a more symmetric index at both 60°/s (prehab: 28.5 ± 9.0 vs. control: 36.5 ± 10.7, $p < .05$, $d = 0.8$) and 180°/s (23.3 ± 9.0 vs. 27.9±12.6, $p < .05$, $d = 0.4$) in the intervention group. Similarly, Shaarani et al. [35] found a trend for a lower reduction of the baseline limb symmetry index in the prehabilitation compared to the control group (-20.3% vs. -24.8%, $p > .05$; based on original data sent by the authors).

Two studies [35, 36] investigated the effects of prehabilitation on the single-leg jump performance from baseline to 12-week post-reconstruction. Kim et al. [36] reported a significant increase of the limb symmetry index for the prehabilitation (75.1 to 85.3%, $p < .05$, $d = 1.1$), but not for the control group (76.5 to 80.5%, $p > .05$, $d = 0.4$). Shaarani et al. [35], found a reduction of the single-leg jump test scores of the injured limb relative to pre-surgery in both groups ($p < .05$). Nevertheless, the prehabilitation group maintained a higher score compared to the control group (144.9 ± 15.5 vs. 113.3 ± 25.5, $p < .05$, $d = 1.5$). Relative to the controls, the prehabilitation group indicated a lower reduction of the baseline limb symmetry (-10.8% vs. -17.6%, $p > .05$; based on original data sent by the authors). However, these changes were not significant.

In terms of hamstring peak torque assessed by one study [35], no significant advantage was found for the prehabilitation group (Table 4).

Self-reported outcomes. One study [35] examined the effects of prehabilitation on 12-weeks postoperative self-reported knee function: the mean modified Cincinnati scores increased significantly from baseline to 12-weeks post-surgery (prehab: 62.6 to 85.3, $p < .05$; controls: 66 to 77.6, $p > .05$) resulting in a significant higher mean score for the prehabilitation compared to the control group (85.3 vs. 77.6, $p < .05$).

Two-year self-reported knee function and return to sport

Two cohort studies [20, 32] compared the level of self-reported knee function ((International Knee Documentation Committee, Knee injury and Osteoarthritis Outcome Score)). Both studies indicated a superior effect of prehabilitation, when compared to usual care. In both studies, the prehabilitation cohort exhibited significantly higher baseline/preoperative [20, 32] scores than the controls. Controlled for this confounder, the prehabilitation cohort continued to have significantly higher Knee injury and Osteoarthritis Outcome Score values in all subscales [20, 32] and International Knee Documentation Committee [20] scores (84 vs. 71) at 2-years post-surgery (Table 4).

The RTS duration and rates were assessed by one study in each case [20, 35]. There was a trend for significant faster RTS of the prehabilitation compared to the control group 34.18 ± 4.14 vs. 42.5 ± 10.46 weeks, $p = .055$) [35]. Nevertheless, no re-injuries occurred in the prehabilitation group during a follow-up period of 15 month after reconstruction [35]. According to another study a significantly higher share of participants from the prehabilitation compared to the control group returned to their preinjury sport at the two years' follow-up [20] (72% vs. 63%; $p < .05$).

Discussion

Low to moderate quality evidence indicates that exercises have a positive impact on pre-operative and postoperative functional performance. Low-level quality evidence supports the superiority of prehabilitation in terms of self-reported knee function at both pre-reconstruction and three months as well as two years after ACLR. The results further provide indications for higher RTS rates and a trend for a shorter time until RTS through prehabilitation.

Quadriceps strength limb symmetry index

Superior intervention effects on quadriceps peak torque LSI were found preoperatively by one study [33], and at three months' follow-up by another study [36]. In contrast, Shaarani et al. [35] reported no significant effects.

The latter findings [35] may be attributed to the fact that the maximum quadriceps strength improved significantly for both the injured and uninjured limb after prehabilitation. These symmetric training-induced improvements may have led to the maintenance of the already existed baseline asymmetries between both limbs. Contrary, in the study of Keays et al. [33], prehabilitation may have resulted in a disproportionate higher preoperative improvement of the quadriceps strength of the injured relative to the unijured limb. Possibly, the prehabilitative intervention in the study of Keays et al. [33] was more specific to quadriceps strength of the injured limb (unilateral strength training) as the intervention by Shaarani et al. [35]. Furthermore, Keays et al. [33] encouraged their participants to perform daily home-based exercises while Shaarani et al. [35] used a gym- and home-based approach with four training sessions per week. However, the influences of the intervention period (6 weeks) and content (strength and balance training) on the treatment effects can be considered low, as both studies were comparable in this regard. An alternative explanation for the higher preoperative LSI observed by Keays et al. [33] may be that the quadriceps peak torque of uninjured limb decreased relative to the injured limb over the preoperative period as a potential result of a lower physical activity level after injury. This mutual approach of both limbs may have resulted in a higher LSI. According to Wellsandt et al. [37], this may consequently lead to an overestimation of the neuromuscular performance of the injured limb.

The conservation of the preoperative neuromuscular performance (lower loss) through prehabilitation beyond the postoperative rehabilitation period may explain both the continuously higher symmetry indices of the prehabilitation compared to the control group indicated by Kim et al. [36] and the still non-significant different limb symmetries between both groups indicated by Shaarani et al. [35] at 12-weeks postoperative.

Single-leg hop for distance limb symmetry index

Kim et al. [36] found an increased single-leg hop for distance LSI in the prehabilitation compared to the control group at three months after ACLR relative to baseline. According to Reid et al. [38] the improvements of the prehabilitation group exceeded the level for a minimal detectable change (MDC: 8.1%). In Shaarani et al. [35], the LSI was reduced in both groups after postoperative rehabilitation compared to baseline. However, there was a trend for a lesser decline for the prehabilitation compared to the control group. The lower decline in the prehabilitation relative to the control group did not completely meet the cut-off for a minimal detectable change [38]. Nevertheless, the prehabilitation group maintained the higher preoperative single-leg hop performance.

Single-legged hop tests are highly reliable in ACL-injured and -reconstructed participants [29, 38]. The single leg hop for distance is a valid and reliable performance-based outcome measure reflecting the combination of leg strength, neuromuscular control and self-confidence in the ACL reconstructed knee [38], as well as the ability to tolerate sports-specific loads [39]. Thus, it may be possible that prehabilitation fostered the restoration of mechanical stability, and patients' confidence, in their knee stability. Consequently, the potentially reduced fear of re-injury may have had positive implications on the postoperative single-leg hop for distance LSI shown by both studies (high effect sizes). Nevertheless, these findings underline, particularly, the specificity of the used prehabilitation contents to jump ability and neuromuscular control. Together with the quadriceps strength, the single leg hop for distance was recently shown to be of prognostic value of an ACL re-injury [10].

Self-reported knee function

Beneficial effects of prehabilitation on both preoperative [33, 34] and two year postoperative self-reported knee function [20, 32] were reported. In terms of the Lysholm score, the improvements in the prehabilitation group from baseline to pre-surgery exceeded the cut-off for the minimal clinical important difference (10 to 17 points [40]). Regarding the Cincinnati knee score, the increases of the prehabilitation group from baseline to pre-surgery and to three month after ACLR met or even exceeded the minimal clinical important difference of 14 points [41] in contrast to the control group. The higher International Knee Documentation Committee scores at both baseline (70 vs. 50 points) and two years post-surgery (84 vs. 71 points) of the prehabilitation compared to the control group exceeded the cut-off for minimal detectable change (8.8 to 15.6 [42]). Similar findings occurred for the Knee injury and

Osteoarthritis Outcome Score: At two years post-surgery, the prehabilitation continued to exhibit higher values as the control group, which exceeded the minimal detectable change in almost all subscales (pain: 6–6.1, symptoms: 5–8.5, activities of daily living: 7–8, sports/recreation: 5.8–12, quality of life: 7–7.2% [42]).

These positive implications may be a consequence of the prehabilitation-related improved pre- and postoperative neuromuscular performance as indicated above. The assumed association between objective and self-reported outcomes are supported by Logerstedt et al. [39] providing evidence for the predictive value of the 6 months' postoperative single-legged jump performance for the self-reported knee function one year after ACL surgery. Furthermore, evidence suggests the predictive value of preoperative neuromuscular performance for postoperative self-reported function and RTS [14–19, 43].

Return to sport time points and rates

The higher RTS rates or shorter time until RTS success found by two studies [20, 35] may be a consequence of the improved postoperative objective and self-reported knee function of the prehabilitation groups as indicated above. The observed RTS rates were, in both the intervention and control groups (in particular in the latter one) slightly below but comparable to such reported in other studies. According to a systematic review of Ardern et al. [44], 81% of the ACL-reconstructed individuals returned to some kind of sports, 65% returned to their preinjury level and 55% returned to competitive sport.

The prehabilitation programs and their practicability

The prehabilitation programmes of the included studies differed in terms of frequency, intensity, time, supervision and setting. Nevertheless, the preoperative training protocols varied less in terms of content: The studies included in the review primarily adopted stretching and balance exercises as well as strengthening and control and co-contraction training with particular focus on the quadriceps. The prehabilitation protocols also included hamstrings strengthening. The hamstring muscles represent a major synergist of the ACL as its contraction reduces anterior tibia translation [45]. In the included cohort studies [20, 32], preoperative treatment was subdivided into two phases. The goal of the initial phase (up to two months after injury) was to resolve inflammatory symptoms and to restore full knee ROM [31]. After impairment resolution (about two month after surgery in average) [16, 20], a 5-week progressive exercise program (second phase) was performed aiming to restore muscle strength and neuromuscular function using intensive muscle strength, plyometric, and advanced neuromuscular exercises. [31]

Based on two of the included studies training compliance appears to be high and dropout rates appear to be low [33, 35]. Therefore, prehabilitation seems to be safe and feasible in participants with ACL injury. This is supported by Eitzen et al. [31]. The authors found high compliance to and tolerance for early staged interventions after ACL injury.

Time between rupture and reconstruction

The average time from injury to surgery 6.5 months [32], 8.2 months [35] and 5 to 9 months [34]. In the remaining two studies, the preoperative timeframes were not reported, but Keays et al. [33] described the inclusion of individuals with chronic ACL deficiencies, which also implies rather longer periods between injury and surgery.

Due to the relatively long preoperative period, some participants may have been engaged in physical training and exercise before or beyond prehabilitation. Except both of the cohort studies in which exercises were already performed early after injury to restore basic knee function, no of the other studies reported if and which exercises/treatment preceded prehabilitation. Therefore, we cannot estimate the effects of these preoperative actions on the results of this review.

Methological quality and limitations of the included studies

A common limitation in exercise trials is the limited possibility to blind participants [46]. This limitation may particularly lead to a biased assessment of self-reported knee function. In order to reduce the risk of bias as much as possible, all investigators shall be blinded to intervention allocation. This was not reported in most of the included studies. The heterogeneity of the study population may further limit the possibility to derive specific practical relevance. Together, with the (partially) randomised study designs and the consideration of known and suggested confounders, the included studies may provide a sufficient statistical power.

However, the included studies have certain limitations:

- Time between diagnosis of ACL tear and surgical reconstruction was not consistently reported across the included studies.
- No consistent definition of standard care treatment (control condition) was provided. Physical activities and therapies potentially performed early after ACL injury have not been reported neither for interventions nor for control groups.
- The heterogeneity across studies in terms of the characteristics of participants was high. For instance, the age of the participants included in one study was quite high (about 40 years) relative to the age groups (18 to 25 years), which are mainly affected by ACL-injuries [47]. The kind of sport and physical activity levels/demands were not described in most studies and one study included only those with chronic ACL deficiency [33].
- Methodological quality of the included studies was moderate and risk of bias ranged from low to high.

Limitations of this review

This systematic review has also certain limitations:

- We included only studies written in English and German. Therefore, relevant literature published in other languages may not have been included.
- The number of the studies included into this systematic review was quite low (n = 6).
- The evaluation of the effects of prehabilitation on long-term self-reported function is based on two prospective cohort studies implying low levels of evidence. Furthermore, both studies referred to the same prehabilitation cohort. The partial overlap of participants may have resulted in a substantial bias. Furthermore, the prehabilitation group exhibited higher self-reported knee function at baseline. Although considered as a covariate in statistical analyses, this may have limited the comparability between both groups.
- Due to the small number of studies available investigating the same outcome at comparable measuring points, the overlap in participants in two studies, the diverse and partly unknown content of the interventions, as well as the variations in study populations, we did not performed qualitative data syntheses (meta-analyses).
- We only screened the databases PubMed (Medline), Web of Knowledge and the Cochrane Library. Considering the topic of our review, almost all manuscripts of interest should be

found therein. However, expanding the search to even more databases, like EMBASE, PEDro, CINAHL, AMED, SportDiscus and CENTRAL may would have led to slightly more hits.

Clinical implications

Our findings apply to physically active adults with primary, unilateral ACL rupture without additional severe injuries to other intraarticular structures who had delayed ACLR. Besides improved preoperative functions, this review provides first evidence that prehabilitation may reduce the decline of postoperative neuromuscular performance of the lower limb and improve self-reported knee function and RTS success. Although more high quality confirmatory RCTs are needed to finally evaluate the clinical importance of prehabilitation, the outcomes found to be impacted by prehabilitation in this review are mostly the same as those found to be predictive for a second ACL injury [10]. Despite these positive implications, the majority of the orthopaedic surgeons seems to consider prehabilitation less important in the preoperative care of ACL-injured individuals [48].

Delayed compared to early ACLR is unlikely to result in postoperative differences in secondary knee pathologies (incidence of meniscal/ chondral lesions, postoperative infection, graft rupture) and functional outcomes [49] as well as two year self-reported knee function [27]. Against this background, it appears plausible that additional preoperative training prior to a delayed surgery may result in better postoperative function compared to early surgery.

Furthermore, the pre-ACLR contact time associated with a delayed surgery can help to identify ACL-deficient individuals without knee instability who may be able to RTS without surgery (copers [50]). This is in line with previous findings, which indicated that, more than half the ACLR can be avoided without aversively affecting outcomes using a delayed ACLR approach [27]. In line with current work applicable in non-professional or leisure time copers [49], delaying surgery for a minimum of 3 months after the ACL tear may be recommended [51].

Based on the studies included in this review, the preoperative or post-injury training protocols (4 to 6 weeks, 2 to 4 times per week) should contain muscle control and co-contraction exercises of the knee muscles with particular attention of the quadriceps as well as strengthening (open and closed chain) and stretching exercises of the lower limb. Moreover, advanced neuromuscular (perturbation, balance, stability, proprioceptive exercises) as well as plyometric exercises (e.g. single leg hops with soft landings) need to be considered. Such more intensive preoperative interventions should be not started before initial impairment resolution (about 2–3 months) in dependence on the functional and tissue repair status as well as concomitant injuries. Although, there are no specific evidence-based guidelines for strength and neuromuscular training in the early stage after ACL injury available yet, we recommend in exemplary the exercise protocols published by Eitzen et al. [31] and Wilk et al. [52] as a guidance.

Conclusion

Low to moderate quality evidence indicates that exercises have a positive impact on pre-operative and postoperative functional performance. Low-level quality evidence supports the superiority of prehabilitation in terms of self-reported knee function at both pre-reconstruction and three months as well as two years after ACLR. Due to the low number of studies included into this systematic review, divergent methodologic quality and high heterogeneity across the studies, high quality RCTs are warranted to finally evaluate the clinical importance of prehabilitation. Specifically, future trials need to investigate, if prehabilitation before delayed compared to early ACLR lead to a faster or improved restoration of postoperative RTS-specific neuromuscular or self-reported function and sport participation. Furthermore, the potential effects of prehabilitation on re-injury incidences need to be assessed.

Supporting information

S1 Checklist.
(DOC)

Author Contributions

Conceptualization: Florian Giesche, Daniel Niederer, Lutz Vogt.

Investigation: Florian Giesche.

Methodology: Florian Giesche, Daniel Niederer, Lutz Vogt.

Writing – original draft: Florian Giesche.

Writing – review & editing: Daniel Niederer, Winfried Banzer, Lutz Vogt.

References

1. van Melick N, van Cingel REH, Brooijmans F, Neeter C, van Tienen T, Hullegie W, et al. Evidence-based clinical practice update: practice guidelines for anterior cruciate ligament rehabilitation based on a systematic review and multidisciplinary consensus. Br J Sports Med. 2016; 50:1506–15. https://doi.org/10.1136/bjsports-2015-095898 PMID: 27539507
2. Wright RW, Haas AK, Anderson J, Calabrese G, Cavanaugh J, Hewett TE, et al. Anterior Cruciate Ligament Reconstruction Rehabilitation: MOON Guidelines. Sports Health. 2015; 7:239–43. https://doi.org/10.1177/1941738113517855 PMID: 26131301
3. Kyritsis P, Bahr R, Landreau P, Miladi R, Witvrouw E. Likelihood of ACL graft rupture: not meeting six clinical discharge criteria before return to sport is associated with a four times greater risk of rupture. Br J Sports Med. 2016; 50:946–51. https://doi.org/10.1136/bjsports-2015-095908 PMID: 27215935
4. Herring SA, Kibler WB, Putukian M. The team physician and the return-to-play decision: a consensus statement-2012 update. Med Sci Sports Exerc. 2012; 44:2446–8. https://doi.org/10.1249/MSS.0b013e3182750534 PMID: 23160348
5. Creighton DW, Shrier I, Shultz R, Meeuwisse WH, Matheson GO. Return-to-play in sport: a decision-based model. Clin J Sport Med. 2010; 20:379–85. https://doi.org/10.1097/JSM.0b013e3181f3c0fe PMID: 20818198
6. Shrier I. Strategic Assessment of Risk and Risk Tolerance (StARRT) framework for return-to-play decision-making. Br J Sports Med. 2015; 49:1311–5. https://doi.org/10.1136/bjsports-2014-094569 PMID: 26036678
7. Barber-Westin SD, Noyes FR. Objective criteria for return to athletics after anterior cruciate ligament reconstruction and subsequent reinjury rates: a systematic review. Phys Sportsmed. 2011; 39:100–10. https://doi.org/10.3810/psm.2011.09.1926 PMID: 22030946
8. Barber-Westin SD, Noyes FR. Factors used to determine return to unrestricted sports activities after anterior cruciate ligament reconstruction. Arthroscopy. 2011; 27:1697–705. https://doi.org/10.1016/j.arthro.2011.09.009 PMID: 22137326
9. Hildebrandt C, Müller L, Zisch B, Huber R, Fink C, Raschner C. Functional assessments for decision-making regarding return to sports following ACL reconstruction. Part I: development of a new test battery. Knee Surg Sports Traumatol Arthrosc. 2015; 23:1273–81. https://doi.org/10.1007/s00167-015-3529-4 PMID: 25682164
10. Ashigbi EYK, Banzer W, Niederer D. Return to Sport Tests' Prognostic Value for Reinjury Risk after ACL Reconstruction: A Systematic Review. Med Sci Sports Exerc. 2019.
11. Paterno MV, Schmitt LC, Ford KR, Rauh MJ, Myer GD, Huang B, et al. Biomechanical measures during landing and postural stability predict second anterior cruciate ligament injury after anterior cruciate ligament reconstruction and return to sport. Am J Sports Med. 2010; 38:1968–78. https://doi.org/10.1177/0363546510376053 PMID: 20702858

12. Paterno MV, Rauh MJ, Schmitt LC, Ford KR, Hewett TE. Incidence of Second ACL Injuries 2 Years After Primary ACL Reconstruction and Return to Sport. Am J Sports Med. 2014; 42:1567–73. https://doi.org/10.1177/0363546514530088 PMID: 24753238

13. Niederer D, Engeroff T, Wilke J, Vogt L, Banzer W. Return to play, performance, and career duration after anterior cruciate ligament rupture: A case-control study in the five biggest football nations in Europe. Scand J Med Sci Sports. 2018; 28:2226–33. https://doi.org/10.1111/sms.13245 PMID: 29927499

14. Eitzen I, Holm I, Risberg MA. Preoperative quadriceps strength is a significant predictor of knee function two years after anterior cruciate ligament reconstruction. Br J Sports Med. 2009; 43:371–6. https://doi.org/10.1136/bjsm.2008.057059 PMID: 19224907

15. Hägglund M, Waldén M, Thomeé R. Should patients reach certain knee function benchmarks before anterior cruciate ligament reconstruction? Does intense 'prehabilitation' before anterior cruciate ligament reconstruction influence outcome and return to sports. Br J Sports Med. 2015; 49:1423–4. https://doi.org/10.1136/bjsports-2015-094791 PMID: 26048899

16. Logerstedt D, Lynch A, Axe MJ, Snyder-Mackler L. Symmetry restoration and functional recovery before and after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc. 2013; 21:859–68. https://doi.org/10.1007/s00167-012-1929-2 PMID: 22349604

17. Scherer JE, Moen MH, Weir A, Schmikli SL, Tamminga R, van der Hoeven H. Factors associated with a more rapid recovery after anterior cruciate ligament reconstruction using multivariate analysis. Knee. 2016; 23:121–6. https://doi.org/10.1016/j.knee.2015.06.009 PMID: 26260243

18. Lepley LK, Palmieri-Smith RM. Pre-operative quadriceps activation is related to post-operative activation, not strength, in patients post-ACL reconstruction. Knee Surg Sports Traumatol Arthrosc. 2016; 24:236–46. https://doi.org/10.1007/s00167-014-3371-0 PMID: 25315083

19. Silkman C, McKeon J. The effect of preoperative quadriceps strength on strength and function after anterior cruciate ligament reconstruction. J Sport Rehabil. 2012; 21:89–93. https://doi.org/10.1123/jsr.21.1.89 PMID: 21904003

20. Failla MJ, Logerstedt DS, Grindem H, Axe MJ, Risberg MA, Engebretsen L, et al. Does Extended Preoperative Rehabilitation Influence Outcomes 2 Years After ACL Reconstruction? A Comparative Effectiveness Study Between the MOON and Delaware-Oslo ACL Cohorts. Am J Sports Med. 2016; 44:2608–14. https://doi.org/10.1177/0363546516652594 PMID: 27416993

21. Hartigan E, Axe MJ, Snyder-Mackler L. Perturbation training prior to ACL reconstruction improves gait asymmetries in non-copers. J Orthop Res. 2009; 27:724–9. https://doi.org/10.1002/jor.20754 PMID: 19023893

22. Alshewaier S, Yeowell G, Fatoye F. The effectiveness of pre-operative exercise physiotherapy rehabilitation on the outcomes of treatment following anterior cruciate ligament injury: a systematic review. Clin Rehabil. 2017; 31:34–44. https://doi.org/10.1177/0269215516628617 PMID: 26879746

23. de Morton NA. The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. Aust J Physiother. 2009; 55:129–33. https://doi.org/10.1016/s0004-9514(09)70043-1 PMID: 19463084

24. Maher CG, Sherrington C, Herbert RD, Moseley AM, Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. Phys Ther. 2003; 83:713–21. PMID: 12882612

25. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

26. Beard DJ, Dodd CA, Trundle HR, Simpson AH. Proprioception enhancement for anterior cruciate ligament deficiency. A prospective randomised trial of two physiotherapy regimes. J Bone Joint Surg Br. 1994; 76:654–9. PMID: 8027158

27. Frobell RB, Roos EM, Roos HP, Ranstam J, Lohmander LS. A randomized trial of treatment for acute anterior cruciate ligament tears. N Engl J Med. 2010; 363:331–42. https://doi.org/10.1056/NEJMoa0907797 PMID: 20660401

28. Frobell RB, Roos HP, Roos EM, Roemer FW, Ranstam J, Lohmander LS. Treatment for acute anterior cruciate ligament tear: five year outcome of randomised trial. Br J Sports Med. 2015; 49:700. https://doi.org/10.1136/bjsports-2014-f232rep PMID: 25926596

29. Fitzgerald GK, Lephart SM, Hwang JH, Wainner RS. Hop tests as predictors of dynamic knee stability. J Orthop Sports Phys Ther. 2001; 31:588–97. https://doi.org/10.2519/jospt.2001.31.10.588 PMID: 11665746

30. Tagesson S, Oberg B, Good L, Kvist J. A comprehensive rehabilitation program with quadriceps strengthening in closed versus open kinetic chain exercise in patients with anterior cruciate ligament deficiency: a randomized clinical trial evaluating dynamic tibial translation and muscle function. Am J Sports Med. 2008; 36:298–307. https://doi.org/10.1177/0363546507307867 PMID: 17940146

31. Eitzen I, Moksnes H, Snyder-Mackler L, Risberg MA. A progressive 5-week exercise therapy program leads to significant improvement in knee function early after anterior cruciate ligament injury. J Orthop Sports Phys Ther. 2010; 40:705–21. https://doi.org/10.2519/jospt.2010.3345 PMID: 20710097

32. Grindem H, Granan LP, Risberg MA, Engebretsen L, Snyder-Mackler L, Eitzen I. How does a combined preoperative and postoperative rehabilitation programme influence the outcome of ACL reconstruction 2 years after surgery? A comparison between patients in the Delaware-Oslo ACL Cohort and the Norwegian National Knee Ligament Registry. Br J Sports Med. 2015; 49:385–9. https://doi.org/10.1136/bjsports-2014-093891 PMID: 25351782

33. Keays SL, Bullock-Saxton JE, Newcombe P, Bullock MI. The effectiveness of a pre-operative home-based physiotherapy programme for chronic anterior cruciate ligament deficiency. Physiother Res Int. 2006; 11:204–18. https://doi.org/10.1002/pri.341 PMID: 17236528

34. Zduński S, Rongies W, Ziółkowski M, Kozieł T, Słomka B, Kazimierski P, et al. Evaluation of the Effectiveness of Preoperative Physiotherapy Using the Lysholm-Gillquist Scale in Patients Qualified for Surgical Arthroscopic Anterior Cruciate Ligament Reconstruction—Pilot Study. Ortop Traumatol Rehabil. 2015; 17:249–58. https://doi.org/10.5604/15093492.1162424 PMID: 26248626

35. Shaarani SR, O'Hare C, Quinn A, Moyna N, Moran R, O'Byrne JM. Effect of prehabilitation on the outcome of anterior cruciate ligament reconstruction. Am J Sports Med. 2013; 41:2117–27. https://doi.org/10.1177/0363546513493594 PMID: 23845398

36. Kim DK, Hwang JH, Park WH. Effects of 4 weeks preoperative exercise on knee extensor strength after anterior cruciate ligament reconstruction. J Phys Ther Sci. 2015; 27:2693–6. https://doi.org/10.1589/jpts.27.2693 PMID: 26504270

37. Wellsandt E, Failla MJ, Snyder-Mackler L. Limb Symmetry Indexes Can Overestimate Knee Function After Anterior Cruciate Ligament Injury. J Orthop Sports Phys Ther. 2017; 47:334–8. https://doi.org/10.2519/jospt.2017.7285 PMID: 28355978

38. Reid A, Birmingham TB, Stratford PW, Alcock GK, Giffin JR. Hop testing provides a reliable and valid outcome measure during rehabilitation after anterior cruciate ligament reconstruction. Phys Ther. 2007; 87:337–49. https://doi.org/10.2522/ptj.20060143 PMID: 17311886

39. Logerstedt D, Grindem H, Lynch A, Eitzen I, Engebretsen L, Risberg MA, et al. Single-legged hop tests as predictors of self-reported knee function after anterior cruciate ligament reconstruction: the Delaware-Oslo ACL cohort study. Am J Sports Med. 2012; 40:2348–56. https://doi.org/10.1177/0363546512457551 PMID: 22926749

40. Chuang L-H, Garratt A, Brealey S. Comparative responsiveness and minimal change of the Knee Quality of Life 26-item (KQoL-26) questionnaire. Qual Life Res. 2013; 22:2461–75. https://doi.org/10.1007/s11136-012-0298-0 PMID: 23468169

41. Smith MV, Klein SE, Clohisy JC, Baca GR, Brophy RH, Wright RW. Lower extremity-specific measures of disability and outcomes in orthopaedic surgery. J Bone Joint Surg Am. 2012; 94:468–77. https://doi.org/10.2106/JBJS.J.01822 PMID: 22398742

42. Collins NJ, Misra D, Felson DT, Crossley KM, Roos EM. Measures of knee function: International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form, Knee Injury and Osteoarthritis Outcome Score (KOOS), Knee Injury and Osteoarthritis Outcome Score Physical Function Short Form (KOOS-PS), Knee Outcome Survey Activities of Daily Living Scale (KOS-ADL), Lysholm Knee Scoring Scale, Oxford Knee Score (OKS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Activity Rating Scale (ARS), and Tegner Activity Score (TAS). Arthritis Care Res (Hoboken). 2011; 63 Suppl 11:S208–28. https://doi.org/10.1002/acr.20632 PMID: 22588746

43. Hartigan EH, Zeni J, Di Stasi S, Axe MJ, Snyder-Mackler L. Preoperative predictors for noncopers to pass return to sports criteria after ACL reconstruction. J Appl Biomech. 2012; 28:366–73. https://doi.org/10.1123/jab.28.4.366 PMID: 22983930

44. Ardern CL, Webster KE, Taylor NF, Feller JA. Return to sport following anterior cruciate ligament reconstruction surgery: a systematic review and meta-analysis of the state of play. Br J Sports Med. 2011; 45:596–606. https://doi.org/10.1136/bjsm.2010.076364 PMID: 21398310

45. More RC, Karras BT, Neiman R, Fritschy D, Woo SL, Daniel DM. Hamstrings–an anterior cruciate ligament protagonist. An in vitro study. Am J Sports Med. 1993; 21:231–7. https://doi.org/10.1177/036354659302100212 PMID: 8465918

46. Moran J, Guinan E, McCormick P, Larkin J, Mockler D, Hussey J, et al. The ability of prehabilitation to influence postoperative outcome after intra-abdominal operation: A systematic review and meta-analysis. Surgery. 2016; 160:1189–201. https://doi.org/10.1016/j.surg.2016.05.014 PMID: 27397681

47. Sanders TL, Maradit Kremers H, Bryan AJ, Larson DR, Dahm DL, Levy BA, et al. Incidence of Anterior Cruciate Ligament Tears and Reconstruction: A 21-Year Population-Based Study. Am J Sports Med. 2016; 44:1502–7. https://doi.org/10.1177/0363546516629944 PMID: 26920430

48. Ebert JR, Webster KE, Edwards PK, Joss BK, D'Alessandro P, Janes G, et al. Current Perspectives of the Australian Knee Society on Rehabilitation and Return to Sport After Anterior Cruciate Ligament Reconstruction. J Sport Rehabil. 2019:1–6. https://doi.org/10.1123/jsr.2019-0291 PMID: 31775119

49. Matthewson G, Kooner S, Rabbani R, Gottschalk T, Old J, Abou-Setta AM, et al. Does a Delay in Anterior Cruciate Ligament Reconstruction Increase the Incidence of Secondary Pathology in the Knee? A Systematic Review and Meta-Analysis. Clin J Sport Med. 2019. https://doi.org/10.1097/JSM.0000000000000762 PMID: 31743219

50. Kaplan Y. Identifying individuals with an anterior cruciate ligament-deficient knee as copers and noncopers: a narrative literature review. J Orthop Sports Phys Ther. 2011; 41:758–66. https://doi.org/10.2519/jospt.2011.3384 PMID: 21979555

51. Salzler MJ, Akgün U, Karahan M, Harner CD. Early Versus Delayed ACL Reconstruction: Why Delayed Surgery Is Our Preferred Choice. In: Nakamura N, Zaffagnini S, Marx RG, Musahl V, editors. Controversies in the Technical Aspects of ACL Reconstruction. Berlin, Heidelberg: Springer Berlin Heidelberg; 2017. pp. 27–33. https://doi.org/10.1016/j.jvs.2017.02.025 PMID: 28502543

52. Wilk KE, Arrigo CA. Preoperative Phase in the Rehabilitation of the Patient Undergoing Anterior Cruciate Ligament Reconstruction. Operative Techniques in Sports Medicine. 2016; 24:12–20. https://doi.org/10.1053/j.otsm.2015.10.003

12

Are heart rate methods based on ergometer cycling and level treadmill walking interchangeable?

Karin Olsson, Jane Salier Eriksson, Hans Rosdahl, Peter Schantz*

Research Unit for Movement, Health and Environment, The Åstrand Laboratory and Laboratory of Applied Sport Science, The Swedish School of Sport and Health Sciences, GIH, Stockholm, Sweden

* peter.schantz@gih.se

Abstract

Introduction

The heart rate (HR) method is a promising approach for evaluating oxygen uptake ($\dot{V}O_2$), energy demands and exercise intensities in different forms of physical activities. It would be valuable if the HR method, established on ergometer cycling, is interchangeable with other regular activities, such as level walking. This study therefore aimed to examine the interchangeability of the HR method when estimating $\dot{V}O_2$ for ergometer cycling and level treadmill walking in submaximal conditions.

Methods

Two models of $\mathrm{HR}-\dot{V}O_2$ regression equations for cycle ergometer exercise (CEE) and treadmill exercise (TE) were established with 34 active commuters. Model 1 consisted of three submaximal intensities of ergometer cycling or level walking, model 2 included also one additional workload of maximal ergometer cycling or running. The regression equations were used for estimating $\dot{V}O_2$ with seven individual HR values based on 25–85% of HR reserve (HRR). The $\dot{V}O_2$ estimations were compared between CEE and TE, within and between each model.

Results

Only minor, and in most cases non-significant, average differences were observed when comparing the estimated $\dot{V}O_2$ levels between CEE and TE. Model 1 ranged from -0.4 to 4.8% (n.s.) between 25–85%HRR. In model 2, the differences between 25–65%HRR ranged from 1.3 to -2.7% (n.s.). At the two highest intensities, 75 and 85%HRR, $\dot{V}O_2$ was slightly lower (3.7%, 4.4%; $P < 0.05$), for CEE than TE. The inclusion of maximal exercise in the $\mathrm{HR}-\dot{V}O_2$ relationships reduced the individual $\dot{V}O_2$ variations between the two exercise modalities.

Editor: Juan M. Murias, University of Calgary, CANADA

Conclusion

The HR methods, based on submaximal ergometer cycling and level walking, are interchangeable for estimating mean $\dot{V}O_2$ levels between 25–85% of HRR. Essentially, the same applies when adding maximal exercise in the $HR-\dot{V}O_2$ relationships. The inter-individual $\dot{V}O_2$ variation between ergometer cycling and treadmill exercise is reduced when using the HR method based on both submaximal and maximal workloads.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Acquiring knowledge of human oxygen uptake ($\dot{V}O_2$), energy demands and intensity levels in physical activities is valuable for health education and promotion as well as from research perspectives. This is since much of the understanding of exercise effects on weight control, morbidity, premature mortality, and physical performance is based on descriptions of intensity levels relative to the maximal oxygen uptake (e.g. [1, 2]), energy expenditure (e.g. [3, 4]) and MET hours (e.g. [5]). In these respects, the heart rate (HR) method can be a promising approach. It is based on the linear relationship between HR and $\dot{V}O_2$ during incremental loads of physical work under steady state conditions [6, 7]. Thanks to this linearity, $\dot{V}O_2$ can, in principle, be estimated by only measuring HR, and based on caloric coefficients [8, p. 104] the energy turnover can be calculated.

The first studies of the $HR-\dot{V}O_2$ relationship in humans were published in the early 1900s [9, 10]. Since then, several studies have used HR monitoring during various physical activities to understand energy requirements and exercise intensity in both humans [7, 11–14] and animals [15]. An accurate way of establishing $HR-\dot{V}O_2$ relationships is to create one for each individual, since there are large inter-individual variations, particularly due to sex, age, body weight and fitness level [16–19]. Today, these relationships are generally established in laboratories with portable HR monitors and stationary automated metabolic systems.

Even though the HR method has been used in several applied studies, a number of methodological issues connected to it appear not to have been sorted out completely, or only to a limited degree. These matters can be grouped into (1) reproducibility of $HR-\dot{V}O_2$ relationships and their outcomes when establishing the HR method under steady state conditions, (2) optimal ways of establishing $HR-\dot{V}O_2$ relationships under steady state conditions, (3) external validity of $HR-\dot{V}O_2$ relationships and the HR method established with one exercise form in relation to another, and (4) external validity of them in relation to both prolonged exercise at various constant exercise intensities as well as under non-steady state conditions.

It is only in recent times that fundamental methodological studies in terms of reproducibility have been carried out [20–22]. In general, these examinations have demonstrated good reproducibility at group level with minor relative differences between test and retest at an intensity range from resting to vigorous intensity levels. Several optimizing issues related to reproducibility and validity do however remain to be further explored. It is, for example, not known how many measuring points are needed, or how spread out they should be to enhance the reproducibility, and thereby the potential validity of the HR method.

As stated above, the interchangeability of the HR method between various forms of exercise to be investigated. So far, it is well known that dynamic exercise, with large muscle groups involved, has a different $HR-\dot{V}O_2$ relationship than static and dynamic exercise with smaller working muscle groups. For instance, $\dot{V}O_2$ has been found to be significantly lower during arm cycling compared to leg cycling as well as leg combined with arm cycling, when compared at equivalent HR values [23–25]. If the comparisons instead are limited to dynamic lower body work, through cycle ergometer and treadmill exercises, differences in the HR$-\dot{V}O_2$ relationships might not be obvious. When specifically comparing ergometer cycling with the simplest form of treadmill exercise, i.e. level walking, there is, to our knowledge, only one previous study. Berggren and Hohwü Christensen [26] observed similar HR$-\dot{V}O_2$ relationships for ergometer cycling compared to horizontal treadmill walking and running. The examination was performed on repeated occasions over a broad measurement range, containing a high number of submaximal steady state workloads for each of the three modalities. However, one considerable limitation was that only one subject, a well-trained man, was investigated.

The HR method can be applied principally in two different ways; one in which the oxygen uptake is measured, the other in which the oxygen uptake is estimated based on knowledge about the oxygen uptake at various workloads. For the latter purpose it is preferable to make use of a cycle ergometer. This is because oxygen consumption during steady state workloads of ergometer cycling is fairly constant between various individuals for both sexes [6] and independent of cycling experience [27]. Results reported by Åstrand [28] and Ryhming [29] demonstrated that two-thirds of healthy and trained individuals (50 men and 62 women) had $\dot{V}O_2$ levels within a range of ± 6% around mean levels at different workloads. Part of this variation can be explained by varying fitness levels, due to various relative positions of physiological thresholds [30–32], and varying muscle fibre type compositions [33]. However, a number of studies have shown that the oxygen cost at submaximal steady state work on cycle ergometers is also dependent on body weight [18, 34–39, p. 87]. Due to the global developments in body weight during the past three decades, this is an important factor to take into consideration when applying this form of exercise. If taking these determining factors into account, and considering that metabolic systems are expensive and technically complicated to use, especially in field exercise conditions [40, 41], it is advantageous that individual HR$-\dot{V}O_2$ relationships can be estimated with various cycle ergometer workloads. This would allow the HR method to be used in health education and promotion, when high measurement accuracy is not a decisive factor. It is also valuable from a research perspective in public health when large samples are to be investigated, which could be difficult to accomplish practically if metabolic measurement devices have to be used.

Considering the advantages in using the HR method, established through incremental constant workloads of ergometer cycling, it would be valuable if its HR$-\dot{V}O_2$ relationship and $\dot{V}O_2$ outcome when using the HR method mimic those of other common forms of dynamic exercise, such as walking. We have therefore investigated this issue further, with focus on the HR method's interchangeability between submaximal ergometer cycling and level walking on a treadmill. At the same time, we have investigated if the HR method, based on ergometer cycling as well as treadmill exercise, can be methodologically optimized if endpoints of maximal exercise are added to the HR$-\dot{V}O_2$ relationships.

The aim of this study was therefore to examine the interchangeability of the HR method when estimating $\dot{V}O_2$ for ergometer cycling and level treadmill walking in submaximal conditions. In accordance with our previous reproducibility studies of the HR method [21, 22], two different models of HR$-\dot{V}O_2$ regression equations for cycle ergometer exercise (CEE) and treadmill exercise (TE), respectively, were constructed. Model 1 consisted of three submaximal steady state workloads of ergometer cycling or level walking, whereas model 2 also included

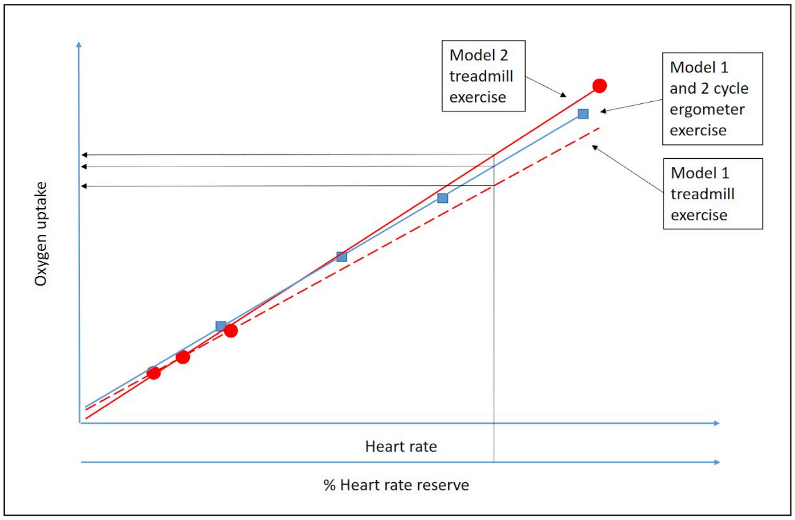

Fig 1. Principal scheme for the analytic models in the study. Two models are used for the HR method to estimate the oxygen uptake in cycle ergometer exercise versus treadmill exercise conditions, respectively. Model 1 is based on three submaximal workloads, whereas model 2 is based on the same three submaximal workloads as well as a workload corresponding to maximal exercise. The figure shows a conceivable example in which model 1 and model 2 in the treadmill exercise results in two different regression equations, whereas in the cycle ergometer exercise, model 1 and model 2 results in the same regression equation. The last step in the HR method is applied here in terms of oxygen uptake being estimated from various percentages of HR reserve and the different regression equations.

one maximal workload of ergometer cycling or running. The HR$-\dot{V}O_2$ regression equations were applied to estimate oxygen uptake, using seven individually derived heart rate values based on % of heart rate reserve (%HRR). The outcomes from CEE and TE were then compared within and between each model (Fig 1).

Methods

Participants

Approval to conduct the study was obtained from the Ethics Committee North of the Karolinska Institute at the Karolinska Hospital (Dnr 03–637), Stockholm, Sweden.

Recruitment of participants. The recruitment of participants started by invitation through advertisements in two major morning newspapers in Stockholm. The inclusion criteria for participation were: being at least 20 years old, living in the County of Stockholm and cycling or walking the whole way, any distance, between home and place of work or study and actively commuting that way at least once a year. A questionnaire (The Physically Active Commuting in Greater Stockholm Questionnaire 1; PACS Q1, supporting information S1 Methods and S2 Methods) was sent to 2148 volunteers [42]. The recruitment of the participants in this particular study was based on those who had their age and commuting distance close to the median values of the overall project's categories of female and male cyclists who only cycled to work (so-called single mode cyclists) and female and male pedestrians who only walked to work (so-called single mode pedestrians), respectively. They also rated their profession as very light or light physically. For details on the recruitment and categorization process as well as the questionnaire used, see Stigell and Schantz [42].

Information describing the physiological studies, tests and standardization procedures as well as a health declaration were sent to the selected volunteers. This information emphasized the right of the participants to cancel at any time during the study, without giving a reason. A signed informed consent of participation was returned. Based on the replies from the health declaration, individuals with high blood pressure or on medication that could affect the normal heart rate were excluded. The remaining were contacted until 10 individuals in each of the four categories (female and male single mode cyclists, and female and male single mode pedestrians) fulfilled the criteria. Due to the fact that there were no systematic differences between the cyclists and the pedestrians in this study's analyses, they were combined into one study group. Finally, with regard to some obstructive health conditions and technical problems with the equipment, there were 34 healthy and physically active commuters who had complete results; these are listed in Table 1.

Equipment

During all metabolic measurements, an automated stationary metabolic gas analysis system, Jaeger Oxycon Pro® (Carefusion GmbH, Hoechberg, Germany) was used in the mixing chamber mode. The software used was JLAB 4.53. The metabolic system was switched on 30 minutes before data collection and calibrated before and after each test using the automated procedures and according to the manufacturer's recommendations. A high precision gas of 15.00% O_2 and 6.00% CO_2 (accuracy: O_2 ± 0.04% and CO_2 ± 0.1%, Air Liquid AB, Kungsängen, Sweden) was used for calibration. A face mask with a non-rebreathing air inlet valve (Combitox, Dräger Safety, Lübeck, Germany) was used, while a tube (inner diameter of 35 mm) was attached to the non-rebreathing valve to lead the exhaled air into the mixing chamber. The measured metabolic variables, including HR, were saved in average values of 15 seconds. Using the mixing chamber mode, Oxycon Pro has been found to be reliable (coefficient of variation for $\dot{V}O_2$: 1.2% between 0.5–6.0 $L \cdot min^{-1}$) [43] and valid against the golden standard method [41, 43]. The HR measurements were performed using Polar Electro S610i Heart Rate Monitor and the associated Polar Wearlink 31 transmitter (Polar Electro Oy, Kempele, Finland).

The cycle ergometer exercise was performed on a manually braked Monark pendulum ergometer cycle 828E (Monark Exercise AB, Vansbro, Sweden). Immediately before each test, the scale was zeroed while the subjects sat on the saddle with their feet resting on the surface between the pedals. A digital metronome (DM70 Seiko S-Yard Co. Ltd, Tokyo, Japan) was used to keep correct cycling cadence. At regular intervals of one minute, the work rate was controlled by checking the cadence and the braking force as indicated on the pendulum scale. The treadmill exercise was conducted on RL2500E treadmill ergometer (Rodby Innovation AB, Vänge, Hagby, Sweden). Its accuracy for velocity and inclination were: ± 1.8% and 0.2°, respectively.

Measurements

Study design and standardization. The subjects visited the laboratory on three different occasions. All occasions followed the same procedure, including measurements of HR at rest, simultaneous measurements of HR and $\dot{V}O_2$ at submaximal and maximal level, with the only difference between the occasions being the type of physical work; twice with cycle ergometer exercise (CEE) and once with treadmill exercise (TE). The first occasion always consisted of

Table 1. Descriptive characteristics of the participants (means ± standard deviations (SD)).

		Age	Height	Weight	BMI	HR_{rest}
		(years)	(m)	(kg)	($kg \cdot m^{-2}$)	($beats \cdot min^{-1}$)
Women	**(n = 17)**	43 ± 4	1.70 ± 0.05	64 ± 9	22 ± 3	62.8 ± 7.9
Men	**(n = 17)**	45 ± 8	1.82 ± 0.06	82 ± 12	25 ± 3	66.7 ± 9.8
All	**(n = 34)**	44 ± 6	1.76 ± 0.08	73 ± 14	23 ± 3	64.7 ± 9.0

CEE for all subjects. For most of the subjects, CEE was also performed at session two and followed by TE at session three. The purpose of conducting repeated sessions with CEE was to familiarize the subjects with the experimental process during the first session (familiarization session). The number of days between the three occasions was 8 ± 9 days (means ± SD). Two educated investigators carried out the experimental tests, all participants had the same investigator for each occasion.

Prior to each test, the participants were instructed to follow some standardization guidelines: 1) not to take part in any vigorous exercise for 24 hours before the tests, 2) not to cycle to the laboratory, 3) to refrain from eating, drinking, smoking and taking snuff for at least one hour before arrival at the laboratory, 4) not to eat a large meal at least three hours before the tests, 5) to avoid stress and 6) to cancel the test if they had fever, an infection or a cold.

On arrival at the laboratory, measurements of body weight and height (only in the familiarization session) were conducted. Thereafter, a resting HR measurement was performed in supine position on a treatment table during ten minutes, and values from the last five minutes were used. Anthropometric and resting HR data were from the familiarization session, while HR and $\dot{V}O_2$ data during exercise were from sessions 2 and 3.

Cycle ergometer exercise. The submaximal ergometer cycling consisted of three different workloads: 50, 100 and 150 watt (W) for the female and 100, 150 and 200 W for the male participants. At each workload, the participants cycled until steady state occurred (approximately six minutes), after which the resistance was increased. The third workload was increased to only 125 W or 175 W for women and men respectively, if after the second workload, the subjects' HR was higher than 150 beats·min^{-1} and their rated perceived exertion (RPE), according to the Borg scale, exceeded 15 for both legs and breathing [44]. A cadence of 50 revolutions per minute (rpm) was used since it was when using this cadence that oxygen consumption has been found fairly constant between individuals [6, 28, p. 19, 29].

Between the second and third workload, the participants continued cycling for one minute at a self-selected low cadence with a resistance of 5 Newton (N). The subjects were then instructed to resume the cadence of 50 rpm while the investigator gradually increased the resistance until, after one minute, the third work rate was reached (resistance was increased to 50 W during the first 15 seconds, to 100 W the second 15 seconds and successively to the third work rate during the last 30 seconds). After the submaximal workloads, and before the maximal test, the participants continued cycling for two minutes at a self-chosen low cadence and a resistance of 5 N.

The maximal test was performed with a cycle cadence of 80 rpm. During the first three minutes, the work rates were set to 60, 100 and 120 or 140 W for one minute each. The latter alternatives depended on which third workload the subjects had achieved during the submaximal phase; 120 W was chosen if the third work rate had been 125 W or 175 W for women and men, respectively, and 140 W was chosen if it had been 150 W or 200 W for women and men, respectively. After these first three minutes, the resistance was increased by 20 W every continued minute until voluntary exhaustion occurred. The increments of work rate were chosen in accordance with our previous evaluation [21].

Treadmill exercise. The submaximal treadmill exercise consisted of three workloads of level (0.0°) walking. Velocities were set to: 4.0, 4.9 and 5.9 km·h^{-1}. The participants continued walking at each workload until steady state was attained (normally five minutes). Immediately after completing the third walking load, the speed was increased to a comfortable level for running, after which the maximal phase was started.

The maximal running test was performed through constant speed (9.0 ± 0.7 km·h^{-1}) and successive increments of inclination. During the first minute of the test, the inclination was set to 0.0°, after one minute it was increased to 1.0° and then by 0.5° every continued minute until voluntary exhaustion occurred and the test was terminated.

For both exercise modalities, rating of RPE for legs and breathing, respectively, was noted at the end of each submaximal workload as well as directly after completion of the maximal tests. In order to ensure that the cycling and running maximal tests achieved their purposes, at least two of the following three criteria were met by each participant: 1) a plateau in $\dot{V}O_2$ despite increasing exercise intensity (defined as a $\dot{V}O_2$ increment of < 150 ml), 2) a respiratory exchange ratio of ≥ 1.10 and 3) a RPE rating of ≥ 17 [45–47].

Data processing and statistical analyses

For determination of HR at rest, values are based on each single time period between the heart rate beats, the average of the last five minutes was transformed to beats per minute. For all forms of submaximal exercise, paired HR and $\dot{V}O_2$ values during the last, of the two consecutive minutes, at steady state have been used as averages for each workload. Since HR has been found to be more variable than $\dot{V}O_2$ at lower intensity levels [48], steady state was defined when HR values were within 2–3 beats·min^{-1}. As recommended by Howley, Bassett [46], maximal values were calculated by averaging the minute with highest continuous paired values of HR and $\dot{V}O_2$. For calculation of a certain percentage level of heart rate reserve (%HRR), both the resting and maximal heart rate values were used in the equation [49]. To exemplify, the HR value of interest was subtracted with HR_{rest} and then divided by HR_{max} minus HR_{rest}. When the submaximal workloads for cycling are expressed in relative levels of HR_{max}, HRR and $\dot{V}O_{2max}$, these levels have been based on the values from the maximal cycling test. Conversely, the maximal values from the treadmill test have been used to describe the percentage levels for treadmill walking.

The HR$-\dot{V}O_2$ relationships, based on the individual paired values of HR and $\dot{V}O_2$ from the three submaximal workloads (model 1) plus one maximal workload (model 2), were described by linear regression analyses for the two forms of exercise within each model. The individual regression equations were then used by the HR method for estimating $\dot{V}O_2$. Individual HR values, based on % of HRR were created according to the American College of Sports Medicine's classification of exercise intensity [50]. The intensity levels: 25 and 35% HRR, (very light to light exercise intensity), 45 and 55%HRR (moderate exercise intensity) and 65, 75 and 85%HRR (vigorous exercise intensity) were selected to cover a broad range of commonly used HR values during submaximal physical activities. In the calculations of individual HR values, HRR was always based on the maximal values from the running test. The individually derived HR values were then used in each individual regression equation for estimating $\dot{V}O_2$ at the seven intensity levels. Confidence intervals (CI) of 95% were calculated for the absolute values of the regression coefficients (y-intercept, slope and r-coefficient) and the $\dot{V}O_2$ estimations, as well as the differences between the modalities. Analyses of both the absolute and relative exercise mode differences were performed with Student´s one-sample t-test and Wilcoxon´s one-sample signed rank test at the group level for all participants (n = 34), since no systematic gender variations were observed.

The estimated mean $\dot{V}O_2$ levels for CEE and TE, respectively, based on all individual values between 25–85% of HRR, have been illustrated through linear line graphs with 95% CI for both models. Linear regression equations, including 95% CI, were created to exemplify the different HR$-\dot{V}O_2$ relationships. All individual estimated $\dot{V}O_2$ values were also graphically pairwise compared between the exercise modalities and models in scatter plots and regressions equations, including 95% CI. The linear line graphs and the scatter plots were created using

Graph-Pad Prism® 8 software package (Graph-Pad Software Inc., San Diego, CA, USA). The other statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics, 25 and 26, Chicago, IL, USA). Values are presented as means ± SD, unless otherwise stated. An alpha level of $P < 0.05$ has been used in all analyses.

Results

Descriptive data on cycle ergometer and treadmill exercises

Descriptive data from the cycle ergometer exercise (CEE) and the treadmill exercise (TE) are presented for the female and male participants separately, and for all participants together in Tables 2 and 3. Mean values of $\dot{V}O_2$ and HR are expressed in both absolute and relative numbers for all workloads (three submaximal and one maximal) within each form of exercise. The submaximal HR levels for all participants, ranged from 103.3 ± 12.7 to 148.7 ± 11.9 beats·min^{-1} during CEE (Table 2) and from 89.1 ± 13.2 to 104.9 ± 12.7 beats·min^{-1} during TE (Table 3). The corresponding maximal HR values were 178.9 ± 9.2 and 181.8 ± 8.3 beats·min^{-1}, respectively. For the $\dot{V}O_2$ and RPE levels of each workload and exercise modality, see Tables 2 and 3.

Regression equations

The constituents of the regression equations (y-intercept, slope and r-coefficient) and their absolute and relative differences between CEE and TE are presented for all participants of model 1 (only submaximal workloads) in Table 4 and model 2 (both submaximal and maximal workloads) in Table 5. There were no absolute or relative significant differences found for either the y-intercept or the slope in model 1 (n.s.). However, the r-coefficient was significantly higher for CEE than TE in both absolute, 0.022 ± 0.045 ($P < 0.01$), and relative numbers, 2.3 ± 4.6% ($P < 0.01$) (Table 4). In model 2, both the absolute and the relative differences between CEE and TE were significant for all regression coefficients. The absolute differences were 0.311 ± 0.532 ($P < 0.01$) for the y-intercept, -0.0025 ± 0.0036 ($P < 0.01$) for the slope and -0.004 ± 0.009 ($P < 0.05$) for the r-coefficient. The corresponding relative differences were 38.1 ± 59.8% ($P < 0.01$), -12.4 ± 16.3% ($P < 0.001$) and -0.4 ± 1.0% ($P < 0.05$)(Table 5).

Table 2. $\dot{V}O_2$, HR, $\dot{V}O_2$/HR and RPE during submaximal and maximal cycle ergometer exercise (means ± SD).

	Workload	**$\dot{V}O_2$**			**HR**			**$\dot{V}O_2$/HR**	**RPE**	
	W	**L·min^{-1}**	**mL·min^{-1}·kg^{-1}**	**%$\dot{V}O_{2max}$**	**beats·min^{-1}**	**%HR$_{max}$**	**%HRR**	**L·min^{-1}/ beats·min^{-1}**	**legs**	**breathing**
Women	**50**	0.83 ± 0.09	13.2 ± 1.6	34.2 ± 4.9	98.2 ± 8.2	54.9 ± 3.7	30.2 ± 5.3	0.0085 ± 0.0010	8.8 ± 1.4	8.8 ± 1.2
(n = 17)	**100**	1.40 ± 0.11	22.3 ± 2.8	57.7 ± 7.3	126.9 ± 9.5	70.9 ± 4.6	54.9 ± 7.7	0.0111 ± 0.0010	12.2 ± 1.8	12.1 ± 1.8
	137 ± 13	1.88 ± 0.19	29.7 ± 3.3	76.9 ± 7.4	151.1 ± 7.7	84.5 ± 3.8	75.9 ± 6.2	0.0125 ± 0.0014	15.3 ± 1.0	15.1 ± 1.3
	Max	2.46 ± 0.28	38.9 ± 4.5	100.0 ± 0.0	179.0 ± 8.0	100.0 ± 0.0	100.0 ± 0.0	0.0137 ± 0.0013	18.6 ± 1.2	18.7 ± 1.1
Men	**100**	1.43 ± 0.17	17.7 ± 2.7	43.5 ± 8.8	108.4 ± 14.6	60.5 ± 6.1	37.1 ± 8.4	0.0134 ± 0.0022	10.9 ± 1.8	10.5 ± 1.8
(n = 17)	**146 ± 11**	2.02 ± 0.24	24.9 ± 3.7	61.0 ± 10.0	129.1 ± 15.8	72.1 ± 6.8	55.7 ± 9.8	0.0159 ± 0.0026	13.5 ± 1.7	12.9 ± 2.2
	179 ± 20	2.46 ± 0.28	30.4 ± 4.7	74.0 ± 9.7	146.2 ± 14.9	81.8 ± 6.1	71.1 ± 8.9	0.0170 ± 0.0027	15.5 ± 1.5	14.4 ± 2.2
	Max	3.38 ± 0.55	41.4 ± 6.3	100.0 ± 0.0	178.7 ± 10.6	100.0 ± 0.0	100.0 ± 0.0	0.0190 ± 0.0033	18.3 ± 1.0	17.9 ± 1.9
All	**75 ± 25**	1.13 ± 0.33	15.4 ± 3.2	38.9 ± 8.5	103.3 ± 12.7	57.7 ± 5.7	33.7 ± 7.7	0.0110 ± 0.0030	9.9 ± 1.9	9.7 ± 1.8
(n = 34)	**123 ± 25**	1.71 ± 0.36	23.6 ± 3.5	59.4 ± 8.8	128.0 ± 12.9	71.5 ± 5.8	55.3 ± 8.7	0.0135 ± 0.0031	12.9 ± 1.9	12.5 ± 2.0
	158 ± 27	2.17 ± 0.38	30.1 ± 4.0	75.5 ± 8.6	148.7 ± 11.9	83.1 ± 5.2	73.5 ± 7.9	0.0148 ± 0.0032	15.4 ± 1.3	14.7 ± 1.8
	Max	2.92 ± 0.64	40.2 ± 5.5	100.0 ± 0.0	178.9 ± 9.2	100.0 ± 0.0	100.0 ± 0.0	0.0163 ± 0.0037	18.4 ± 1.1	18.3 ± 1.6

Table 3. $\dot{V}O_2$, HR, $\dot{V}O_2$/HR and RPE during submaximal (walking) and maximal (running) treadmill exercise (means ± SD).

	Workload	$\dot{V}O_2$			HR			$\dot{V}O_2$/HR	RPE	
	$km\cdot h^{-1}$	$L\cdot min^{-1}$	$mL\cdot min^{-1}\cdot kg^{-1}$	$\%\dot{V}O_{2max}$	$beats\cdot min^{-1}$	$\%HR_{max}$	%HRR	$L\cdot min^{-1}$/ $beats\cdot min^{-1}$	legs	breathing
Women	**4.0**	0.64 ± 0.09	10.0 ± 1.1	24.1 ± 3.3	87.8 ± 11.7	48.1 ± 6.5	21.0 ± 6.3	0.0073 ± 0.0009	7.5 ± 1.2	7.5 ± 1.2
(n = 17)	**4.9**	0.74 ± 0.09	11.7 ± 1.2	28.0 ± 3.2	94.8 ± 10.3	51.9 ± 5.6	26.7 ± 5.5	0.0079 ± 0.0010	9.1 ± 2.1	9.1 ± 1.9
	5.9	0.93 ± 0.13	14.7 ± 1.4	35.2 ± 3.9	105.2 ± 11.3	57.6 ± 5.8	35.4 ± 6.6	0.0089 ± 0.0012	10.8 ± 2.2	10.9 ± 2.0
	Max	2.66 ± 0.31	42.0 ± 4.4	100.0 ± 0.0	182.9 ± 8.1	100.0 ± 0.0	100.0 ± 0.0	0.0145 ± 0.0015	17.4 ± 2.1	18.6 ± 1.0
Men	**4.0**	0.86 ± 0.16	10.5 ± 1.2	24.1 ± 4.6	90.5 ± 14.8	50.0 ± 7.2	20.8 ± 9.7	0.0097 ± 0.0021	8.3 ± 1.2	8.1 ± 1.5
(n = 17)	**4.9**	1.01 ± 0.20	12.3 ± 1.4	28.3 ± 5.1	95.9 ± 13.3	53.0 ± 6.1	25.6 ± 8.1	0.0107 ± 0.0024	9.8 ± 1.6	9.5 ± 1.7
	5.9	1.27 ± 0.26	15.4 ± 1.8	35.4 ± 6.5	104.6 ± 14.3	57.8 ± 6.3	33.2 ± 8.4	0.0123 ± 0.0026	11.4 ± 1.5	11.2 ± 2.3
	Max	3.60 ± 0.54	44.3 ± 6.8	100.0 ± 0.0	180.7 ± 8.7	100.0 ± 0.0	100.0 ± 0.0	0.0200 ± 0.0031	17.4 ± 1.1	17.6 ± 1.8
All	**4.0**	0.75 ± 0.17	10.3 ± 1.2	24.1 ± 3.9	89.1 ± 13.2	49.0 ± 6.8	20.9 ± 8.1	0.0085 ± 0.0020	7.9 ± 1.3	7.8 ± 1.4
(n = 34)	**4.9**	0.88 ± 0.21	12.0 ± 1.3	28.1 ± 4.2	95.4 ± 11.7	52.4 ± 5.8	26.1 ± 6.8	0.0093 ± 0.0023	9.4 ± 1.9	9.3 ± 1.8
	5.9	1.10 ± 0.27	15.0 ± 1.7	35.3 ± 5.3	104.9 ± 12.7	57.7 ± 6.0	34.3 ± 7.5	0.0106 ± 0.0027	11.1 ± 1.9	11.1 ± 2.1
	Max	3.13 ± 0.65	43.2 ± 5.8	100.0 ± 0.0	181.8 ± 8.3	100.0 ± 0.0	100.0 ± 0.0	0.0173 ± 0.0037	17.4 ± 1.6	18.1 ± 1.5

Estimation of oxygen uptake using the HR method

The estimated $\dot{V}O_2$ levels, based on the individual regression equations and seven individual HR values, derived from % of HRR, are presented for all participants in Tables 6 (model 1) and 7 (model 2). There were no significant absolute or relative differences between the estimated $\dot{V}O_2$ levels when comparing CEE with TE for any of the intensity levels between 25–85% of HRR (94.0–164.2 $beats\cdot min^{-1}$) in model 1. The $\dot{V}O_2$ differences between CEE and TE ranged from 0.05 ± 0.24 to 0.10 ± 0.51 $L\cdot min^{-1}$ and -0.4 ± 34.9 to 4.8 ± 17.1%, respectively (n.s.) (Table 6). When including the maximal workloads in the regression equations (model 2), the estimated $\dot{V}O_2$ differences between CEE and TE varied from 0.07 ± 0.26 to -0.05 ± 0.20 $L\cdot min^{-1}$ and 1.3 ± 40.3 to -2.7 ± 10.0%, respectively, (n.s.) in the intensity range of 25–65% of HRR (94.0–140.8 $beats\cdot min^{-1}$) (Table 7). At the two highest exercise intensities, 75 and 85% (152.5 and 164.2 $beats\cdot min^{-1}$), the $\dot{V}O_2$ levels were slightly lower during the cycle ergometer exercise than the treadmill exercise. These absolute $\dot{V}O_2$ differences were -0.07 ± 0.20 (P = T-test: 0.041, Wilcoxon: 0.071) and -0.10 ± 0.22 $L\cdot min^{-1}$ ($P < 0.05$), respectively, the corresponding relative differences were -3.7 ± 9.0% ($P < 0.05$) and -4.4 ± 8.6% ($P < 0.05$).

Illustrations of the estimated mean $\dot{V}O_2$ levels and the 95% confidence intervals, based on all individual values, are presented for all exercise intensity levels in Fig 2A (model 1) and 2B (model 2). Although these figures show that the average $\dot{V}O_2$ levels between the two exercise modalities differ to a very low extent, when instead comparing the individual $\dot{V}O_2$ values, the spreading between CEE and TE is generally greater in model 1 compared to model 2 (Fig 3A

Table 4. Regression equations of model 1, and the absolute and relative exercise mode differences (n = 34, means ± SD, (95% CI) and P-values).

	y-intercept	slope	r
Cycle ergometer exercise (CEE)	-1.356 ± 0.743 (-1.615 to -1.097)	0.0240 ± 0.0073 (0.0215 to 0.0266)	0.995 ± 0.008 (0.992 to 0.998)
Treadmill exercise (TE)	-1.364 ± 0.842 (-1.658 to -1.070)	0.0235 ± 0.0099 (0.0201 to 0.0270)	0.972 ± 0.045 (0.957 to 0.988)
Abs. diff. CEE-TE	0.009 ± 0.821 (-0.278 to 0.295)	0.0005 ± 0.0077 (-0.0022 to 0.0032)	0.022 ± 0.045 (0.007 to 0.038)
P-values T-test/Wilcoxon	0.952/0.952	0.738/0.611	0.007/0.008
Rel. diff. CEE-TE (%)	32.8 ± 180.1 (-30.0 to 95.7)	1.2 ± 35.8 (-11.3 to 13.7)	2.3 ± 4.6 (0.7 to 3.8)
P-values T-test/Wilcoxon	0.296/0.765	0.848/0.614	0.007/0.008

Table 5. Regression equations of model 2, and the absolute and relative exercise mode differences (n = 34, means ± SD, (95% CI) and P-values).

	y-intercept	slope	r
Cycle ergometer exercise (CEE)	-1.310 ± 0.609 (-1.523 to -1.098)	0.0236 ± 0.0060 (0.0215 to 0.0257)	0.994 ± 0.009 (0.991 to 0.997)
Treadmill exercise (TE)	-1.621 ± 0.559 (-1.816 to -1.426)	0.0261 ± 0.0058 (0.0241 to 0.0282)	0.998 ± 0.002 (0.997 to 0.999)
Abs. diff. CEE-TE	0.311 ± 0.532 (0.125 to 0.496)	-0.0025 ± 0.0036 (-0.0038 to -0.0013)	-0.004 ± 0.009 (-0.007 to -0.001)
P-values T-test/Wilcoxon	0.002/0.003	0.000/0.001	0.015/0.024
Rel. diff. CEE-TE (%)	38.1 ± 59.8 (17.2 to 58.9)	-12.4 ± 16.3 (-18.1 to -6.7)	-0.4 ± 1.0 (-0.8 to -0.1)
P-values T-test/Wilcoxon	0.001/0.000	0.000/0.000	0.015/0.024

and 3B). Additionally, when comparing the same individual $\dot{V}O_2$ values between the two models within each exercise modality, the individual scattering is clearly less during the cycle ergometer exercise than the treadmill exercise (Fig 4A and 4B).

Discussion

This is, to our knowledge, the first systematic study on the potential interchangeability of the HR method when estimating oxygen uptake in cycle ergometer exercise (CEE) and treadmill exercise (TE) with special reference to level walking. Another novel aspect was a search for pathways that can optimize the HR method. This was analysed through comparing two different models of HR—$\dot{V}O_2$ relationships. Model 1 consisted of three submaximal steady state workloads of ergometer cycling or level walking on the treadmill, while model 2 included one additional workload of maximal exercise in CEE and TE.

In order to apply the HR method, the development of regression equations is a necessary first step. A number of previous studies [18, 20, 51, 52] investigating issues related to the HR method have primarily based their evaluations on separate regression coefficients. This division of analyses can, however, lead to misinterpretations of the consequences of the HR method. We have therefore instead applied the HR method by estimating different levels of $\dot{V}O_2$ based on a broad range of HR values, and combined it with analyses of ingredients from the regression equations.

Table 6. Estimation of $\dot{V}O_2$ in model 1, and the absolute and relative exercise mode differences (n = 34, means ± SD, (95% CI) and P-values).

	Exercise intensity						
	Very light–light		**Moderate**		**Vigorous**		
	25%HRR	**35%HRR**	**45%HRR**	**55%HRR**	**65%HRR**	**75%HRR**	**85%HRR**
HR (beats·min^{-1})	94.0 ± 7.5	105.7 ± 7.1	117.4 ± 6.8	129.1 ± 6.8	140.8 ± 6.8	152.5 ± 7.1	164.2 ± 7.5
Estimation of $\dot{V}O_2$ (L·min^{-1})							
Cycle ergometer exercise (CEE)	0.91 ± 0.31 (0.80 to 1.02)	1.19 ± 0.33 (1.07 to 1.30)	1.47 ± 0.37 (1.34 to 1.60)	1.75 ± 0.42 (1.60 to 1.89)	2.03 ± 0.48 (1.86 to 2.19)	2.30 ± 0.55 (2.11 to 2.50)	2.58 ± 0.62 (2.37 to 2.80)
Treadmill exercise (TE)	0.86 ± 0.26 (0.77 to 0.95)	1.13 ± 0.33 (1.01 to 1.24)	1.40 ± 0.42 (1.25 to 1.54)	1.67 ± 0.52 (1.49 to 1.85)	1.94 ± 0.62 (1.73 to 2.16)	2.21 ± 0.72 (1.96 to 2.47)	2.48 ± 0.83 (2.19 to 2.77)
Abs. diff. CEE-TE (L·min^{-1})	0.05 ± 0.24 (-0.03 to 0.13)	0.06 ± 0.22 (-0.02 to 0.14)	0.07 ± 0.25 (-0.02 to 0.15)	0.08 ± 0.29 (-0.03 to 0.18)	0.08 ± 0.36 (-0.04 to 0.21)	0.09 ± 0.43 (-0.06 to 0.24)	0.10 ± 0.51 (-0.08 to 0.28)
P-values T-test/ Wilcoxon	0.207/0.164	0.127/0.135	0.116/0.118	0.142/0.149	0.183/0.158	0.226/0.185	0.265/0.228
Rel. diff. CEE-TE (%)	-0.4 ± 34.9 (-12.6 to 11.8)	3.7 ± 19.0 (-2.9 to 10.4)	4.6 ± 16.5 (-1.2 to 10.4)	4.8 ± 17.1 (-1.2 to 10.7)	4.7 ± 18.3 (-1.7 to 11.1)	4.6 ± 19.6 (-2.2 to 11.4)	4.5 ± 20.8 (-2.8 to 11.7)
P-values T-test/ Wilcoxon	0.952/0.343	0.260/0.161	0.114/0.081	0.112/0.144	0.140/0.153	0.179/0.164	0.220/0.222

Table 7. Estimation of $\dot{V}O_2$ in model 2, and the absolute and relative exercise mode differences (n = 34, means ± SD, (95% CI) and P-values).

	Exercise intensity						
	Very light–light		Moderate		Vigorous		
	25%HRR	35%HRR	45%HRR	55%HRR	65%HRR	75%HRR	85%HRR
HR (beats·min^{-1})	94.0 ± 7.5	105.7 ± 7.1	117.4 ± 6.8	129.1 ± 6.8	140.8 ± 6.8	152.5 ± 7.1	164.2 ± 7.5
Estimation of $\dot{V}O_2$ (L·min^{-1})							
Cycle ergometer exercise (CEE)	0.91 ± 0.33 (0.80 to 1.02)	1.19 ± 0.34 (1.07 to 1.30)	1.46 ± 0.36 (1.34 to 1.59)	1.74 ± 0.40 (1.60 to 1.88)	2.01 ± 0.45 (1.86 to 2.17)	2.29 ± 0.50 (2.11 to 2.46)	2.57 ± 0.56 (2.37 to 2.76)
Treadmill exercise (TE)	0.84 ± 0.29 (0.74 to 0.94)	1.15 ± 0.32 (1.04 to 1.26)	1.45 ± 0.36 (1.33 to 1.58)	1.76 ± 0.40 (1.62 to 1.90)	2.06 ± 0.45 (1.90 to 2.22)	2.36 ± 0.51 (2.19 to 2.54)	2.67 ± 0.56 (2.47 to 2.86)
Abs. diff. CEE-TE (L·min^{-1})	0.07 ± 0.26 (-0.02 to 0.16)	0.04 ± 0.23 (-0.04 to 0.12)	0.01 ± 0.21 (-0.06 to 0.09)	-0.02 ± 0.20 (-0.09 to 0.05)	-0.05 ± 0.20 (-0.12 to 0.02)	-0.07 ± 0.20 (-0.14 to 0.00)	-0.10 ± 0.22 (-0.18 to -0.03)
P-values T-test/ Wilcoxon	0.136/0.114	0.339/0.215	0.777/0.590	0.615/0.955	0.189/0.379	0.041/0.071	0.009/0.011
Rel. diff. CEE-TE (%)	1.3 ± 40.3 (-12.8 to 15.3)	1.2 ± 21.8 (-6.4 to 8.8)	-0.3 ± 15.3 (-5.6 to 5.0)	-1.6 ± 11.9 (-5.8 to 2.5)	-2.7 ± 10.0 (-6.2 to 0.7)	-3.7 ± 9.0 (-6.8 to -0.5)	-4.4 ± 8.6 (-7.5 to -1.4)
P-values T-test/ Wilcoxon	0.857/0.270	0.752/0.388	0.912/0.700	0.429/0.765	0.119/0.281	0.024/0.047	0.005/0.011

The main results are that only small, and in most cases (24 of 28) non-significant differences in estimated $\dot{V}O_2$ levels (range of relative means: -4.4 to 4.8%) were noted between CEE and TE when applying the HR method at intensity levels between 25–85% of HRR in both models. When interpreting the results, we observe that these relative mean $\dot{V}O_2$ differences between CEE and TE are of the same order of magnitude as the relative test-retest differences (range of means: 1.0–3.6%) of the HR method using ergometer cycling [21, 22]. At the same time, rather large standard deviations and confidence intervals were occasionally seen among the $\dot{V}O_2$ differences between the two exercise modalities in both models. Thus, there may be some undetected exercise mode differences due to the limited power of these results. If there are any further significant differences, they are, however, most likely of rather low magnitude and will therefore be of minor importance at the group level, but they may still be critical for validity at the individual level.

The absence of significant differences in estimated $\dot{V}O_2$ levels between submaximal ergometer cycling and level walking (model 1; Table 6) is in line with the only previous study that we can find that has specifically compared the HR—$\dot{V}O_2$ relationship of ergometer cycling with horizontal treadmill exercise [26]. Although, Berggren and Hohwü Christensen [26] did not apply the HR method, they observed similar $\dot{V}O_2$ levels at equivalent HR values in ergometer cycling and walking in one trained man.

Whether the HR-method is interchangeable between ergometer cycling and other forms of treadmill exercise, such as level running, as well as walking or running using inclination are also relevant questions. After studying previous literature [18, 26, 53–55], our analyses of appropriate studies [18, 54, 55], indicate that the HR method, based on ergometer cycling, is, at group level, interchangeable with level treadmill running, but not with treadmill exercise using inclination. For instance, our analysis of Lafortuna, Agosti [18] demonstrated between 16–24% (obese women) and 14–19% (normal weight women) lower $\dot{V}O_2$ levels at equivalent HR values during submaximal ergometer cycling when compared with treadmill walking using work load increments of both increased speed and inclination. All our analyses, results and supplementary texts in this literature survey can be found in the supporting information in S1 Discussion. The overall picture indicates a clear need for further studies of these matters.

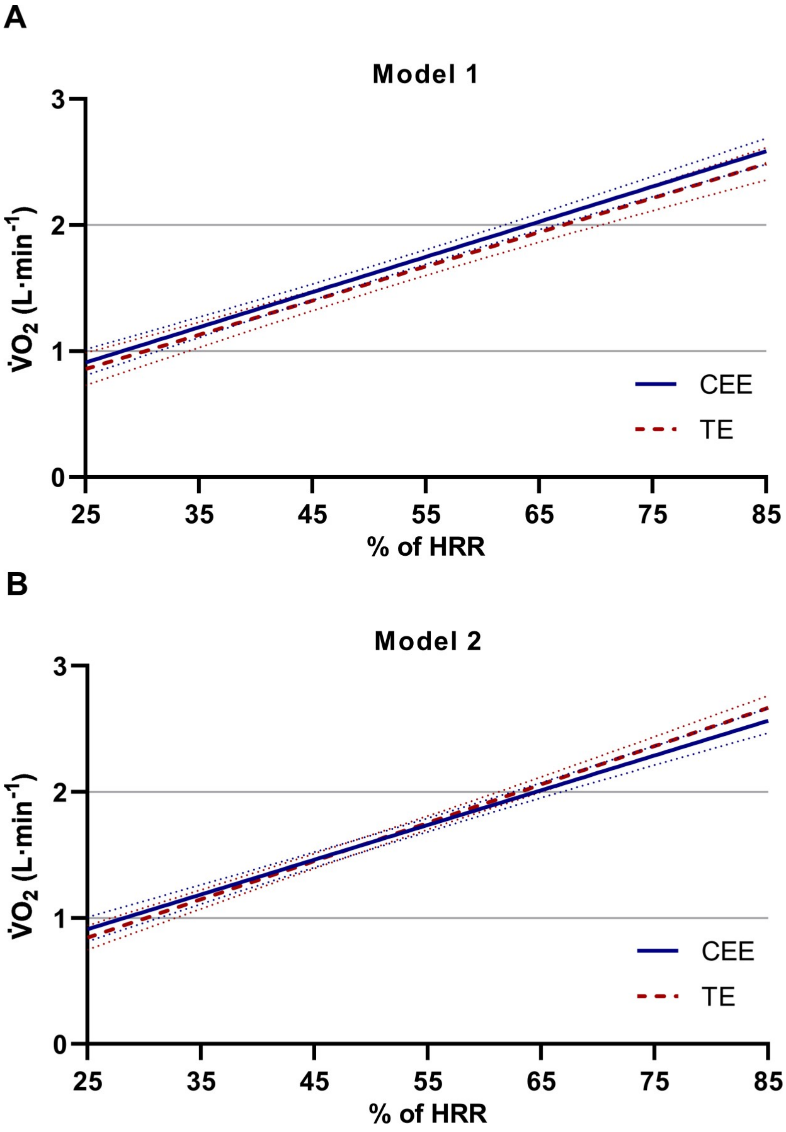

Fig 2. Estimated mean $\dot{V}O_2$ levels between 25–85% of HRR in cycle ergometer exercise (CEE) and treadmill exercise (TE) in model 1 and model 2. Based on all participants (n = 34) individual values. CEE: blue solid line. TE: red dashed line. The linear regression equations with 95% CI and r-coefficients were: Model 1 **(A)** y($\dot{V}O_2$CEE) = 0.210 (0.042—0.377) + 0.0279(0.0251—0.0308) * x(%HRR), r-coefficient = 0.781, y($\dot{V}O_2$TE) = 0.178(-0.030—0.386) + 0.0271 (0.0236—0.0307) * x(%HRR) and r-coefficient = 0.700. Model 2 **(B)** y($\dot{V}O_2$CEE) = 0.222(0.064—0.380) + 0.0276 (0.0249—0.0303) * x(%HRR), r-coefficient = 0.795, y($\dot{V}O_2$TE) = 0.084(-0.072—0.241) + 0.0304(0.0277—0.0331) * x(% HRR) and r-coefficient = 0.825.

Issues of optimization of the HR method protrude when moving the focus from effects on mean values (cf. Fig 2) to effects on individual values (cf. Figs 3 and 4). It is notable that the deviances between the two exercise modalities are generally greater in model 1 than in model 2 (Fig 3 hin and 3B). Thus, the differences between CEE and TE seem to be reduced when the maximal workloads are added to the HR—$\dot{V}O_2$ relationships. Interestingly, when comparing model 1 with model 2 within CEE, in contrast to TE, the values are well aligned along the line of identity (cf. Fig 4A and 4B). Thus, the HR method in CEE, in itself, does not gain anything through adding maximal exercise. Thereby it cannot explain the reduction in spreading when

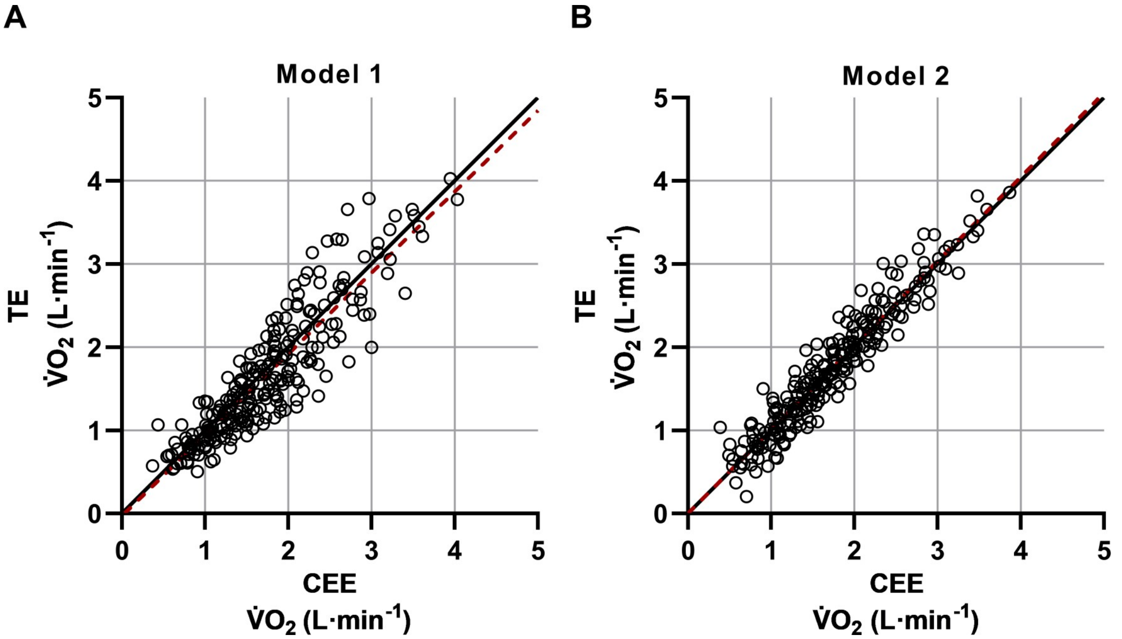

Fig 3. Comparison of the estimated individual $\dot{V}O_2$ values between cycle ergometer exercise (CEE) and treadmill exercise (TE) in model 1 and model 2. Based on all participants (n = 34), ranging between 25–85% of HRR. The overall linear regression equations (red dashed lines) with 95% CI and r-coefficients were: Model 1 **(A)** y($\dot{V}O_2$TE) = -0.033(-0.148—0.082) + 0.9753 (0.9144—1.0362) * x($\dot{V}O_2$CEE) and r-coefficient = 0.899. Model 2 **(B)** y($\dot{V}O_2$TE) = -0.004(-0.081—0.073) + 1.0125(0.9714—1.0536) * x($\dot{V}O_2$CEE) and r-coefficient = 0.953.

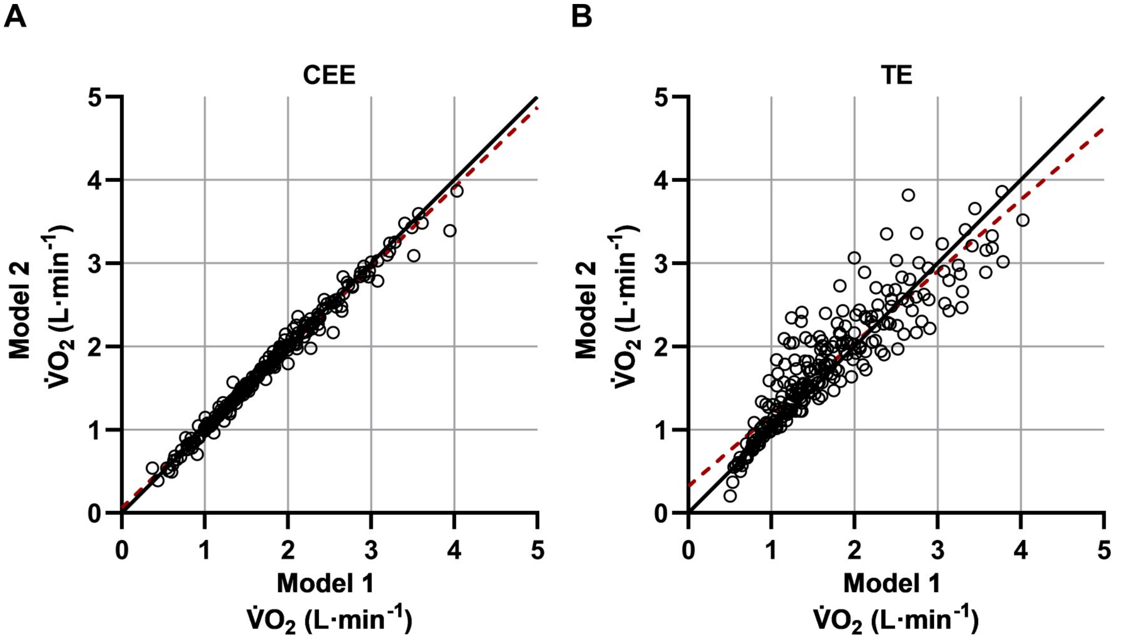

Fig 4. Comparison of the estimated individual $\dot{V}O_2$ values between model 1 and model 2 in cycle ergometer exercise (CEE) and treadmill exercise (TE). Based on all participants (n = 34), ranging between 25–85% of HRR. The overall linear regression equations (red dashed lines) with 95% CI and r-coefficients were: CEE **(A)** y($\dot{V}O_2$Model 2) = 0.056(0.028—0.084) + 0.9632 (0.9483—0.9780) * x($\dot{V}O_2$Model 1) and r-coefficient = 0.993. TE **(B)** y($\dot{V}O_2$Model 2) = 0.318(0.223—0.413) + 0.8606 (0.8091—0.9122) * x($\dot{V}O_2$Model 1) and r-coefficient = 0.906.

comparing CEE and TE in model 1 with model 2 (Fig 3A and 3B). It is more likely that it is due to changes between model 1 and model 2 within TE, as indicated by a higher r-coefficient in model 2 (r = 0.998) compared to model 1 (r = 0.972) (Tables 4 and 5).

How can this be understood? We think that a reasonable starting point in explaining the overall pattern of differences between CEE and TE, as well as between the two models, is to view them in relation to the positions and widths of the submaximal measurement ranges applied, as well as their distances to the maximal HR and $\dot{V}O_2$ values. In model 1, the submaximal ergometer cycling was performed at average HR levels ranging between 103.3–148.7 beats·min^{-1} (based on all participants), whereas the corresponding range of level walking was between 89.1–104.9 beats·min^{-1}. The rather narrow submaximal range in TE, as compared to CEE, increases the sensitivity to variations in HR and $\dot{V}O_2$ values on the linear regression equations. This is mirrored in the significantly different r-coefficients in ergometer cycling (0.995) and level walking (0.972) for model 1 (Table 4). An additional explanation to this instability in the individual submaximal TE values is that activities of light intensity, such as sedentary activities and slow walking, have been demonstrated to generate more varying HR—$\dot{V}O_2$ relationships than activities of moderate exercise intensity [11, 56, 57]. The wide gap between the submaximal and maximal HR and $\dot{V}O_2$ values add further to the instability within the TE models. To include a broader range of submaximal measurement points in level walking may assist in curing the instability noted in this study, contribute to optimizing the HR method for level walking, and thereby enhance its interchangeability with ergometer cycling.

As stated in the Introduction, a number of methodological issues related to the HR method need to be sorted out. Previously we have studied the reproducibility of the HR method in ergometer cycling [21, 22]. In the present study we have evaluated the interchangeability of the HR method between two exercise modalities, and searched for ways to optimize the method. In future studies, we will evaluate the external validity of the HR method in relation to exercise in field conditions. It is, in this context, important to gain knowledge about if slow components of HR and $\dot{V}O_2$ kinetics [48, 58, 59] lead to drifts in HR—$\dot{V}O_2$ relationships with time, and in such cases under which conditions. If, for example, HR starts to increase disproportionately more than $\dot{V}O_2$, then this must be compensated for as part of the HR method. One way to evaluate a need for such a correction would be to study each individual in the laboratory during prolonged exercise at the average intensity that is anticipated in the measurement context of interest. Additionally, this matter deserves to be studied under conditions of prolonged intermittent exercise that mimic applied measurement conditions. In fact, ocular inspections of HR and $\dot{V}O_2$ recordings during active commuting point in the direction of that if slow components exist, they increase to the same relative degree in HR and $\dot{V}O_2$ [41].

In conclusion, the current study demonstrates that the HR methods based on submaximal ergometer cycling and level walking are interchangeable for estimating mean $\dot{V}O_2$ levels between very light and vigorous exercise intensities corresponding to 25–85% of HRR. Fundamentally, the same finding is observed when including maximal exercise in the HR—$\dot{V}O_2$ relationships. Finally, the inter-individual variation in $\dot{V}O_2$ between ergometer cycling and treadmill exercise is reduced when using the HR method based on both submaximal and maximal workloads.

Supporting information

S1 Methods. The Physically Active commuting in Greater Stockholm Questionnaire 1 (PACS Q1). The original version in Swedish.
(DOC)

S2 Methods. The Physically Active commuting in Greater Stockholm Questionnaire 1 (PACS Q1). The original version in Swedish translated into English.
(DOC)

S1 Discussion. Analyses of previous literature using the HR method for estimating oxygen uptake in ergometer cycling, and treadmill walking and running.
(DOCX)

Acknowledgments

We are grateful to the volunteers for participating in the study and for the technical assistance of Phoung Pihlträd. We also want to thank the reviewers for their constructive comments.

Author Contributions

Conceptualization: Karin Olsson, Peter Schantz.

Data curation: Karin Olsson, Jane Salier Eriksson.

Formal analysis: Karin Olsson, Peter Schantz.

Funding acquisition: Peter Schantz.

Investigation: Jane Salier Eriksson, Hans Rosdahl, Peter Schantz.

Methodology: Karin Olsson, Peter Schantz.

Project administration: Jane Salier Eriksson, Peter Schantz.

Supervision: Hans Rosdahl, Peter Schantz.

Validation: Hans Rosdahl.

Visualization: Karin Olsson, Peter Schantz.

Writing – original draft: Karin Olsson, Jane Salier Eriksson, Peter Schantz.

Writing – review & editing: Karin Olsson, Hans Rosdahl, Peter Schantz.

References

1. Wenger HA, Bell GJ. The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness. Sports Med. 1986; 3(5):346–56. Epub 1986/09/01. https://doi.org/10.2165/00007256-198603050-00004 PMID: 3529283.
2. Montero D, Lundby C. Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. J Physiol. 2017; 595(11):3377–87. Epub 2017/01/31. https://doi.org/10.1113/JP273480 PMID: 28133739.
3. Paffenbarger RS Jr., Hyde RT, Wing AL, Hsieh CC. Physical activity, all-cause mortality, and longevity of college alumni. N Engl J Med. 1986; 314(10):605–13. Epub 1986/03/06. https://doi.org/10.1056/NEJM198603063141003 PMID: 3945246.
4. Ross R, Janssen I. Physical activity, total and regional obesity: dose-response considerations. Med Sci Sports Exerc. 2001; 33(6 Suppl):S521–7; discussion S8-9. Epub 2001/06/28. https://doi.org/10.1097/00005768-200106001-00023 PMID: 11427779.
5. Hu FB, Sigal RJ, Rich-Edwards JW, Colditz GA, Solomon CG, Willett WC, et al. Walking compared with vigorous physical activity and risk of type 2 diabetes in women: a prospective study. Jama. 1999; 282(15):1433–9. Epub 1999/10/27. https://doi.org/10.1001/jama.282.15.1433 PMID: 10535433.
6. Åstrand P-O, Ryhming I. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during sub-maximal work. J Appl Physiol. 1954; 7(2):218–21. Epub 1954/09/01. https://doi.org/10.1152/jappl.1954.7.2.218 PMID: 13211501.
7. Achten J, Jeukendrup AE. Heart rate monitoring: applications and limitations. Sports Med. 2003; 33(7):517–38. Epub 2003/05/24. https://doi.org/10.2165/00007256-200333070-00004 PMID: 12762827.

8. Carpenter TM. Tables, factors, and formulas for computing respiratory exchange and biological transformations of energy. 4st ed. Washington, DC: Carnegie Instituton of Washington Publication; 1964.

9. Boothby WM. A determination of the circulation rate in man at rest and at work. American Journal of Physiology-Legacy Content. 1915; 37(2):383–417. https://doi.org/10.1152/ajplegacy.1915.37.2.383

10. Krogh A, Lindhard J. A comparison between voluntary and electrically induced muscular work in man. J Physiol. 1917; 51(3):182–201. Epub 1917/07/03. https://doi.org/10.1113/jphysiol.1917.sp001795 PMID: 16993364.

11. Booyens J, Hervey G. The pulse rate as a mean of measuring metabolic rate in man. Canadian Journal of Biochemistry and Physiology. 1960; 38:1301–9. https://doi.org/10.1139/o60-162

12. Åstrand I. Estimating the energy expenditure of housekeeping activities. Am J Clin Nutr. 1971; 24 (12):1471–5. Epub 1971/12/01. https://doi.org/10.1093/ajcn/24.12.1471 PMID: 5118021.

13. Montoye HJ. Measuring physical activity and energy expenditure. Champaign, IL: Human Kinetics; 1996.

14. Shephard RJ, Aoyagi Y. Measurement of human energy expenditure, with particular reference to field studies: an historical perspective. Eur J Appl Physiol. 2012; 112(8):2785–815. Epub 2011/12/14. https://doi.org/10.1007/s00421-011-2268-6 PMID: 22160180.

15. Green JA. The heart rate method for estimating metabolic rate: review and recommendations. Comp Biochem Physiol A Mol Integr Physiol. 2011; 158(3):287–304. Epub 2010/09/28. https://doi.org/10.1016/j.cbpa.2010.09.011 PMID: 20869457.

16. Fairbarn MS, Blackie SP, McElvaney NG, Wiggs BR, Pare PD, Pardy RL. Prediction of heart rate and oxygen uptake during incremental and maximal exercise in healthy adults. Chest. 1994; 105(5):1365–9. Epub 1994/05/01. https://doi.org/10.1378/chest.105.5.1365 PMID: 8181321.

17. Ekelund U, Poortvliet E, Yngve A, Hurtig-Wennlov A, Nilsson A, Sjostrom M. Heart rate as an indicator of the intensity of physical activity in human adolescents. Eur J Appl Physiol. 2001; 85(3–4):244–9. Epub 2001/09/19. https://doi.org/10.1007/s004210100436 PMID: 11560077.

18. Lafortuna CL, Agosti F, Galli R, Busti C, Lazzer S, Sartorio A. The energetic and cardiovascular response to treadmill walking and cycle ergometer exercise in obese women. Eur J Appl Physiol. 2008; 103(6):707–17. Epub 2008/05/23. https://doi.org/10.1007/s00421-008-0758-y PMID: 18496708.

19. Farinatti PT, Soares PP. Cardiac output and oxygen uptake relationship during physical effort in men and women over 60 years old. Eur J Appl Physiol. 2009; 107(6):625–31. Epub 2009/08/28. https://doi.org/10.1007/s00421-009-1162-y PMID: 19711096.

20. McCrory MA, Mole PA, Nommsen-Rivers LA, Dewey KG. Between-day and within-day variability in the relation between heart rate and oxygen consumption: effect on the estimation of energy expenditure by heart-rate monitoring. Am J Clin Nutr. 1997; 66(1):18–25. Epub 1997/07/01. https://doi.org/10.1093/ajcn/66.1.18 PMID: 9209164.

21. Schantz P, Salier Eriksson J, Rosdahl H. The heart rate method for estimating oxygen uptake: Analyses of reproducibility using a range of heart rates from cycle commuting. PLoS One. 2019; 14(7):e0219741. Epub 2019/07/25. https://doi.org/10.1371/journal.pone.0219741 PMID: 31339909.

22. Schantz P, Salier Eriksson J, Rosdahl H. The heart rate method for estimating oxygen uptake: analyses of reproducibility using a range of heart rates from commuter walking. Eur J Appl Physiol. 2019; 119 (11–12):2655–71. Epub 2019/10/20. https://doi.org/10.1007/s00421-019-04236-0 PMID: 31628539.

23. Åstrand P-O. Quantification of exercise capability and evaluation of physical capacity in man. Prog Cardiovasc Dis. 1976; 19(1):51–67. Epub 1976/07/01. https://doi.org/10.1016/0033-0620(76)90008-6 PMID: 785542.

24. Vokac Z, Bell H, Bautz-Holter E, Rodahl K. Oxygen uptake/heart rate relationship in leg and arm exercise, sitting and standing. J Appl Physiol. 1975; 39(1):54–9. Epub 1975/07/01. https://doi.org/10.1152/jappl.1975.39.1.54 PMID: 1150592.

25. Eston RG, Brodie DA. Responses to arm and leg ergometry. Br J Sports Med. 1986; 20(1):4–6. Epub 1986/03/01. https://doi.org/10.1136/bjsm.20.1.4 PMID: 3697603.

26. Berggren G, Hohwü Christensen E. Heart rate and body temperature as indices of metabolic rate during work. Arbeitsphysiologie. 1950; 14(3):255–60. Epub 1950/01/01. https://doi.org/10.1007/BF00933843 PMID: 14783388.

27. Ekblom B, Gjessing E. Maximal oxygen uptake of the Easter Island population. J Appl Physiol. 1968; 25 (2):124–9. Epub 1968/08/01. https://doi.org/10.1152/jappl.1968.25.2.124 PMID: 5666085.

28. Åstrand P-O. Experimental studies of physical working capacity in relation to sex and age. Doctoral Thesis, Copenhagen: Munksgaard; 1952. Available from: http://urn.kb.se/resolve?urn=urn:nbn:se:gih:diva-5581

29. Ryhming I. A modified Harvard step test for the evaluation of physical fitness. Arbeitsphysiologie. 1953; 15(3):235–50. Epub 1953/01/01. https://doi.org/10.1007/BF00933320 PMID: 13139422.

30. Sun XG, Hansen JE, Garatachea N, Storer TW, Wasserman K. Ventilatory efficiency during exercise in healthy subjects. Am J Respir Crit Care Med. 2002; 166(11):1443–8. Epub 2002/11/27. https://doi.org/10.1164/rccm.2202033 PMID: 12450934.

31. Poole DC, Burnley M, Vanhatalo A, Rossiter HB, Jones AM. Critical Power: An Important Fatigue Threshold in Exercise Physiology. Med Sci Sports Exerc. 2016; 48(11):2320–34. Epub 2016/10/19. https://doi.org/10.1249/MSS.0000000000000939 PMID: 27031742.

32. Iannetta D, Inglis EC, Mattu AT, Fontana FY, Pogliaghi S, Keir DA, et al. A Critical Evaluation of Current Methods for Exercise Prescription in Women and Men. Med Sci Sports Exerc. 2020; 52(2):466–73. Epub 2019/09/04. https://doi.org/10.1249/MSS.0000000000002147 PMID: 31479001.

33. Coyle EF, Sidossis LS, Horowitz JF, Beltz JD. Cycling efficiency is related to the percentage of type I muscle fibers. Med Sci Sports Exerc. 1992; 24(7):782–8. Epub 1992/07/01. PMID: 1501563.

34. Åstrand I, Åstrand P-O, Stunkard A. Oxygen intake of obese individuals during work on a bicycle ergometer. Acta Physiol Scand. 1960; 50:294–9. Epub 1960/12/30. https://doi.org/10.1111/j.1748-1716.1960.tb00183.x PMID: 13684885.

35. Cotes JE. Relationships of oxygen consumption, ventilation and cardiac frequency to body weight during standardized submaximal exercise in normal subjects. Ergonomics. 1969; 12(3):415–27. Epub 1969/05/01. https://doi.org/10.1080/00140136908931065 PMID: 5345650.

36. Berry MJ, Storsteen JA, Woodard CM. Effects of body mass on exercise efficiency and VO2 during steady-state cycling. Med Sci Sports Exerc. 1993; 25(9):1031–7. Epub 1993/09/01. PMID: 8231771.

37. Lafortuna CL, Proietti M, Agosti F, Sartorio A. The energy cost of cycling in young obese women. Eur J Appl Physiol. 2006; 97(1):16–25. Epub 2006/02/08. https://doi.org/10.1007/s00421-006-0137-5 PMID: 16463044.

38. Lafortuna CL, Agosti F, Busti C, Galli R, Sartorio A. The energy cost of cycling and aerobic performance of obese adolescent girls. J Endocrinol Invest. 2009; 32(8):647–52. Epub 2009/06/06. https://doi.org/10.1007/BF03345735 PMID: 19494705.

39. Björkman FME. Validity and reliability of a submaximal cycle ergometer test for estimation of maximal oxygen uptake. Doctoral Thesis, The Swedish School of Sport and Health Sciences, GIH, Stockholm, Sweden. 2017. Available from: http://urn.kb.se/resolve?urn=urn:nbn:se:gih:diva-5095

40. Salier Eriksson J, Rosdahl H, Schantz P. Validity of the Oxycon Mobile metabolic system under field measuring conditions. Eur J Appl Physiol. 2012; 112(1):345–55. Epub 2011/05/12. https://doi.org/10.1007/s00421-011-1985-1 PMID: 21559947.

41. Schantz P, Salier Eriksson J, Rosdahl H. An overview, description and synthesis of methodological issues in studying oxygen consumption during walking and cycling commuting using a portable metabolic system (Oxycon Mobile). An appendix in: Jane Salier Eriksson. The heart rate method for estimating oxygen uptake in walking and cycle commuting. Evaluations based on reproducibility and validity studies of the heart rate method and a portable metabolic system. Doctoral Thesis, The Swedish School of Sport and Health Sciences, GIH, Stockholm, Sweden. 2018. Available from: http://urn.kb.se/resolve?urn=urn%3Anbn%3Ase%3Agih%3Adiva-5413

42. Stigell E, Schantz P. Active Commuting Behaviors in a Nordic Metropolitan Setting in Relation to Modality, Gender, and Health Recommendations. Int J Environ Res Public Health. 2015; 12(12):15626–48. Epub 2015/12/23. https://doi.org/10.3390/ijerph121215008 PMID: 26690193.

43. Foss O, Hallén J. Validity and stability of a computerized metabolic system with mixing chamber. Int J Sports Med. 2005; 26(7):569–75. Epub 2005/10/01. https://doi.org/10.1055/s-2004-821317 PMID: 16195991.

44. Borg G. Psychophysical scaling with applications in physical work and the perception of exertion. Scand J Work Environ Health. 1990; 16 Suppl 1:55–8. Epub 1990/01/01. https://doi.org/10.5271/sjweh.1815 PMID: 2345867.

45. Borg G. Perceived exertion as an indicator of somatic stress. Scand J Rehabil Med. 1970; 2(2):92–8. Epub 1970/01/01. PMID: 5523831.

46. Howley ET, Bassett DR Jr., Welch HG. Criteria for maximal oxygen uptake: review and commentary. Med Sci Sports Exerc. 1995; 27(9):1292–301. Epub 1995/09/01. PMID: 8531628.

47. Midgley AW, McNaughton LR, Polman R, Marchant D. Criteria for determination of maximal oxygen uptake: a brief critique and recommendations for future research. Sports Med. 2007; 37(12):1019–28. Epub 2007/11/22. https://doi.org/10.2165/00007256-200737120-00002 PMID: 18027991.

48. Zuccarelli L, Porcelli S, Rasica L, Marzorati M, Grassi B. Comparison between Slow Components of HR and V O2 Kinetics: Functional Significance. Med Sci Sports Exerc. 2018; 50(8):1649–57. Epub 2018/03/24. https://doi.org/10.1249/MSS.0000000000001612 PMID: 29570539.

49. Karvonen MJ, Kentala E, Mustala O. The effects of training on heart rate; a longitudinal study. Ann Med Exp Biol Fenn. 1957; 35(3):307–15. Epub 1957/01/01. PMID: 13470504.

50. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc. 2011; 43(7):1334–59. Epub 2011/06/23. https://doi.org/10.1249/MSS.0b013e318213fefb PMID: 21694556.

51. Christensen CC, Frey HM, Foenstelien E, Aadland E, Refsum HE. A critical evaluation of energy expenditure estimates based on individual O2 consumption/heart rate curves and average daily heart rate. Am J Clin Nutr. 1983; 37(3):468–72. Epub 1983/03/01. https://doi.org/10.1093/ajcn/37.3.468 PMID: 6829489.

52. Lafortuna CL, Lazzer S, Agosti F, Busti C, Galli R, Mazzilli G, et al. Metabolic responses to submaximal treadmill walking and cycle ergometer pedalling in obese adolescents. Scand J Med Sci Sports. 2010; 20(4):630–7. Epub 2009/08/27. https://doi.org/10.1111/j.1600-0838.2009.00975.x PMID: 19706003.

53. Hermansen L, Saltin B. Oxygen uptake during maximal treadmill and bicycle exercise. J Appl Physiol. 1969; 26(1):31–7. Epub 1969/01/01. https://doi.org/10.1152/jappl.1969.26.1.31 PMID: 5762873.

54. Hermansen L, Ekblom B, Saltin B. Cardiac output during submaximal and maximal treadmill and bicycle exercise. J Appl Physiol. 1970; 29(1):82–6. Epub 1970/07/01. https://doi.org/10.1152/jappl.1970.29.1.82 PMID: 4912876.

55. Abrantes C, Sampaio J, Reis V, Sousa N, Duarte J. Physiological responses to treadmill and cycle exercise. Int J Sports Med. 2012; 33(1):26–30. Epub 2011/11/05. https://doi.org/10.1055/s-0031-1285928 PMID: 22052028.

56. Spurr GB, Prentice AM, Murgatroyd PR, Goldberg GR, Reina JC, Christman NT. Energy expenditure from minute-by-minute heart-rate recording: comparison with indirect calorimetry. Am J Clin Nutr. 1988; 48(3):552–9. Epub 1988/09/01. https://doi.org/10.1093/ajcn/48.3.552 PMID: 3414570.

57. Li R, Deurenberg P, Hautvast JG. A critical evaluation of heart rate monitoring to assess energy expenditure in individuals. Am J Clin Nutr. 1993; 58(5):602–7. Epub 1993/11/01. https://doi.org/10.1093/ajcn/58.5.602 PMID: 8237863.

58. Coyle EF, Gonzalez-Alonso J. Cardiovascular drift during prolonged exercise: new perspectives. Exerc Sport Sci Rev. 2001; 29(2):88–92. Epub 2001/05/08. https://doi.org/10.1097/00003677-200104000-00009 PMID: 11337829.

59. Jones AM, Grassi B, Christensen PM, Krustrup P, Bangsbo J, Poole DC. Slow component of VO2 kinetics: mechanistic bases and practical applications. Med Sci Sports Exerc. 2011; 43(11):2046–62. Epub 2011/05/10. https://doi.org/10.1249/MSS.0b013e31821fcfc1 PMID: 21552162.

13

The effects of post activation potentiation warm-up and pre-shot routine programs on driving performance in amateur golfers

Wichai Yeemin*, Supatcharin Kemarat, Apiluk Theanthong

Faculty of Allied Health Sciences, Thammasat University, Bangkok, Thailand

* wichai.yee@allied.tu.ac.th

Abstract

The purpose of this study was to assess the effects of three different programs, i.e. active dynamic warm-up program plus functional resistance warm-up using Theraband plus pre-shot routine program (AFPR); pre-shot routine program (PR); and active dynamic warm-up program plus functional resistance warm-up using Theraband (AF) on driver club head speed, driving distance, and driving accuracy in the amateur golfers. Fifteen amateur golfers with an average age of 19.67 ± 0.89 years and 4.87 ± 1.77 points of average handicap were assigned to participate in either AFPR, PR or AF program. All participants in the three programs practiced three sessions on non-consecutive days per week during the intervention phase. Each participant's performance was assessed before and after six weeks of the program through hitting ten maximal drives with the ball flight and swing analyzed using the P3ProSwing Golf Simulator and recorded for the driver club head speed, driving distance, and driving accuracy. Multivariate analysis of variance showed no statistically significant differences ($P < .05$) of the performances of the golfers participated in the 3 programs (club head speed: $F = 1.02$, $P = 0.33$; accuracy: $F = 0.32$, $P = 0.72$; distance: $F = 0.18$, $P = 0.83$). Furthermore, a paired t-tests also showed no statistically significant ($P < .05$) improvement occurred in the 3 programs after the six-week training. Although the effect of the 3 programs did not show statistically significant increase in the performance of the amateur golfers, however, the three parameters of the performance, i.e. the driver club head speed, the driving distance and the driving accuracy showed certain improvements. The 3 training programs may have benefit to the amateur golfers with certain increases of their performance.

Editor: Feng Chen, Tongii University, CHINA

Competing interests: The authors have declared that no competing interests exist.

Introduction

Golf is an extremely popular sport played across the world regardless of gender, skill level or age. It also involves a wide variety of shots to master (e.g., driving, chipping, and putting) [1]. Extended periods between shots, and competitive situations that could be distracting and destructive performance [2]. Therefore, golf presents participants with both cognitive and behavioral challenges [3]. Successful golfers have been identified as having the ability to hit the ball effectively. An effective driving depends on many factors such as driver club head speed, driving distance, and driving accuracy. These factors can be developed through the thorough physical and mental training programs. For example, flexibility, power, and strength training can improve driving distance and driver club head speed [4]. A short-term activation in a warm-up program can improve driving distance and driver club head speed [5]. Moreover, sports science studies found that warming-up combining with post-activation potentiation (PAP) technique can improve the efficiency of the movement [6–10]. The effect of utilizing the PAP technique combined with a warm-up on golf performance can be seen by Tilley and Macfarlane's study [11]. The study found that Active Dynamic warm-up program PLUS 10-minute functional resistance warm-up using Theraband leads to a significant increase in immediate performance of certain factors of the golf drive such as driving distance, drivers club head speed, and driving accuracy. In addition to utilizing the PAP technique combined with a warm-up, effective driving can be improved by controlling the psychological state while hitting the ball. However, it was usually found that the golfers had the difficulty to control the psychological state during the competition due to the stress and the anxiety that they had to face. Tanaka and Sekiya [12] revealed that the pressure condition increased physiological arousal, decreased the amplitudes of arms and club movements on the backswing, and decreased the club movement speed on the fore-swing. One specific cognitive-behavioral strategy used in golfing is a pre-performance routine or pre-shot routine [13]. The use of pre-shot routines is effective in improving the performance of skilled participants across several sports [2, 13–24]. Particularly, the pre-shot routine that focuses on both physical and mental (imagery, breathing control, and self-talk) preparation will be a good effect on driving performance [13].

As mentioned above, it could be assumed that the development of an effective driving could be achieved through the collaboration among the active dynamic warm-up, the post-activation potentiation (PAP) technique, and the pre-shot routine that focused on physical and mental preparation. Currently, however, there has been no studies on the effect of utilizing dynamic warm-up programs combined with the post-activation potentiation (PAP) technique and the pre-shot routine on driving performance of the golfers. Therefore, the purpose of this study was to assess the effects of three different programs, i.e. active dynamic warm-up program plus functional resistance warm-up using Theraband plus pre-shot routine program (AFPR), pre-shot routine program (PR), and active dynamic warm-up program plus functional resistance warm-up using Theraband (AF) on driver club head speed, driving distance and driving accuracy in the amateur golfers. It was hoped that the results of the study can be used as data and guidelines for improving and increasing the efficiency of driving of the golf players.

Materials and methods

Participants

The Ethics Committee of Thammasat University certified that the study was accomplished according to the Declaration of Helsinki as revised in 2013. The G*Power software version 3.1 was used to determine the sample size by using data of Tilley and Macfarlane's study [11]. The following parameters were selected: large effect size (f = 0.85), an alpha level of .05, a power level of 0.8, and three groups. The total sample size was determined to be at least 12. However, the total sample size of this study was 15 in case of any dropout of the participants and to conform to the Tilley and Macfarlane's study [11]. Participants in this study included 15 amateur golfers (14 males, 1 female) who were an average of 19.67 ± 0.89 years of age and 4.87 ± 1.77 of the golf club handicap (Table 1). All participants met the inclusion criteria which included being a member of the Thammasat University golf club, aged between 18–23 years old, possessing an official golf club handicap at the time of the study between 0–10, competing for at least 3 times per year in the golf events organized by the public or private sector, no current

Table 1. Participant characteristics.

Experimental group	N	Age (yrs.)	Handicap	Experience (yrs.)
AFPR	5	20.20±1.10	4.80±1.64	8.20±1.64
PR	5	19.60±0.55	5.00±2.55	7.00±0.71
AF	5	19.20±1.10	4.80±1.48	8.40±1.52
Total	15	19.67±0.89	4.87±1.77	7.87±1.46

physical injuries, no medical problem history of the upper and the lower limbs during 3 months before the start of the study, be willing to participate in the study, no barriers to communication. All participants were assigned either to AFPR (n = 5) or PR (n = 5) or AF (n = 5) group by sorting the average value of club head speed, which was derived from 10 times driving (thirty-second rest intervals between each shot) and randomly assigned to the experimental groups.

Measurements

The pre- and post-test of golf driving measurements were analyzed by a P3ProSwing Golf Simulator located in a golf lab at the Sports Science and Sports Development Department, Thammasat University. The participant performed a full swing and hit a real golf ball to a screen. The screen presented a fairway, on which the ball was placed. It also presented a green and a hole with a pin and a flag. A visual trajectory line of the golf ball flied to the final position was displayed on the screen.

The golf ball was shot from a 22.9 cm x 35.6 cm sensing platform with 1.5 cm high artificial grass on top. The platform contained 65 optical sensors that detected the information about the speed and direction of the club head at the ball impact. The simulator estimated the distance and direction for each shot. The simulator accurately monitored the ball flight with 99% precision [25].

Procedures

For each golf shot, the distance, the club head speed at the ball impact [11] and the accuracy in terms of the direction [25] were measured. The collection of data of the pre-test was one week before participating in the program and the post-test was one week after participating in the program. All participants used their own drivers. They were informed that the same driver and under the same conditions would apply for the post-test. Before the pre-test measurement started, they could take practice shots with their drivers to familiarize themselves with the new surface and the artificial environment. At the start of the measurement, the participants were instructed to aim for the pin and to proceed at their own pace. All golfers performed 10 test shots with thirty-second rest intervals between each shot. The same procedure was used during the post-test. After the pre-test, the participants attended the study venue on three separate, non-consecutive days (maximum of 1 hour required at each session) over a maximum of 6-week period at the Sports Science and Sports Development Department, Thammasat University. The set up for the exercises was identical at each session for each participant. At every session, there was at least one researcher present for safety and supervising the program with the participants. The participants carried out one of the three programs shown below (Table 2), during each session:

Statistical analysis

A Shapiro-Wilk test was conducted to verify if all the data met the normality test assumption. Comparing driver club head speed, driving distance and driving accuracy among the three

Table 2. Experimental programs.

Activity details	Experimental group		
	AFPR	PR	AF
Active dynamic warm-up program PLUS functional resistance warm-up using Theraband	✓	-	✓
1. Running on a treadmill at 60% of maximum heart rate for 5 minutes			
2. Theraband (red) exercises (Each exercise conducted for 10 times and repeated for 2 sets with 30-second rest between set).			
• trunk rotation movement in standing • standing lunge and trunk rotation movement • right arm cross chest adduction and internal rotation with body rotation • left arm external rotation and shoulder abduction with rotation • wood chops from the right and left trunk rotation			
3. Ten practice swing shots with a driver (slow to speed pace and 30-second rest between shot).			
Pre-shot routine program	✓	✓	-
1. Taking a deep breath with tempo 1-2-3-4 during inhalation and 8-7-6-5-4-3-2-1 during exhalation and aiming to the pin			
2. Performing the practice swings at their own pace			
3. Addressing the ball and the set-up position, grip checking, and eye contact with the ball.			
4. Aiming to the pin and taking a deep breath with tempo 1-2-3-4 during inhalation and 8-7-6-5-4-3-2-1 during exhalation			
5. Performing the full swing (back swing, down swing, follow through)			
Twenty practice swings shot with a driver in the Golf Simulator	✓	✓	✓

programs by using a multivariate analysis of variance MANOVA test [26, 27]. Paired t-tests were then performed between each of the three sets of data to identify within-group differences between the data for each program [25]. An alpha value of $p < 0.05$ was set as the criterion level of significance.

Results

The subjects in this study were tested for driver club head speed, driving distance, and driving accuracy after AFPR, PR and AF treatments. The result of MANOVA revealed no statistically significant differences for the driver club head speed ($p = 0.33$), driving distance ($p = 0.83$), and driving accuracy ($p = 0.72$) between the three programs (Table 3). Paired t-tests between pre and post-treatment were used to further investigate the three factors of performance that showed no statistically significant differences. The result showed that no statistically significant differences of the three factors were seen between the three programs for the pre- and post-tests. However, the mean results did show improved figures for these three performance factors with post-intervention. The analysis showed that the AFPR had increased driving distance by 4.1 yards ($p = 0.70$) and driving accuracy by 3.87 yards over the pre-treatment ($p = 0.82$). The PR had increased the driver club head speed by 0.19 miles/hour ($p = 0.82$), the driving distance by 10.79 yards ($p = 0.17$), and the driving accuracy by 1.18 yards compared to the pre-

Table 3. Comparison of treatments on the golf performance variables after 6 weeks of training.

	Experimental group			F	P
	AFPR	PR	AF		
Club head speed	110.47±5.33	107.78±6.00	103.19±10.22	1.02	0.33
Distance	259.89±16.18	257.21±16.48	251.93±28.11	0.18	0.83
Accuracy	23.58±20.58	30.85±22.33	21.29±14.66	0.32	0.72

Table 4. Comparison of the golf performance variables prior to training and after 6 weeks of training.

Club head speed				
Experiment group	**Prior to training**	**After 6 weeks**	**t**	**P**
AFPR	110.63±5.53	110.47±5.33	0.33	0.75
PR	107.59±7.01	107.78±6.00	-0.23	0.82
AF	102.87±9.75	103.19±10.22	0.47	0.66
Distance				
Experiment group	**Prior to training**	**After 6 weeks**	**t**	**P**
AFPR	255.79±12.53	259.89±16.18	-0.40	0.70
PR	246.42±27.52	257.21±16.48	-1.64	0.17
AF	229.54±34.27	251.93±28.11	-2.77	0.05
Accuracy				
Experiment group	**Prior to training**	**After 6 weeks**	**t**	**P**
AFPR	27.45±26.09	23.58±20.58	0.23	0.82
PR	32.03±9.80	30.85±22.33	0.13	0.90
AF	43.89±27.45	21.29±14.66	2.04	0.11

treatment (p = 0.90). The AF had increased the driver club head speed by 0.32 miles/hour (p = 0.66), the driving distance by 22.39 yards (p = 0.05), and the driving accuracy by 22.60 yards (p = 0.11) over the pre-treatment (Table 4).

Discussion

The purpose of this study was to assess the effects of three different programs, i.e. AFPR, PR and AF on driver club head speed, driving distance and driving accuracy in amateur golfers. The results of this study revealed improvement in the driver club head speed, the driving distance, and the driving accuracy when compared to the pre-intervention. As there has been no previous research on the performance of utilizing dynamic warm-up programs combined with the post-activation potentiation (PAP) technique and the pre-shot routine on later driving performance, therefore, it is difficult to draw a comparison to the other studies. Driving performance has been shown to have a positive relationship with the driver club head speed, the driving distance, and the driving accuracy. Several studies have used these factors as an indicator of driving performance [4, 11, 28, 29]. The development of each factor can be achieved through physical and mental training. For the club head speed, it can be achieved through training warm-up programs [5–8, 11, 28] and pre-physical and mental routine [13, 14, 16]. However, there is no study focuses on a combination of both programs. Therefore, this study tried to explore the effect of training warm-up and pre-shot routine together and compared to solely train either warm-up program or pre-shot routine program. The result of this study showed that the three programs did not significantly affect the club head speed. The difference between this study and the previous research is the duration of the test in which conducted after ending the one-week programs. This may be a reason why training the three programs in this study led to little improvement in the parameters of the driving performance. Usually, the study of the effect of pre-shot routine and warm-up programs were done in an acute phase, which was tested immediately after the end of the program [6, 7, 11, 13]. An increase in the training period for more than six weeks could be a technique to obtain a different result. Cohn et al. suggested that the training routine of physical and mental skills should be practiced for 16 weeks or more because it would show the change in the performance of the golf players [2]. Although there was no significant difference after the sixth weeks, however, this study found that the club head speed was increased in the PR and AF groups. This suggested that the three programs developed as part of this study helped to increase the club head speed score. This supports the previous study that had suggested an increment in the club head speed score with the implementation of the active dynamic warm-up, warm-up with post-activation potentiation (PAP) technique, and the pre-shot routine [4, 5, 11, 13, 30] and increasing the athletes performance [6–8, 31].

Another factor related to the driving performance is the driving distance which varies according to the club head speed score [29]. This study found that the driving distance had little improvement according to the club head speed score after participating for six weeks in the programs. In addition, the findings of this study are similar to the previous research which found that the driving distance before practicing driving skills, pre-shot routine, and practicing driving skills in conjunction with pre-shot routine programs were not significantly different after the five-week training [13]. Based on the previous research, the amateur golf player's ability to play golf would not increase or change immediately after a 14-week physical and psychological skill training, but after 16 weeks [2]. It would appear that the duration of the training of the three programs in this study should be increased in order to allow the amateur golfers for a more adaptation. Although there was no statistical significant difference in the performances of the golfers after the sixth weeks, however, this study found that the driving distance was increased in all experimental groups especially the AF and PR programs with a rather distinct improvement (22.39 yards and 10.79 yards, respectively). An explanation for the improvement in the driving distance seen in this study by adding of AF program is that the effect of the warm-up training program throughout the sixth weeks led to the improved physiological factors such as strength, balance, and flexibility [4, 10, 28] and neurological activity of the skeletal muscles [11]. This, in turn, may have resulted in the greater coordination, the force production, and the driving performance in relation to the driving distance. This finding is similar to the previous study which found that the distance in the golf driving increased after the warm-up program [11]. An explanation for the improvement in the driving distance seen in this study by adding of the PR program is that focusing on various activities associated with the driving led to the improved concentration during the driving. Besides, the practice of pre-shot routine also allowed the movement mechanism while hitting the ball to work more automatically [32]. As a result, swinging the ball to the sweet spot was more accurate and the smash factor was increased. These two factors affected the increase in the driving distance [11]. As mentioned above, it can be seen that the effect of AF and PR program trainings is consistent with the results of the past studies. However, when the training program which combined these two types of the exercises, a little improvement in the driving distance (4.1 yards) was found. There are a number of factors that could have contributed to this outcome. The complexity of the training program, the number of the activities, and the duration of the training could cause the amateur golfers not to connect the skills and led to the obvious changes. This supports the previous research which suggested that the amateurs may immediately recognize and learn new strategies and tactics that excellent athletes used but obvious developments and changes would occur after more than 14 weeks of training [33].

The last factor of this study related to the driving performance is the driving accuracy. Results showed no statistically significant differences between the three programs. Thus, the cumulative effects of training the three programs for six weeks on the driving accuracy cannot be supported. An existing study reported that the advantageous effect of the 14-week cognitive-behavioral intervention program did not immediately improve the performance in the elite collegiate golfers. Improvements in the performance were reported in this particular study; however, in a 4-month follow-up, the researchers acknowledged that intervening variables may have confounded these improvements [2]. It has been suggested that the extended periods may be required for the internalization of cognitive-behavioral performance strategies

[2, 33]. This may explain the findings in the present study, in that extended time may be required to relegate well-established strategies, and learn and adjust to new ones [2]. The previous study reported the significant improvements in putting performance among the novice golfers, utilizing a cognitive-behavioral intervention in the later stages of a 14-week study [33]. These improvements were maintained over a period, with a change in the behavior indicative of motor skill learning [13]. Although there was no statistically significant difference after the sixth weeks in which PR program according to previous research [20, 21, 23], however, this study found that the driving accuracy was increased in all experimental groups especially in the AF program, which had a rather distinct improvement (22.60 yards). An explanation for the improvement in the driving accuracy seen in this study by adding of AF program is that the effect of training the warm-up program throughout the six weeks led to improved physiological factors such as strength, balance, and flexibility [4, 28]. These are factors that create the power to hit the ball with distance and accuracy [34]. A disparate result for the cumulative effects of AFPR program may be the complexity of the training program and the amount of activity that was abundant. Thus, the training duration was not enough to connect the skills and change. Reasons for the ineffectiveness of the PR program may be the duration of the post-test which was determined one week after the training program to assess the accuracy that occurred after the training program had stopped. This study is different from the previous studies, which typically studied the effects of the pre-shot routine program in an acute phase by testing immediately after the program ended [13, 23]. Thus, the results of driving accuracy in this study are different from the previous studies.

Our results are in line with the later evidence showing that the limited time and the complex intervention dose inhibited a cumulative benefit. This implies that providing an amateur golfer with a single, easy-to-understand technique with limited time depends on their learning is a good option in the applied settings for intervention development because it is likely to give the amateur golfer to relegate the well-established strategies, and learn and adjust to the new ones. As noted above, the results suggested that the AFPR, PR, and AF developed as part of this study helped to improve the club head speeds, the driving distance, and the driving accuracy. Although the results of this study showed small increases in the club head speed, the driving distance, and the driving accuracy of the amateur golf players, however, the improvement was in a good direction and also supported the previous studies. The results of this study can be incorporated into the daily practice of the coaches with the amateur golfers when helping them to devise their driving training program. More research is required to identify the latent effect of these programs, the optimum duration of the warm-up and pre-shot routine, and the optimum components of the exercises such as the repetitions, the level of resistance, the equipment used, and the mental techniques used. Additionally, further research involving the use of these types of exercises in structured training programs over a longer period and their effect on the performance would also be beneficial.

Conclusions

The findings of the present study showed that amateur golfers were able to improve the speed of the club head, the driving distance and the driving accuracy following a 6-week acquisition phase utilizing either AFPR, PR or AF program. However, statistically significant improvements in performance were not found in the 6-week programs. Although this study attempted to provide a robust design, there are limitations that may have affected our results such as the small number of the participants within the experimental groups, the complexity of the intervention, and the limited time of the training. Increasing the duration of the training is an option to improve the club head speed, the driving distance, and the driving accuracy.

Moreover, the introduction of the program that combines pre-shot routine and warm-up training should be done after the amateur golfers have solely learned a pre-shot routine and warm-up programs, as the golfers have known how to use both techniques and have the skills to use them. Thus, the connection between the two techniques is more facile.

Further research may be carried out by increasing the duration of the training for more than 6 weeks to see the cumulative effects over the longer periods. The sample size should be increased to infer to the population more reliable. Besides, further study may be extended to the professional golfers to explore the effects on the different levels of the performance.

Acknowledgments

The authors would like to gratefully acknowledge the assistance provided by the Department of Sport Science and Sport Development, Faculty of Allied Health Sciences, Thammasat University. We also gratefully acknowledge the support of all volunteers participated in this study.

Author Contributions

Conceptualization: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

Data curation: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

Formal analysis: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

Funding acquisition: Wichai Yeemin.

Investigation: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

Methodology: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

Project administration: Wichai Yeemin.

Resources: Wichai Yeemin.

Supervision: Wichai Yeemin.

Validation: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

Visualization: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

Writing – original draft: Wichai Yeemin.

Writing – review & editing: Wichai Yeemin, Supatcharin Kemarat, Apiluk Theanthong.

References

1. Gryc T, Zahalka F, Maly T, Mala L, Hrasky P. Movement's analysis and weight transfer during the golf swing. Journal of Physical Education and Sport. 2015; 15(4):781–7. https://doi.org/10.7752/jpes.2015.04119
2. Cohn PJ, Rotella RJ, Lloyd JW. Effects of a cognitive-behavioral intervention on the preshot routine and performance in golf. Sport Psychol. 1990; 4(1):33–47. https://doi.org/10.1123/tsp.4.1.33
3. Singer RN. Strategies and metastrategies in learning and performing self-paced athletic skills. Sport Psychol. 1988; 2(1):49–68. https://doi.org/10.1123/tsp.2.1.49
4. Gordon BS, Moir GL, Davis SE, Witmer CA, Cummings DM. An Investigation into The Relationship of Flexibility, Power, and Strength to Club Head Speed in Male Golfers. J Strength Cond Res. 2009; 23 (5):1606–10. https://doi.org/10.1519/JSC.0b013e3181a3c39d PMID: 19620901
5. Fradkin A, Sherman C, Finch C. Improving golf performance with a warm up conditioning programme. Br J Sports med. 2004; 38(6):762–5. https://doi.org/10.1136/bjsm.2003.009399 PMID: 15562177
6. Hodgson M, Docherty D, Robbins D. Post-activation potentiation: underlying physiology and implications for motor performance. Sports Med. 2005; 35(7):585–95. https://doi.org/10.2165/00007256-200535070-00004 PMID: 16026172

7. Rixon KP, Lamont HS, Bemben MG. Influence of type of muscle contraction, gender, and lifting experience on postactivation potentiation performance. J Strength Cond Res. 2007; 21(2):500–5. https://doi.org/10.1519/R-18855.1 PMID: 17530946
8. Zois J, Bishop DJ, Ball K, Aughey RJ, Zois J, Bishop DJ, et al. High-intensity warm-ups elicit superior performance to a current soccer warm-up routine. J Sci Med Sport. 2011; 14(6):522–8. https://doi.org/10.1016/j.jsams.2011.03.012 PMID: 21907619
9. Anthi X, Dimitrios P, Christos K. On the mechanisms of post-activation potentiation: the contribution of neural factors. Journal of physical education and sport. 2014; 14(2):134–7. https://doi.org/10.7752/jpes.2014.02021
10. Xenofondos A, Laparidis K, Kyranoudis A, Galazoulas C, Bassa E, Kotzamanidis C. Post-activation potentiation: Factors affecting it and the effect on performance. Journal of Physical Education and Sport. 2010; 28(3):32–8.
11. Tilley NR, Macfarlane A. Effects of different warm-up programs on golf performance in elite male golfers. Int J Sports Phys Ther. 2012; 7(4):388–95. PMID: 23936749
12. Tanaka Y, Sekiya H. The influence of audience and monetary reward on the putting kinematics of expert and novice golfers. Res Q Exerc Sport. 2010; 81(4):416–24. https://doi.org/10.1080/02701367.2010.10599702 PMID: 21268465
13. McCann P, Lavallee D, Lavallee RM. The Effect of Pre-Shot Routines on Golf Wedge Shot Performance. Euro J Sport Sci. 2001; 1(5):1–10. https://doi.org/10.1080/17461390100071503
14. Boutcher SH, Crews DJ. The effect of a preshot attentional routine on a well-learned skill. Int J Sport Psychol. 1987; 18(1):30–9.
15. Crews DJ, Boutcher SH. Effects of structured preshot behaviors on beginning golf performance. Percept Mot Skills. 1986; 62(1):291–4. https://doi.org/10.2466/pms.1986.62.1.291
16. Lobmeyer D, Wasserman E. Preliminaries to free throw shooting: Superstitious behavior. J Sport Behav. 1986; 9(2):70–8.
17. Cotterill S. Pre-performance routines in sport: Current understanding and future directions. Int Rev Sport Exerc Psychol. 2010; 3(2):132–53. https://doi.org/10.1080/1750984X.2010.488269
18. Cotterill ST. Experiences of developing pre-performance routines with elite cricket players. J Sport Psychol Action. 2011; 2(2):81–91. https://doi.org/10.1080/21520704.2011.584245
19. Cotterill ST, Sanders R, Collins D. Developing effective pre-performance routines in golf: Why don't we ask the golfer? J Appl Sport Psychol. 2010; 22(1):51–64. https://doi.org/10.1080/10413200903403216
20. Hazell J, Cotterill ST, Hill DM. An exploration of pre-performance routines, self-efficacy, anxiety and performance in semi-professional soccer. Eur J Sport Sci. 2014; 14(6):603–10. https://doi.org/10.1080/17461391.2014.888484 PMID: 24559097
21. Wergin VV, Beckmann J, Gröpel P, Mesagno C. Investigating cumulative effects of pre-performance routine interventions in beach volleyball serving. PloS one. 2020; 15(1):e0228012. https://doi.org/10.1371/journal.pone.0228012 PMID: 31968003
22. Lee S, Lee K, Kwon S. Developing and Instructing Pre-Performance Routines for Tenpin Bowling Competitions. Percept Mot Skills. 2015; 120(3):673–86. https://doi.org/10.2466/30.25.PMS.120v14x5 PMID: 25932945
23. Mesagno C, Hill DM, Larkin P. Examining the accuracy and in-game performance effects between pre- and post-performance routines: A mixed methods study. Psychol Sport Exerc. 2015; 19:85–94. https://doi.org/10.1016/j.psychsport.2015.03.005
24. Mesagno C, Marchant D, Morris T. A pre-performance routine to alleviate choking in "choking-susceptible" athletes. Sport Psychol. 2008; 22(4):439–57. https://doi.org/10.1123/tsp.22.4.439
25. Sommer M, Rönnqvist L. Improved motor-timing: effects of synchronized metronome training on golf shot accuracy. J Sports Sci Med. 2009; 8:648–56. PMID: 24149608
26. Wen H, Sze N, Zeng Q, Hu S. Effect of music listening on physiological condition, mental workload, and driving performance with consideration of driver temperament. Int J Environ Res Public health. 2019; 16(15):2766. https://doi.org/10.3390/ijerph16152766 PMID: 31382474
27. Chen F, Peng H, Ma X, Liang J, Hao W, Pan X. Examining the safety of trucks under crosswind at bridge-tunnel section: A driving simulator study. Tunn. Undergr. Space Technol. 2019; 92:103034. https://doi.org/10.1016/j.tust.2019.103034
28. Marshall KJ, Llewellyn TL. Effects of Flexibility and Balance on Driving Distance and Club Head Speed in Collegiate Golfers. Int J Exerc Sci. 2017; 10(7):954–63. PMID: 29170697
29. Gergley JC. Acute effects of passive static stretching during warm-up on driver clubhead speed, distance, accuracy, and consistent ball contact in young male competitive golfers. J Strength Cond Res. 2009; 23(3):863–7. https://doi.org/10.1519/JSC.0b013e3181a00c67 PMID: 19387392

30. Perkins-Ceccato N, Passmore SR, Lee TD. Effects of focus of attention depend on golfers' skill. J Sports Sci. 2003; 21(8):593–600. https://doi.org/10.1080/0264041031000101980 PMID: 12875310

31. Pochai P, Ruangthai R, Phimphaphorn P. The effect of post activation potentiation warm up on speed and h-reflex. J Sports Sci. Technol. 2015; 15(2):123–32. (Thai).

32. Boutcher SH. Attention and athletic performance: An integrated approach. In: Horn TS, editor. Advances in sport psychology: Human Kinetics Publishers; 1992. p. 251–65.

33. Beauchamp PH, Halliwell WR, Fournier JF, Koestner R. Effects of cognitive-behavioral psychological skills training on the motivation, preparation, and putting performance of novice golfers. Sport Psychol. 1996; 10(2):157–70. https://doi.org/10.1123/tsp.10.2.157

34. Lephart SM, Smoliga JM, Myers JB, Sell TC, Tsai Y-S. An eight-week golf-specific exercise program improves physical characteristics, swing mechanics, and golf performance in recreational golfers. J Strength Cond Res. 2007; 21(3):860–9. https://doi.org/10.1519/R-20606.1 PMID: 17685707

14

Subconcussive head impact exposure between drill intensities in U.S. high school football

Kyle Kercher[1], **Jesse A. Steinfeldt**[2], **Jonathan T. Macy**[1], **Keisuke Ejima**[3], **Keisuke Kawata**[4,5]*

1 Department of Applied Health Science, School of Public Health-Bloomington, Indiana University, Bloomington, Indiana, United States of America, **2** Department of Counseling and Educational Psychology, School of Education, Indiana University, Bloomington, Indiana, United States of America, **3** Department of Epidemiology and Biostatistics, School of Public Health-Bloomington, Indiana University, Bloomington, Indiana, United States of America, **4** Department of Kinesiology, School of Public Health-Bloomington, Indiana University, Bloomington, Indiana, United States of America, **5** Program in Neuroscience, College of Arts and Sciences, Indiana University, Bloomington, Indiana, United States of America

* kkawata@indiana.edu

Editor: Markus Geßlein, Paracelsus Medizinische Privatuniversitat - Nurnberg, GERMANY

Abstract

USA Football established five levels-of-contact to guide the intensity of high school football practices. The objective of this study was to examine head impact frequency and magnitude by levels-of-contact to determine which drills had the greatest head impact exposure. Our primary hypothesis was that there would be an incremental increase in season-long head impact exposure between levels-of-contact: *air*<*bags*<*control*<*thud*<*live*. This observational study included 24 high-school football players during all 46 practices, 1 scrimmage, 9 junior varsity and 10 varsity games in the 2019 season. Players wore a sensor-installed mouthguard that monitored head impact frequency, peak linear acceleration (PLA), and rotational acceleration (PRA). Practice/game drills were filmed and categorized into five levels-of-contact (*air*, *bags*, *control*, *thud*, *live*), and head impact data were assigned into one of five levels-of-contact. Player position was categorized into lineman, hybrid, and skill. A total of 6016 head impacts were recorded during 5 levels-of-contact throughout the season. In the overall sample, total number of impacts, sum of PLA, and PRA per player increased in a near incremental manner (*air*<*bag*s<c*ontrol* = *thud*<*live*), where *live* drills had significantly higher cumulative frequency (113.7±17.8 hits/player) and magnitude [2,657.6±432.0 *g* (PLA), and 233.9 ± 40.1 krad/s^2 (PRA)] than any other levels-of-contact, whereas air drills showed the lowest cumulative frequency (7.7±1.9 hits/player) and magnitude [176.9±42.5 *g* (PLA), PRA 16.7±4.2 krad/s^2 (PRA)]. There was no significant position group difference in cumulative head impact frequency and magnitude in a season. Although there was no difference in average head impact magnitude across five levels-of-contact and by position group PLA (18.2–23.2*g*) and PRA (1.6–2.3krad/s^2) per impact], high magnitude (60-100*g* and >100*g*) head impacts were more frequently observed during *live* and *thud* drills. Level-of-contact influences cumulative head impact frequency and magnitude in high-school football, with players incurring frequent, high magnitude head impacts during *live*, *thud*, and *control*. It is important to consider level-of-contact to refine clinical exposure guidelines to minimize head impact burden in high-school football.

Competing interests: The authors have declared that no competing interests exist.

Introduction

The long-term consequence of sport-related head injury is a complex public health issue with no concrete solution [1, 2]. Despite inherent risk of head injury in contact sports (e.g., American football, hockey, soccer), participating in these team sports, especially during developmental age, provides well-documented benefits, including higher levels of physical activity, improved mental health, and lower likelihood of smoking cigarettes and using illegal substances [3]. In 2017, in an attempt to promote a safer football environment, USA Football (the national governing body over amateur football) developed a modified version of tackle football to introduce kids to the sport by reducing the field size and number of players, as well as rule changes to increase activity, game play, and learning [4]. These types of safety modifications are further substantiated in high school, college, and professional football. For example, in 2018 kickoff rules were adjusted to reduce injuries due to high concussion incidence during kickoff plays [5], players must wear helmets that meet certain laboratory safety standards, and a hit to the head or neck area and blindside blocking are prohibited [6, 7]. Consistent with these adjustments, concussion and catastrophic injury rates have been reduced [8, 9]; however, subconcussive head impact exposure has proven more complex.

Subconcussive head impact is defined as a hit to the head that does not induce overt concussion symptoms [10]. These head impacts are most common in American football, where athletes can experience several hundred impacts with some exceeding 1,000 head impacts in a single season [11]. Evidence has emerged to indicate that both high school and college football players with frequent experience of subconcussive head impacts exhibit neuronal microstructural damage [12, 13], abnormal brain activation [14, 15], ocular-motor impairment [16], and elevation in brain-injury blood biomarkers [17]. One line of research suggests that long-term exposure to these hits is a key factor in developing neurodegenerative disorders later in life [18, 19]. Although USA Football (for high school) and the NCAA (for college) have eliminated practicing two times in the same day (two-a-days) to minimize head impact frequency, one study found that total head impact frequency during a summer camp increased by 26% [20]. Similarly, a policy change to reduce the number of preseason practices from 29 to 25 failed to reduce head impact frequency in college football players, with one team's cumulative head impacts increasing up to 35% [21]. These mixed results highlight the need to dissect football practices to increase our understanding of what type of contact drills and intensities cause the greatest frequency and magnitude of subconcussive head impacts.

USA Football has identified five levels-of-contact (*air*, *bag*, *control*, *thud*, *live*) that define the intensities and structure of football practices nationwide. The levels-of-contact were designed to guide effective practice schedules through a step-by-step approach to teach fundamental football skills [22]. The National Federation of State High School Associations implemented the levels-of-contact in their high school football practice guidelines beginning in 2014. However, it remains unknown whether, and to what extent, different levels-of-contact influence head impact frequency and magnitude in high school football players across an entire season and between position groups.

Therefore, we conducted a longitudinal observational study to examine cumulative head impact frequency and magnitude across different drill intensities in high school football players over the course of a single season. Our primary hypothesis was that there would be an incremental increase in season-long head impact frequency and magnitude between levels-of-contact, with *live* recording the greatest head impact exposure and air recording the lowest: *air* < *bags* < *control* < *thud* < *live*. Since the proximity to opponents and nature of contact during nearly every play for linemen [23], we also tested our secondary hypothesis that there would be a group difference in head impact frequency, in which linemen would have greater head impact frequency in most levels-of-contact, compared to the hybrid and skill positions. Our exploratory aim was to identify the average head impact magnitudes by levels-of-contact and position group, as well as to identify frequency of head impacts that were within 25–60 *g*, 60–100 *g*, or >100 *g* in each level-of-contact.

Methods

Participants

This single-site, observational study included 24 male high school football players at Bloomington High School-North. The study was conducted during the 2019 football season including practices and games during the pre-season, in-season, and playoffs. None of the 24 players was diagnosed with a concussion during the study period as confirmed by team athletic trainer and physician. Inclusion criterion was being an active football team member which was defined as any player, freshmen through seniors, planning to participate in the 2019 season. Exclusion criteria included a history of head neck injury (including concussion) in the previous year or any neurological disorders, although no participant met any exclusionary criteria. The Indiana University Institutional Review Board and the Monroe County Community School Corporation Research Review Board approved the study, and all participants and their legal guardians gave written informed consent.

Study procedures

At the preseason data collection, self-reported demographic information (age, race/ethnicity, height, weight, number of previously diagnosed concussions, and years of experience in various contact sports, including tackle football) were obtained. Participants were custom-fitted with the Vector mouthguard (Athlete Intelligence, Inc.) that measured the number of hits and magnitude of head linear and rotational acceleration. Participants wore the mouthguard for all practices (n = 46), scrimmage (n = 1), and all games (n = 9 junior varsity, n = 10 varsity) from pre-season training camp (August 13, 2019) to the end of the season (November 1, 2019). The mean (SD) practice duration was 105 (20.5) minutes in duration. Video data were collected using Hudl (Agile Sports Technologies, Inc.) during the same timeframe as subconcussive head impact data collection. Participants' playing positions were verified by team coaches and categorized into three groups as follows: 11 linemen athletes (defensive lineman, offensive lineman), 7 hybrid athletes (tight end, linebacker, running back), and 6 skill athletes (wide receiver, defensive back), which is in line with prior literature [24, 25]. No quarterbacks participated in this study. In accordance with USA Football guidelines [26], head impacts were categorized by levels-of-contact: *air*, *bags*, *control*, *thud*, and *live*. See Levels-of-Contact and Film Review section for more details and supplemental file A for example video for each level-of-contact.

Head impact measurement

This study used an instrumented Vector mouthguard for measuring frequency of head impacts as well as linear and rotational head accelerations during impacts, as previously described [27].

The mouthguard employs a triaxial accelerometer (ADXL377, Analog Devices) with 200 *g* maximum per axis to sense linear acceleration. For rotational acceleration, a triaxial gyroscope (L3GD20H, ST Microelectrics) was employed. An impact is detected when a linear acceleration magnitude exceeds 10.0 *g* for three consecutive samples (sampling every 0.2 milliseconds). All impact with a standard hit duration of 96 milliseconds were transmitted wirelessly through the antenna transmitter to the sideline antenna and computer, then stored on a secure internet database. The Vector mouthguard is installed with an in-mouth sensor to ensure that data acquisition occurs only when the mouthguard is securely fitted in one's mouth. Linear acceleration data were transformed within the Athlete Intelligence software to the head's center of gravity based on the 50th percentile male. From raw impact data extracted from the server, the number of hits, peak linear acceleration (PLA) of each hit, and peak rotational acceleration (PRA) of each hit were used for analyses. Kinematic accuracy of the prototype of Vector mouthguard [16] showed an excellent correlation with the matched data from an anthropomorphic testing device (crash test dummy) [28, 29]. When the mouthguard, headgear-mounted, and skin-patch sensors were compared to high speed video during soccer headings, the mouthguard showed superior skull coupling (displacement < 1 mm) compared to headgear ($<$ 13mm) and skin patch ($<$ 4mm) for the ear canal reference point [30]. A researcher was present during all practices to track when the practice shifted between levels-of-contact, and head impact data were categorized into each level-of-contact by corresponding timestamps of head impact to timeframes of each level-of-contact.

Levels-of-contact and film review

The five levels-of-contact are *air*, *bags*, *control*, *thud*, and *live* with *air* being estimated to have the lowest intensity and *live* being the highest [26]. *Air* is defined as drills being run unopposed and without contact. *Bags* is defined as drills being run against a bag or soft-contact surface. *Control* is defined as drills being run at an assigned speed until the moment of contact. It does not involve tackling, rather contact is above the waist and players stay on their feet. *Thud* is defined as drills being run at a competitive, fast speed through the moment of contact. It does not involve full tackling, rather contact is above the waist and players stay on their feet and a quick whistle ends the drills. *Live* is defined as drills being run in game-like conditions that include live-drill during practice as well as real games. *Live* should be the only time players are allowed to fully tackle another player to the ground. All head impacts in *air*, *bags*, *control*, and *thud* were during practices, whereas *live* occurred in both practices and games.

Statistical analysis

Our primary aim was to examine whether cumulative head impact frequency, PLA, and PRA from a single season differ between 5 levels-of-contact (*air*, *bags*, *control*, *thud*, and *live*). Our secondary aim further examined the difference in these head impact measures between 3 position groups (lineman, hybrid, and skill). Three-way repeated measures ANOVA models were used to compare outcome variables (season-long cumulative head impact frequency, PLA, and PRA normalized per player) on 5 levels-of-contact and 3 groups. The assumption of sphericity was assessed with Mauchly's test and resulted in violation of sphericity ($p<0.01$), thus the Greenhouse-Geisser correction was used to report within-subject outputs, followed by the effect size (Partial Eta Squared). When there was a significant effect for levels-of-contact and/or group, then Bonferroni post-hoc tests were used to determine where the difference in head impact outcome occurred. For the exploratory aim, we similarly assessed whether average head impact magnitude differed by levels-of-contact and group using repeated measures ANOVA. Lastly, a total number of head impacts within ranges of 25–60 *g*, 60–100 *g*, or >100 *g* in each level-of-contact was descriptively assessed in the overall sample. These exploratory thresholds are modeled based on the published papers that suggested < 25 *g* as minimal magnitude [11, 31], 60 *g* being previously thought to be a cut-off threshold to induce concussion [32], and 100 *g* (precisely, 102.5 + 33.8 *g*) being an average magnitude leading to concussion diagnosis [24, 33]. All the data were analyzed using SPSS Statistics Version 25, and the level of statistical significance was set to $p<0.05$. Data are presented per player.

Results

Demographics and overall head impact exposure

A total of 6016 head impacts were recorded during 5 levels-of-contact in 24 high school football players throughout the season, resulting in a median of 203 hits, 4310.5 *g*, and 415.5 krad/s^2. Consistent with previous reports [13, 34], the distribution of head impact count was strongly right skewed with a median PLA of 19.7 *g* (interquartile range: 15.3–27.8 *g*) and PRA of 1.8 krad/s^2 (interquartile range: 1.2–2.6 krad/s^2) per impact (Fig 1). These data are not reflective of head impacts that occurred outside the 5 levels-of-contact, such as walk-through and pre-practice/game conditioning. For comparison purposes, a driver can experience 30 *g* to 40 *g* of head and chest acceleration when a car collides into a fixed wall at 30 mph [35, 36]. Demographics and head impact data in the overall sample are detailed in Table 1.

Level-of-contact-dependent cumulative head impact exposure

Levels-of-contact displayed an influence on cumulative head impact frequency and sum of PLA and PRA sustained during a season, as illustrated by a statistically significant main effect and medium effect size on levels-of-contact in the overall sample [Frequency, $F(1.95, 40.88) = 22.44$, $p<0.001$, $\eta_p^2 = 0.517$; PLA, $F(1.77, 37.08) = 21.70$, $p<0.001$, $\eta_p^2 = 0.508$; PRA, $F(1.62, 34.15) = 20.56$, $p<0.001$, $\eta_p^2 = 0.494$]. Bonferroni post-hoc tests revealed incremental increases in head impact frequency and sum of PLA and PRA as levels-of-contact intensify, except for between control and thud (*air* < *bags* < *control* = *thud* < *live*). For example, a football player experienced an average of 113.7±17.8 hits, 2,657.6±432.0 *g* (PLA), and 233.9 ± 40.1 krad/s^2 (PRA) during *live* drills throughout a season, whereas 7.7±1.9 hits, 176.9±42.5 *g* (PLA), PRA 16.7±4.2 krad/s^2 (PRA) during *air* drills throughout a season. See Fig 2A–2C for the visual trend of the outcomes and S1 Table for Bonferroni post-hoc results.

While all 3 position groups exhibited similar incremental patterns of head impact frequency and magnitude (*air* < *bags* < *control* < *thud* < *live*), there was no significant group difference in cumulative frequency as well as sums of PLA and PRA (Fig 3A–3C). Head impact kinematics for each level-of-contact are detailed in S2 Table.

Head impact magnitudes by levels-of-contact and position group

Median head impact magnitudes (PLA and PRA) were similar across all levels-of-contact and all groups, ranging between 18.2 and 23.2 g for PLA (Fig 4A) and 1.6 and 2.3 krad/s^2 for PRA (Fig 4B) per head impact. See S3 Table for median PLA and PRA in each level-of-contact. Consistent with published papers [13, 23, 34], it was evident that a large number of head impacts across all levels-of-contact fell within 10 to 30 *g*, which might have diluted the minority of high magnitude head impacts. Our exploratory descriptive analysis identified that levels-of-contact also influence the number of high magnitude head impacts, whereby 60–100 *g* and >100 *g* were most prevalent in *live*, followed by *thud* and *control*, whereas very few hits were observed in *bags* and *air* (Fig 5A–5C).

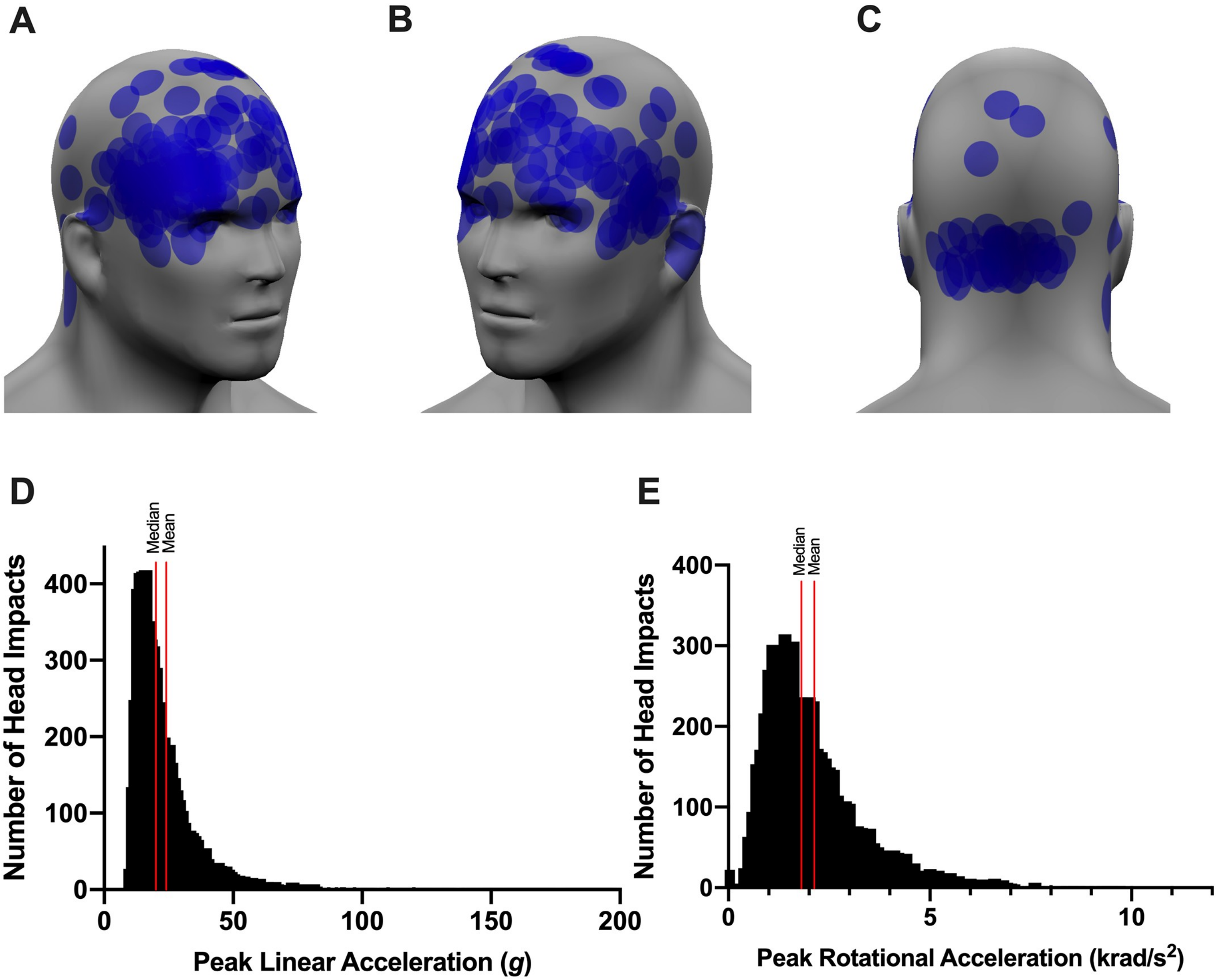

Fig 1. Head impact distribution in 24 high school football players in a single season. A representative data from a defensive lineman demonstrates the estimated locations of hits based on the data from the Vector mouthguard (A, front right; B, front left; C, back). Histogram of distribution of peak linear acceleration (D) and peak rotational acceleration (E) for all hits occurred during 5 levels-of-contact.

Discussion

Subconcussion research is still at its infancy, but it is a rapidly growing area of concern in sport injury prevention. To contribute to this emerging field, this study examined whether cumulative subconcussive head impact frequency and magnitude in a single season differed across levels-of-contact and between player position groups. There were four key findings in this study. First, cumulative head impact frequency and magnitude increased as the level-of-contact increased, with the greatest head impact burden observed during *live*, followed by *thud* and *control* drills, and minimal head impacts during *bags* and *air* drills. Second, there were notable position group differences in head impact measures, where median values of head impacts in the linemen and hybrid players were greater in all levels-of-contact than those of the skill players. However, this was not supported by statistically significant group differences

Table 1. Group demographics and head impact kinematics.

Variables	Overall	Linemen	Hybrid	Skill
N (%)	24 (100)	11 (46)	7 (29)	6 (25)
Age, y	15.7 ± 1.1	16.1 ± 0.9	15.1 ± 1.2	15.7 ± 1.0
BMI, kg/m^2	27.3 ± 6.3	31.6 ± 7.0	24.4 ± 2.5	23.0 ± 0.8
No. of previous concussion				
0, *n* (%)	16 (66.7)	6 (54.5)	0 (0)	3 (50.0)
1, *n* (%)	6 (25.0)	4 (36.4)	0 (0)	2 (33.3)
2, *n* (%)	2 (8.3)	1 (9.1)	0 (0)	1 (16.7)
Tackle football experience, y	4.9 ± 2.7	5.8 ± 2.7	3.6 ± 2.5	4.8 ± 3.4
Race, *n* (%)				
White	21 (88)	9 (82)	7 (100)	5 (83)
Black/African American	0 (0)	0 (0)	0 (0)	0 (0)
Asian	0 (0)	0 (0)	0 (0)	0 (0)
American Indian/Alaska	1 (4)	0 (0)	0 (0)	1 (17)
Multiracial	2 (8)	2 (18)	0 (0)	0 (0)
Ethnicity, *n* (%)				
Not Latino/Hispanic	20 (83)	9 (82)	5 (71)	6 (100)
Latino/Hispanic	4 (17)	2 (18)	2 (29)	0 (0)
Impact Kinematics for season, median (IQR)				
Median cumulative impact count	203 (118.0–350.0)	213 (139.0–478.0)	204 (153.5–314.5)	131.5 (51.3–263.5)
Median cumulative peak linear acceleration, *g*	4310.5 (2686.3–8616.8)	4289.5 (3178.6–11143.9)	5262.7 (3445.1–7203.1)	2837.7 (1117.2–6269.6)
Median cumulative peak rotational acceleration, krad/s^2	415.5 (179.2–828.0)	358.7 (203.2–919.3)	438.6 (293.0–655.9)	296.5 (132.6–569.6)

Data are reported as either mean (SD) or *n* (%), except for head impact data using median (IQR). BMI, body mass index. IQR, interquartile range. OL, offensive lineman. DL, defensive lineman. TE, tight end. LB, linebacker. RB, running back. WR, wide receiver. DB, defensive back.

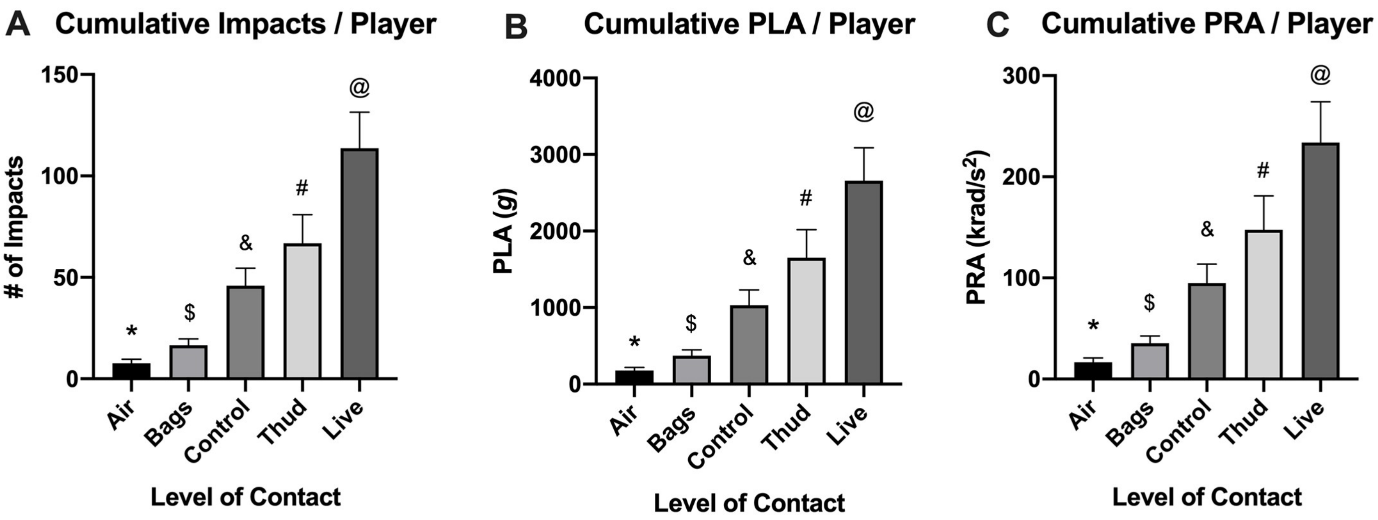

Fig 2. Cumulative head impact kinematics between levels-of-contact throughout a season. Cumulative (A) head impact count, (B) peak linear acceleration, and (C) peak rotational acceleration per player was influenced by the level-of-contact in an incremental manner, with *live* being the highest and *air* being the lowest. Data are presented as mean ± SD. Bonferroni post-hoc results are listed below: please refer to S1 Table for exact p-values of all possible comparisons. Please refer to S2 Table for median (IQR) values of head impact kinematics. @ Live is greater than thud ($p = 0.045$), control ($p<0.001$), bags ($p<0.001$) and air ($p<0.001$). # Thud is lesser than live ($p = 0.045$), no difference from control ($p = 0.095$), and greater than bags ($p = 0.013$) and air ($p = 0.008$). & Control is lesser than live ($p<0.001$), no difference from control ($p = 0.095$), and greater than bags ($p = 0.008$) and air ($p = 0.003$). $ Bags is lesser than live ($p<0.001$), thud ($p<0.01$), and control ($p = 0.008$), and greater than air ($p = 0.041$). * Air is lesser than live ($p<0.001$), thud ($p = 0.007$), control ($p = 0.003$), and bags ($p = 0.041$).

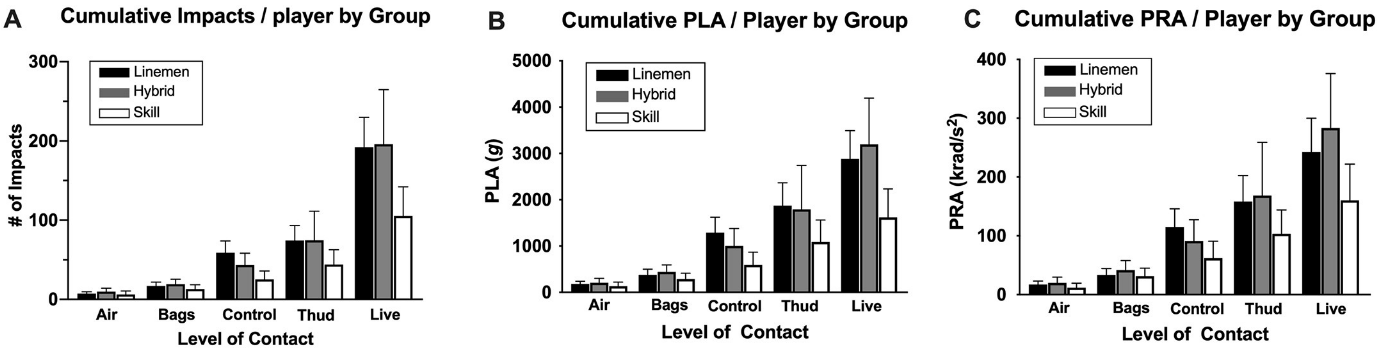

Fig 3. Group-dependent cumulative head impact exposure between levels-of-contact. All 3 groups shared similar incremental pattern in cumulative (A) head impact count, (B) peak linear acceleration, and (C) peak rotational acceleration per player, with *live* being the highest and *air* being the lowest. There was no group difference in the cumulative head impact frequency or magnitude. Data are presented as mean ± SD. Please refer to S2 Table for median (IQR) values of head impact kinematics.

likely due to the lack of sample size in each group. Third, the mean head impact magnitude was similar (18 to 23 *g*) across all levels-of-contact. Lastly, very high impact magnitudes (>100 *g*) were small in number overall but were more frequent in *thud* and *live* than other levels-of-contact. Taken together, our data, for the first time, empirically support the USA football's categorization of levels-of-contact while calling for a need to dissect football practice guidelines to make more specific recommendations for practice and games to minimize cumulative head impact burden on adolescents' brain health.

Owing to the sensor-installed helmets, mouthguards, headbands, and skin patches, our knowledge of head impact exposure in American football has drastically improved in the past 15 years. These technological advancements allowed researchers to evaluate head impact frequency and magnitude in various position groups, practice types (e.g., shell-only, full-gear),

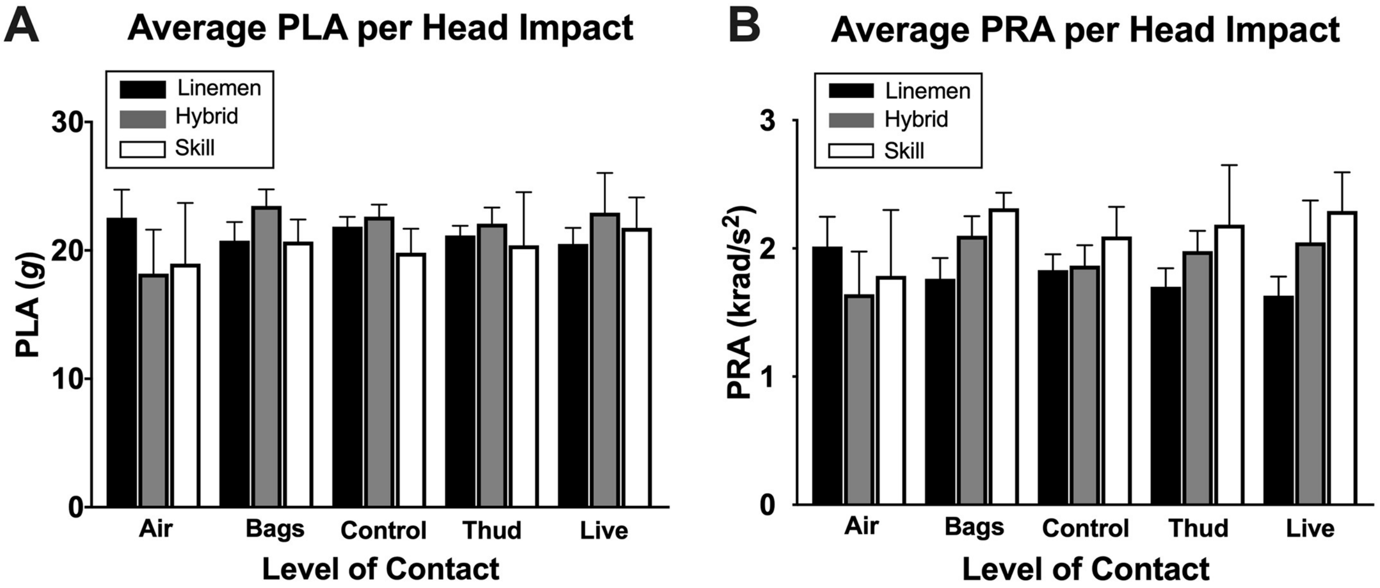

Fig 4. Average head impact magnitude per impact between levels-of-contact and group. There was no significant difference in average peak linear acceleration (A) and peak rotational acceleration (B) across 5 levels-of-contact and 3 groups. Data are presented as mean ± SD. See S3 Table for median (IQR) values of head impact kinematics.

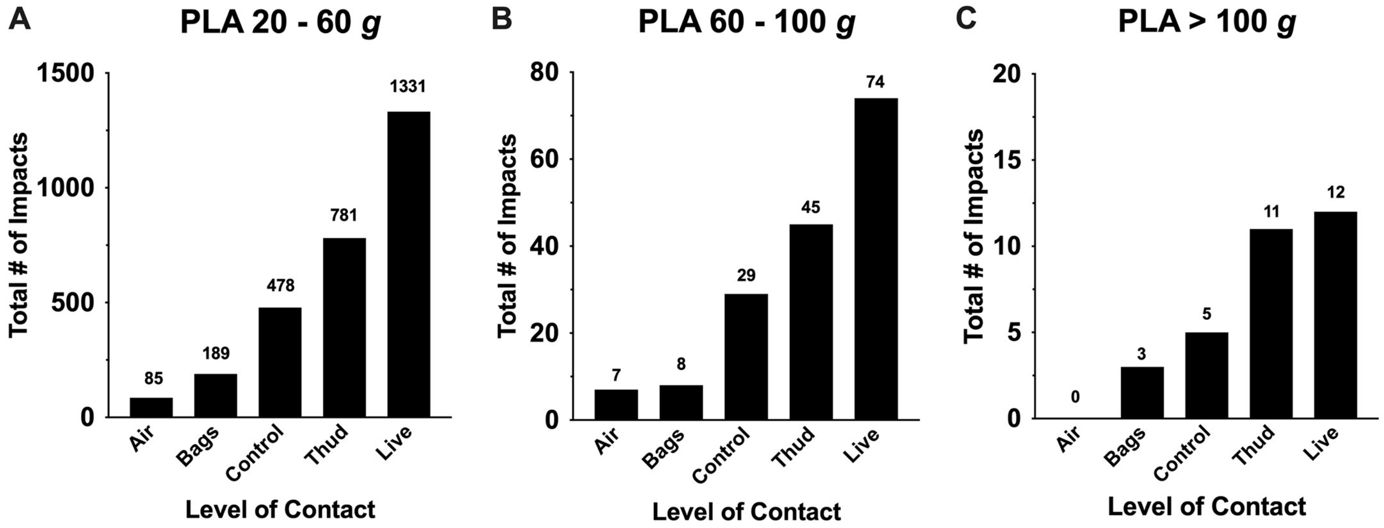

Fig 5. Frequency of head impacts within various magnitude range. A total number of head impacts from the overall sample throughout a season was categorized into peak linear acceleration ranging (A) 20–60 *g*, (B) 60–100 *g*, and (C) > 100 *g*. *Live* and *thud* consistently showed frequency head impacts in high impact magnitudes.

play types (e.g., running, passing, special teams), and time-based hit rates [37–43]. Previous research has suggested that overall head impact exposure elevates in relation to increased practice duration, contact intensity, and time spent in high risk drills [21, 39]. However, these variables differ greatly between players and position groups. For example, previous literature demonstrated that the differences between individual players accounted for 48% of the variance in head impact exposure during practice [44]. Additionally, recent research suggests different types of plays (e.g., running, passing, special teams) also have different average head impact magnitudes which will influence cumulative head impact exposure [41]. Despite this high degree of variance between players and play types, previous studies reported that the linemen and hybrid position groups consistently have higher head impact exposure compared to skill positions such as receivers, defensive backs, and quarterbacks [37, 38]. In our sample, we were able to observe a similar trend in the context of five different levels-of-contact, where the linemen and hybrid positions sustained a minimum of 27% more head impacts in *live* drills, with upwards of 3 to 4 fold higher head impact exposure in *air*, *bags*, and *control* than those of the skill position (see S2 Table). However, these group differences did not reach statistical significance likely due to the heterogeneous head impact exposure within each group (e.g., some skill players sustain many hits while several linemen experienced less hits), pointing to the issue of lack of sample size.

Another important finding from this study was that the average head impact magnitude (PLA and PRA) was similar (18 to 23 *g*) across 5 levels-of-contact. However, the frequency of strong magnitudes of head impacts (60–100 *g* and >100 *g*) were greater during *live* and *thud* drills than other drills. These observations illustrate the fact that the majority of head impacts in high school football are considered mild, but the minority high magnitude impacts are evident in contact-prone drills. This evidence fills the critical gap in knowledge that the restriction of practice frequency and shortening of a season may not be effective unless considering the intensity of practice drills. Nevertheless, there have been attempts by various football governing bodies to restrict the amount of full contact in practices [20, 21, 26, 38, 45]. For example, USA Football's National Practice Guidelines for Youth Tackle Football suggest that full-contact should not be done for more than 30 minutes per day and no more than 90 minutes per week during the regular season [26]. By limiting full contact practices from 3 days to 2 days per week, there was an average decline of 42% in head impact frequency (~250 hits per player) in high school football players [38]. In the same study, although the overall average head impact magnitude remained unchanged before and after reduction of contact practice, researchers found that a frequency of high magnitude head impacts were elevated in linemen and hybrid positions [38], suggesting that the team might have implemented higher intensity drills (i.e., *live*, *thud*, *control*) to compensate for the reduction of practice frequency. This compensatory trend was more conspicuous in the college setting. Even though the NCAA eliminated two-a-day practices and reduced practice frequency from 29 to 25 practices during summer camp, to compensate for the loss of practice times, football teams tended to incorporate more high-intensity, contact-prone practice drills, leading to increased head impact frequency [20, 21]. These data further substantiate the importance of regulating the duration of specific drill types, rather than restricting practice type and frequency.

Several studies suggest that football players who go on to sustain concussion tend to experience frequent subconcussive head impacts [24, 33]. Since none of the players in the current study were diagnosed with concussion, we were unable to suggest the potential preventive effect of levels-of-contact on concussion incidence. However, it is noteworthy that our exploratory analysis revealed no correlation (r = 0.07, p = 0.71) between previous number of concussion and subconcussive head impacts sustained during this season. This finding suggests that previous concussion history has almost no influence in how players perform, in the context of head impact exposure, during the season.

Clinical implications

Pending confirmation by a larger-scale study, these results may have important implications for the clinical management and guidelines regulating subconcussive head impact exposure in high school football. If a restriction of contact-prone drills (i.e., live, thud) during practice is indeed effective in reducing head impact frequency and associated magnitude, establishing a policy or guideline to minimize head impact burden would be a logical next step. In addition to the consideration of level-of-contact, the most effective method for informing clinical guidelines may be a blend of the different strategies. For example, it is important to implement guidelines informed by head impact data derived not only from entire teams or position groups, but also from more targeted and specific practice structural variables such as drill type and hit per player per minute rates. Additionally, individual-level variables such as tackling technique, starting status, or count of repetitions players participate in would be imperative to determine who might be at risk for sustaining many head impacts in a short window. Fortunately, coaches across the nation have the ability to implement the USA Football levels-of-contact within their practice structures with relatively minimal effort. This strategy may allow coaches to directly influence the total subconcussive head impact exposure in their athletes.

Limitations

There are several limitations to this study. Our examination of head impacts in high school football is limited in that it was conducted on a single high school football team in the Midwest composed of primarily white males. Because of lack of racial and ethnical diversity in our sample, we were unable to conduct any analysis to identify whether race/ethnicity played a role in subconcussive head impact exposure. This should be addressed in a future study along with whether race/ethnical background influence one's neurologic resiliency and susceptibility to subconcussive head impacts. Therefore, the results from the current study are not generalizable to the broader U.S. population of high school football teams. A second limitation of the study is that the USA Football levels-of-contact guidelines are just that, guidelines. They are not legislation, and it is unknown what percentage of high schools currently utilize the USA Football level-of-contact system; thus, this further limits the generalizability. Implementation of the levels-of-contact is not a perfectly reliable variable in that situations occur in practice that may have overlapping levels-of-contact. This overlap made cumulative calculations of time spent in each level-of-contact difficult during certain drills and the time spent in each level is likely to influence cumulative head impact exposure. Coaches in this high school attempted to implement the levels-of-contact consistently, but to control up to 22 athletes participating in a single drill at the same time is not always feasible. An additional limitation is that the Vector mouthguard has not been validated for true positive versus false positive data. These limitations portend a follow-up research question as to whether regulating the number of repetitions in each level-of-contact is a more effective approach than teamwide contact restrictions for minimizing the frequency and magnitude of subconcussive head impacts in individual players (or position groups). Hence, for future research we suggest examining subject-specific head impact data and playing style (run-first vs. pass-first offense) [46] that incorporate multiple predictor variables such as time spent in each level-of-contact, the repetitions per player per drill, levels-of-contact, position group, and impacts per event (i.e., drill, practice/game, or week) for head impact outcomes.

Conclusion

Levels-of-contact may influence cumulative head impact frequency and magnitude in high school football players, with players incurring frequent head impacts during *live*, *thud*, and *control*. Strong magnitudes of head impacts ($> 60\ g$) were frequently observed especially during *live* and *thud*. It is important to consider levels-of-contact to refine football practice guidelines/policies to minimize cumulative head impact burden in high school football players.

Supporting information

S1 Table. Bonferroni post-hoc results on 5 levels of contact.
(DOCX)

S2 Table. Cumulative head impact frequency and magnitude for the entire season.
(DOCX)

S3 Table. Average peak linear acceleration and peak rotation acceleration.
(DOCX)

S1 Video.
(MP4)

Author Contributions

Conceptualization: Kyle Kercher, Jesse A. Steinfeldt, Jonathan T. Macy, Keisuke Kawata.

Data curation: Kyle Kercher.

Formal analysis: Kyle Kercher, Keisuke Ejima, Keisuke Kawata.

Funding acquisition: Keisuke Kawata.

Investigation: Kyle Kercher, Jesse A. Steinfeldt, Jonathan T. Macy, Keisuke Ejima, Keisuke Kawata.

Methodology: Kyle Kercher, Jesse A. Steinfeldt, Jonathan T. Macy, Keisuke Kawata.

Project administration: Kyle Kercher, Jesse A. Steinfeldt, Keisuke Kawata.

Resources: Kyle Kercher, Jesse A. Steinfeldt, Keisuke Ejima, Keisuke Kawata.

Supervision: Jonathan T. Macy, Keisuke Kawata.

Visualization: Kyle Kercher, Keisuke Kawata.

Writing – original draft: Kyle Kercher, Keisuke Kawata.

Writing – review & editing: Jesse A. Steinfeldt, Jonathan T. Macy, Keisuke Ejima, Keisuke Kawata.

References

1. Broglio SP, Eckner JT, Paulson HL, Kutcher JS. Cognitive decline and aging: the role of concussive and subconcussive impacts. Exerc Sport Sci Rev. 2012; 40(3):138–44. Epub 2012/06/26. https://doi.org/10.1097/JES.0b013e3182524273 PMID: 22728452.
2. Hutchison MG, Di Battista AP, McCoskey J, Watling SE. Systematic review of mental health measures associated with concussive and subconcussive head trauma in former athletes. Int J Psychophysiol. 2018; 132(Pt A):55–61. Epub 2017/11/28. https://doi.org/10.1016/j.ijpsycho.2017.11.006 PMID: 29175229.
3. Logan K, Cuff S. Organized Sports for Children, Preadolescents, and Adolescents. Pediatrics. 2019; 143(6):1–20. https://doi.org/10.1542/peds.2019-0997 PMID: 136776529.
4. USA Football—Rookie Tackle. Indianapolis, IN [cited 2020 3/12]. https://usafootball.com/rookietackle/.
5. National Collegiate Athletic Association. New kickoff rule approved for 20182018 [cited 2020 3/12]. https://www.ncaa.com/news/football/article/2018-04-13/alteration-football-kickoff-rule-approved.
6. National Federation of State High School Associations. New Blocking, Kicking Rules Address Risk Minimization in High School Football. NFHS News [Internet]. 2018 [cited 2020 3/12]. https://www.nfhs.org/articles/new-blocking-kicking-rules-address-risk-minimization-in-high-school-football/.
7. National Football League. NFL Video Rulebook: Defenseless Player New York, NY2020. https://operations.nfl.com/the-rules/nfl-video-rulebook/defenseless-player/.
8. Wiebe DJ, D'Alonzo BA, Harris R, Putukian M, Campbell-McGovern C. Association Between the Experimental Kickoff Rule and Concussion Rates in Ivy League Football. JAMA. 2018; 320(19):2035–6. Epub 2018/10/05. https://doi.org/10.1001/jama.2018.14165 PMID: 30285061.
9. McIntosh AS, McCrory P. Preventing head and neck injury. Br J Sports Med. 2005; 39(6):314–8. Epub 2005/05/25. https://doi.org/10.1136/bjsm.2005.018200 PMID: 15911597.
10. McCrory P, Meeuwisse W, Dvorak J, Aubry M, Bailes J, Broglio S, et al. Consensus statement on concussion in sport-the 5(th) international conference on concussion in sport held in Berlin, October 2016. Br J Sports Med. 2017; 51(11):838–47. Epub 2017/04/28. https://doi.org/10.1136/bjsports-2017-097699 PMID: 28446457.
11. Bailes JE, Petraglia AL, Omalu BI, Nauman E, Talavage T. Role of subconcussion in repetitive mild traumatic brain injury. J Neurosurg. 2013; 119(5):1235–45. Epub 2013/08/27. https://doi.org/10.3171/2013.7.JNS121822 PMID: 23971952.
12. Bahrami N, Sharma D, Rosenthal S, Davenport EM, Urban JE, Wagner B, et al. Subconcussive Head Impact Exposure and White Matter Tract Changes over a Single Season of Youth Football. Radiology. 2016; 281(3):919–26. Epub 2016/10/25. https://doi.org/10.1148/radiol.2016160564 PMID: 27775478.
13. Hirad AA, Bazarian JJ, Merchant-Borna K, Garcea FE, Heilbronner S, Paul D, et al. A common neural signature of brain injury in concussion and subconcussion. Sci Adv. 2019; 5(8):eaau3460. Epub 2019/08/29. https://doi.org/10.1126/sciadv.aau3460 PMID: 31457074.
14. Breedlove EL, Robinson M, Talavage TM, Morigaki KE, Yoruk U, O'Keefe K, et al. Biomechanical correlates of symptomatic and asymptomatic neurophysiological impairment in high school football. Journal of biomechanics. 2012; 45(7):1265–72. Epub 2012/03/03. https://doi.org/10.1016/j.jbiomech.2012.01.034 PMID: 22381736.
15. Talavage TM, Nauman EA, Breedlove EL, Yoruk U, Dye AE, Morigaki KE, et al. Functionally-detected cognitive impairment in high school football players without clinically-diagnosed concussion. Journal of neurotrauma. 2014; 31(4):327–38. https://doi.org/10.1089/neu.2010.1512 PMID: 20883154.

16. Kawata K, Rubin LH, Lee JH, Sim T, Takahagi M, Szwanki V, et al. Association of Football Subconcussive Head Impacts With Ocular Near Point of Convergence. JAMA Ophthalmol. 2016; 134(7):763–9. https://doi.org/10.1001/jamaophthalmol.2016.1085 PMID: 27257799.

17. Kawata K, Liu CY, Merkel SF, Ramirez SH, Tierney RT, Langford D. Blood Biomarkers for Brain Injury: What Are We Measuring? Neuroscience and biobehavioral reviews. 2016; 68:460–73. Epub 2016/05/18. https://doi.org/10.1016/j.neubiorev.2016.05.009 PMID: 27181909.

18. McKee AC, Stern RA, Nowinski CJ, Stein TD, Alvarez VE, Daneshvar DH, et al. The spectrum of disease in chronic traumatic encephalopathy. Brain: a journal of neurology. 2013; 136(Pt 1):43–64. Epub 2012/12/05. https://doi.org/10.1093/brain/aws307 PMID: 23208308.

19. Mez J, Daneshvar DH, Kiernan PT, Abdolmohammadi B, Alvarez VE, Huber BR, et al. Clinicopathological Evaluation of Chronic Traumatic Encephalopathy in Players of American Football. Jama. 2017; 318(4):360–70. https://doi.org/10.1001/jama.2017.8334 PMID: 28742910.

20. Stemper BD, Shah AS, Harezlak J, Rowson S, Duma S, Mihalik JP, et al. Repetitive Head Impact Exposure in College Football Following an NCAA Rule Change to Eliminate Two-A-Day Preseason Practices: A Study from the NCAA-DoD CARE Consortium. Ann Biomed Eng. 2019; 47(10):2073–85. Epub 2019/08/08. https://doi.org/10.1007/s10439-019-02335-9 PMID: 31388849.

21. Stemper BD, Shah AS, Mihalik JP, Harezlak J, Rowson S, Duma S, et al. Head Impact Exposure in College Football following a Reduction in Preseason Practices. Med Sci Sports Exerc. 2020. Epub 2020/01/25. https://doi.org/10.1249/MSS.0000000000002283 PMID: 31977633.

22. USA Football—Levels of Contact. Indianapolis, IN. [cited 2020 3/12]. https://usafootball.com/resources-tools/coach/levels-of-contact/.

23. Duma SM, Manoogian SJ, Bussone WR, Brolinson PG, Goforth MW, Donnenwerth JJ, et al. Analysis of real-time head accelerations in collegiate football players. Clin J Sport Med. 2005; 15(1):3–8. Epub 2005/01/18. https://doi.org/10.1097/00042752-200501000-00002 PMID: 15654184.

24. Beckwith JG, Greenwald RM, Chu JJ, Crisco JJ, Rowson S, Duma SM, et al. Timing of concussion diagnosis is related to head impact exposure prior to injury. Med Sci Sports Exerc. 2013; 45(4):747–54. Epub 2012/11/09. https://doi.org/10.1249/MSS.0b013e3182793067 PMID: 23135364.

25. Broglio SP, Eckner JT, Martini D, Sosnoff JJ, Kutcher JS, Randolph C. Cumulative head impact burden in high school football. Journal of neurotrauma. 2011; 28(10):2069–78. Epub 2011/07/27. https://doi.org/10.1089/neu.2011.1825 PMID: 21787201.

26. USA Football—National Practice Guidelines for Youth Tackle Football. Indianapolis, IN: USA Football; 2014 [cited 2020 3/12]. https://assets.usafootball.com/documents/practice-guidelines-youth.pdf.

27. Wu LC, Nangia V, Bui K, Hammoor B, Kurt M, Hernandez F, et al. In Vivo Evaluation of Wearable Head Impact Sensors. Ann Biomed Eng. 2016; 44(4):1234–45. Epub 2015/08/21. https://doi.org/10.1007/s10439-015-1423-3 PMID: 26289941.

28. Camarillo DB, Shull PB, Mattson J, Shultz R, Garza D. An instrumented mouthguard for measuring linear and angular head impact kinematics in American football. Ann Biomed Eng. 2013; 41(9):1939–49. Epub 2013/04/23. https://doi.org/10.1007/s10439-013-0801-y PMID: 23604848.

29. Bartsch A, Samorezov S, Benzel E, Miele V, Brett D. Validation of an "Intelligent Mouthguard" Single Event Head Impact Dosimeter. Stapp car crash journal. 2014; 58:1–27. Epub 2015/07/21. PMID: 26192948.

30. Wu LC, Nangia V, Bui K, Hammoor B, Kurt M, Hernandez F, et al. In Vivo Evaluation of Wearable Head Impact Sensors. Ann Biomed Eng. 2015. Epub 2015/08/21. https://doi.org/10.1007/s10439-015-1423-3 PMID: 26289941.

31. Barber Foss KD, Yuan W, Diekfuss JA, Leach J, Meehan W, DiCesare CA, et al. Relative Head Impact Exposure and Brain White Matter Alterations After a Single Season of Competitive Football: A Pilot Comparison of Youth Versus High School Football. Clin J Sport Med. 2019; 29(6):442–50. Epub 2019/11/07. https://doi.org/10.1097/JSM.0000000000000753 PMID: 31688173.

32. Guskiewicz KM, Mihalik JP, Shankar V, Marshall SW, Crowell DH, Oliaro SM, et al. Measurement of head impacts in collegiate football players: relationship between head impact biomechanics and acute clinical outcome after concussion. Neurosurgery. 2007; 61(6):1244–52; discussion 52–3. Epub 2007/12/29. https://doi.org/10.1227/01.neu.0000306103.68635.1a PMID: 18162904.

33. Beckwith JG, Greenwald RM, Chu JJ, Crisco JJ, Rowson S, Duma SM, et al. Head impact exposure sustained by football players on days of diagnosed concussion. Med Sci Sports Exerc. 2013; 45(4):737–46. Epub 2012/11/09. https://doi.org/10.1249/MSS.0b013e3182792ed7 PMID: 23135363.

34. Crisco JJ, Fiore R, Beckwith JG, Chu JJ, Brolinson PG, Duma S, et al. Frequency and location of head impact exposures in individual collegiate football players. J Athl Train. 2010; 45(6):549–59. Epub 2010/11/11. https://doi.org/10.4085/1062-6050-45.6.549 PMID: 21062178.

35. Wagner R. A 30 mph front/rear crash with human test persons. SAE Transactions. 1979; 88(4):3587–95. https://www.jstor.org/stable/44648350.

36. Nave CR. Force on driver in example car crash HyperPhysics: Georgia State University; 2016 [cited 2020 June 20]. http://hyperphysics.phy-astr.gsu.edu/hbase/carcr2.html#cc2.

37. Broglio SP, Martini D, Kasper L, Eckner JT, Kutcher JS. Estimation of head impact exposure in high school football: implications for regulating contact practices. The American journal of sports medicine. 2013; 41(12):2877–84. Epub 2013/09/05. https://doi.org/10.1177/0363546513502458 PMID: 24001576.

38. Broglio SP, Williams RM, O'Connor KL, Goldstick J. Football Players' Head-Impact Exposure After Limiting of Full-Contact Practices. Journal of athletic training. 2016; 51(7):511–8. Epub 2016/06/23. https://doi.org/10.4085/1062-6050-51.7.04 PMID: 27333460.

39. Asken BM, Brooke ZS, Stevens TC, Silvestri PG, Graham MJ, Jaffee MS, et al. Drill-Specific Head Impacts in Collegiate Football Practice: Implications for Reducing "Friendly Fire" Exposure. Ann Biomed Eng. 2019; 47(10):2094–108. Epub 2018/07/11. https://doi.org/10.1007/s10439-018-2088-5 PMID: 29987539.

40. Reynolds BB, Patrie J, Henry EJ, Goodkin HP, Broshek DK, Wintermark M, et al. Practice type effects on head impact in collegiate football. Journal of neurosurgery. 2015:1–10. Epub 2015/08/05. https://doi.org/10.3171/2015.5.JNS15573 PMID: 26238972.

41. Urban JE, Flood WC, Zimmerman BJ, Kelley ME, Espeland MA, McNamara L, et al. Evaluation of head impact exposure measured from youth football game plays. J Neurosurg Pediatr. 2019:1–10. Epub 2019/05/11. https://doi.org/10.3171/2019.2.PEDS18558 PMID: 31075762.

42. Mihalik JP, Bell DR, Marshall SW, Guskiewicz KM. Measurement of head impacts in collegiate football players: an investigation of positional and event-type differences. Neurosurgery. 2007; 61(6):1229–35; discussion 35. Epub 2007/12/29. https://doi.org/10.1227/01.neu.0000306101.83882.c8 PMID: 18162902.

43. Crisco JJ, Wilcox BJ, Beckwith JG, Chu JJ, Duhaime AC, Rowson S, et al. Head impact exposure in collegiate football players. Journal of biomechanics. 2011; 44(15):2673–8. https://doi.org/10.1016/j.jbiomech.2011.08.003 PMID: 21872862.

44. Campolettano ET, Rowson S, Duma SM, Stemper B, Shah A, Harezlak J, et al. Factors Affecting Head Impact Exposure in College Football Practices: A Multi-Institutional Study. Ann Biomed Eng. 2019; 47(10):2086–93. Epub 2019/06/27. https://doi.org/10.1007/s10439-019-02309-x PMID: 31240507.

45. Cranmer GA, Sanderson J. "Rough Week for Testosterone": Public Commentary around the Ivy League's Decision to Restrict Tackle Football in Practice. Western Journal of Communication. 2018; 82(5):631–47. https://doi.org/10.1080/10570314.2018.1441431 PMID: 131966020.

46. Martini D, Eckner J, Kutcher J, Broglio SP. Subconcussive head impact biomechanics: comparing differing offensive schemes. Med Sci Sports Exerc. 2013; 45(4):755–61. Epub 2012/11/09. https://doi.org/10.1249/MSS.0b013e3182798758 PMID: 23135370.

15

Effects of high intensity interval exercise on cerebrovascular function: A systematic review

**Alicen A. Whitaker[1☯], Mohammed Alwatban[1‡], Andrea Freemyer[1‡], Jaime Perales-Puchalt[2,3‡], Sandra A. Billinger[1,3,4☯]*

1 Department of Physical Therapy and Rehabilitation Science, University of Kansas Medical Center, Kansas City, KS, United States of America, **2** University of Kansas Alzheimer's Disease Center, Fairway, KS, United States of America, **3** Department of Neurology, University of Kansas Medical Center, Kansas City, KS, United States of America, **4** Department of Physical Medicine and Rehabilitation, University of Kansas Medical Center, Kansas City, KS, United States of America

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* sbillinger@kumc.edu

Editor: Chris Harnish, Mary Baldwin University Murphy Deming College of Health Sciences, UNITED STATES

Abstract

High intensity interval exercise (HIIE) improves aerobic fitness with decreased exercise time compared to moderate continuous exercise. A gap in knowledge exists regarding the effects of HIIE on cerebrovascular function such as cerebral blood velocity and autoregulation. The objective of this systematic review was to ascertain the effect of HIIE on cerebrovascular function in healthy individuals. We searched PubMed and the Cumulative Index to Nursing and Allied Health Literature databases with apriori key words. We followed the Preferred Reporting Items for Systematic Reviews. Twenty articles were screened and thirteen articles were excluded due to not meeting the apriori inclusion criteria. Seven articles were reviewed via the modified Sackett's quality evaluation. Outcomes included middle cerebral artery blood velocity (MCAv) (n = 4), dynamic cerebral autoregulation (dCA) (n = 2), cerebral de/oxygenated hemoglobin (n = 2), cerebrovascular reactivity to carbon dioxide (CO_2) (n = 2) and cerebrovascular conductance/resistance index (n = 1). Quality review was moderate with 3/7 to 5/7 quality criteria met. HIIE acutely lowered exercise MCAv compared to moderate intensity. HIIE decreased dCA phase following acute and chronic exercise compared to rest. HIIE acutely increased de/oxygenated hemoglobin compared to rest. HIIE acutely decreased cerebrovascular reactivity to higher CO_2 compared to rest and moderate intensity. The acute and chronic effects of HIIE on cerebrovascular function vary depending on the outcomes measured. Therefore, future research is needed to confirm the effects of HIIE on cerebrovascular function in healthy individuals and better understand the effects in individuals with chronic conditions. In order to conduct rigorous systematic reviews in the future, we recommend assessing MCAv, dCA and CO_2 reactivity during and post HIIE.

Introduction

Competing interests: The authors have declared that no competing interests exist.

High intensity interval exercise (HIIE) has emerged at the forefront of exercise regimens due to the shorter activity time needed to benefit [1–3]. HIIE confers similar or significant increased aerobic fitness compared to conventional moderate intensity continuous exercise [1, 4–7]. While aerobic fitness is a measure of increased cardiovascular health, the entire vascular system (including the cerebral vascular system) may be improved following increased aerobic fitness [8]. With aging, higher aerobic fitness is associated with a lower risk of stroke and dementia [9, 10]. A review and meta-analysis of HIIE in healthy adults has shown significant increases in aerobic fitness [1, 5, 6, 11]. Preliminary evidence has also shown HIIE may improve cognitive function [12]. However, the effects of HIIE on cerebrovascular function have not been systematically reviewed.

Cerebrovascular function is the ability of the cerebral blood vessels to deliver oxygen and nutrients for neuronal metabolism and maintain cerebral blood flow through dynamic autoregulation (dCA). dCA is the ability of the brain to sustain a constant cerebral blood flow despite large fluctuations in peripheral blood pressure [13, 14]. During resting conditions, cerebral blood flow responds to arterial blood pressure fluctuations, neuronal metabolism, cortical activation, arterial blood gases and cardiac output [15]. Cerebral blood flow can be measured at rest using magnetic resonance imaging or transcranial Doppler ultrasound (TCD). Middle cerebral artery blood velocity (MCAv) measured by TCD is the only technique to measure cerebral blood flow during exercise, with high temporal resolution [16]. MCAv is linearly related to cerebral blood flow with the caveat that the MCA diameter remains unchanged [17].

A normal cerebrovascular response to submaximal moderate continuous exercise results in increased MCAv [18–20], increased cerebral oxygenation [21, 22] and sustained dCA [23, 24]. MCAv has been shown to concomitantly increase as exercise intensity increases, up to moderate intensity [15, 18, 25–28]. MCAv is affected differently during high intensity exercise. During continuous high intensity exercise and hyperventilation, MCAv is decreased due to a reduction in arterial carbon dioxide (CO_2) [29, 30] causing downstream arteriole constriction [15, 31]. Cerebrovascular reactivity is the ability of the small vessels in the brain to vasodilate and vasoconstrict in response to fluctuating CO_2 levels [32, 33]. The cerebrovascular response to HIIE may differ from continuous high intensity exercise due to the repetitive short interval bouts that rapidly increase blood pressure which may cause cerebrovascular hyper-perfusion [34, 35]. If neuroprotective mechanisms of the brain, such as dCA, do not respond quickly to the repetitive and rapid increases in blood pressure, HIIE could elevate the risk for leakage within the blood brain barrier [34, 36]. For clinical populations with cerebrovascular impairment, such as stroke [37–39], the cerebrovascular response to HIIE may play an important role in guiding exercise prescription [36].

Previous scientific statements and narrative reviews have recounted the molecular, hemodynamic and structural processes (i.e. CO_2, nitric oxide, systemic blood pressure, vessel compliance, glial cell integrity) associated with the cerebrovascular response that may occur during HIIE [36, 40]. However, these detailed narrative reviews [41, 42] did not report the statistical findings of previous studies showing cerebrovascular function during HIIE. To our knowledge, our current systematic review is the first to systematically search and report the results of

during HIIE is important because it provides objective results to support the previously described narrative statements on hemodynamic processes during HIIE [41, 42]. The purpose of this systematic review was to address the gap in knowledge and report the various study results of HIIE on cerebrovascular function compared to moderate continuous exercise or rest conditions. We systematically examined the results of HIIE studies in healthy individuals based on the operationalization of cerebrovascular outcomes.

Methods

This review follows the guidelines for Preferred Reporting Items for Systematic Reviews [43]. Literature searches and reviews were performed using PubMed and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) databases. The University of Kansas Medical Center Online Library system was used to access these databases in February, March, and June 2020. In this systematic review, we included peer-reviewed manuscripts written in English from January 2010 to June 2020.

Key words used to search the databases included "high intensity interval training", "HIIT", "high intensity interval exercise", "HIIE" AND "cerebral blood flow", "cerebral blood velocity", "dynamic autoregulation". We believe these key words primarily reflect the high intensity interval intervention and cerebrovascular function outcome measures. The main outcomes of this systematic review were MCAv, an indirect measure of cerebral blood flow, and dCA, a measure of cerebrovascular homeostasis during peripheral blood pressure changes [30, 44–48]. MCAv supplies oxygen and nutrients to neurons while dCA maintains stable perfusion [49]. However, additional cerebrovascular measures were also included such as oxygenated hemoglobin [50–52], cerebrovascular reactivity [46, 53], cerebrovascular conductance index and cerebrovascular resistance index [45, 54]. Oxygenated hemoglobin is an important measure of aerobic metabolism within cerebral tissue using near-infrared spectrometry [55]. Cerebrovascular reactivity is a measure of cerebrovascular regulation [56] and shows the ability of the vessels to vasodilate or vasoconstrict to a stimulus [57]. Cerebrovascular conductance index is a measure of the conductance of peripheral blood pressure to cerebral blood velocity and is calculated as MCAv/mean arterial pressure (MAP) [45]. Cerebrovascular resistance index (MAP/MCAv) measures the resistance of cerebral perfusion pressure to cerebral blood velocity [45].

The identified abstracts from PubMed and CINAHL were screened using the following inclusion criteria: 1) experimental or quasi-experimental, 2) aerobic exercise identified as the primary means of performing HIIE, 3) cerebrovascular measures were primary or secondary outcomes and 4) human subjects across the lifespan with no current disease. After the removal of duplicates, two researchers screened titles/abstracts for inclusion criteria (A.W. and M.A.). The full texts were examined, and data extracted (A.W. and M.A.). If the authors were unable to come to an agreement, a third author moderated incongruity (A.F.).

A quality review was performed for each article using the modified version of Sackett's 1981 criteria [58]. We critically analyzed each article's study design, population, HIIE protocols, cerebrovascular outcomes and results. If an article did not report enough information to determine sufficient quality criteria a "No" rating was given. Articles were rated based on the level of evidence including level I for large randomized control trials, level II for small randomized trial, level III for nonrandomized design, Level IV for case series and Level V for case reports [59].

The search methods resulted in 67 articles. After removal of duplicates, 15 [45, 46, 50, 53, 60–69] articles were identified in PubMed and 5 [47, 51, 70–72] new articles in CINAHL. During the initial screening of titles/abstracts, 11 articles were excluded due to HIIE not being the primary experimental protocol performed (n = 6), studies not measuring cerebral arteries (n = 4) and an animal study (n = 1). Studies that combined other interventions with HIIE were excluded due to the confounding variables that could affect cerebrovascular outcomes (see S1 Table). After the full text assessment, two articles were excluded due to not meeting experimental or quasi experimental criteria (n = 2). See Fig 1 for flow diagram of article selection.

We included seven articles describing cerebrovascular outcomes following HIIE within this review [44–47, 50, 51, 53]. The full texts are described in Table 1. Of the articles reviewed, six were small, randomized trials and one nonrandomized cross-over trial. All the studies

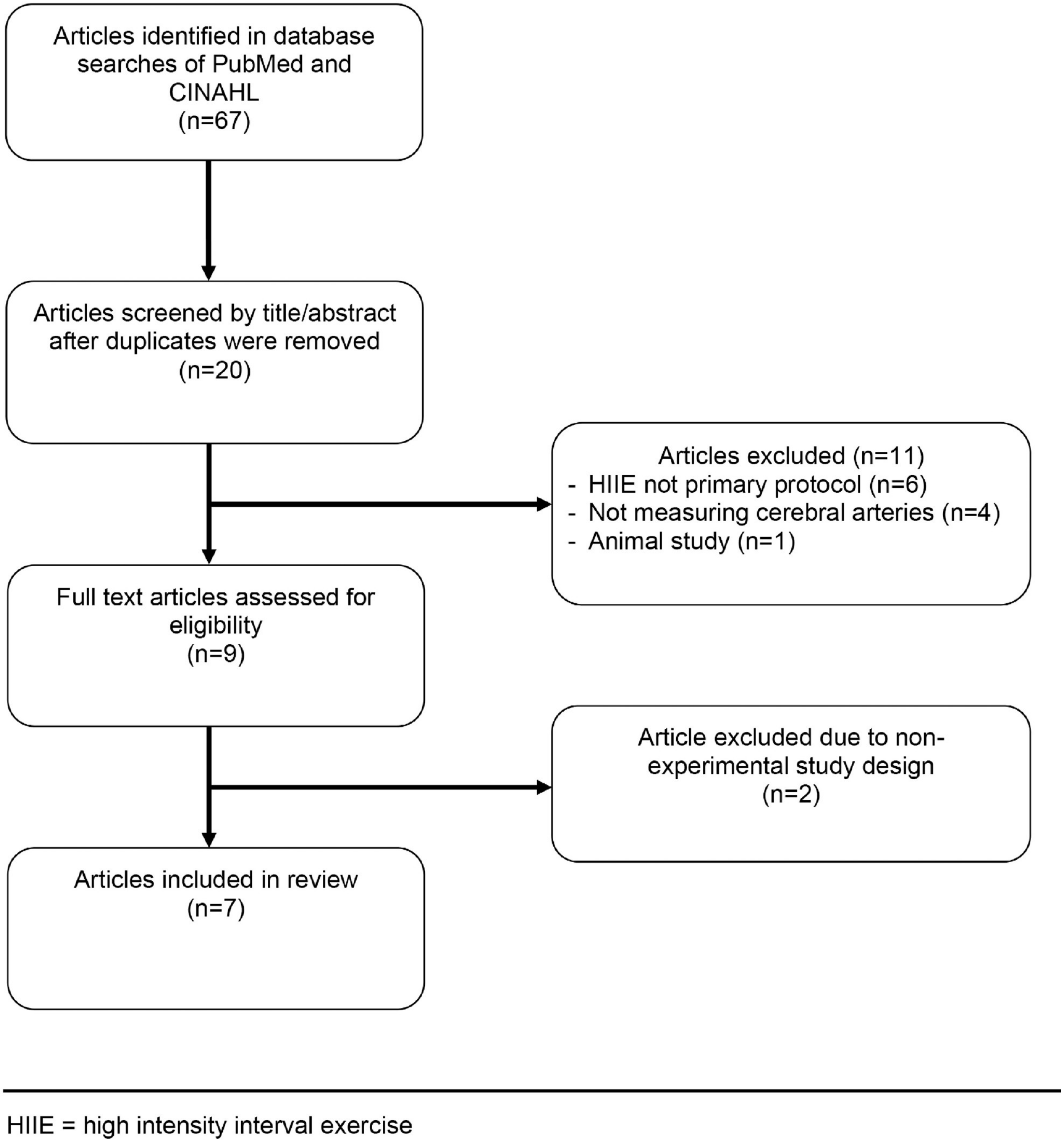

Fig 1. Flow diagram of article selection.

Table 1. Summary of reviewed articles.

Study	Design	Level of Evidence	Subjects	Intervention	Outcome measures	Results
Burma et al, March 2020	Small Randomized Cross-Over Trial	II	9 Young Adults (age 26 ± 5 years old)	3 cycling conditions: • High intensity interval training (HIIT, 1-min interval at 85–90% predicted heart rate reserve with 1-min active recovery 15% power output for 10 intervals) • Moderate intensity continuous exercise (MICT, 50–60% predicted heart rate reserve for 45 min) • No-exercise control	TCD measured: Average Exercise MCAv dCA via Transfer Function Analysis during forced MAP oscillations (repeated squat-stand maneuver)	Significant increase in exercise MCAv during MICT compared to HIIT ($p<0.05$) and baseline ($p<0.05$). No change in exercise MCAv during HIIT compared to baseline ($p>0.05$). Significantly higher systolic gain/phase compared to diastolic/mean gain/phase at 0.05 and 0.10 Hz during control ($p<0.05$). Decreased systolic phase in 0.05 Hz immediately following HIIT ($p>0.102$) and MICT until hour 4 ($p>0.079$). Decreased systolic phase in 0.10 Hz immediately following HIIT until hour 2 ($p>.11$) and immediately following MICT until hour 4 ($p>0.079$). No change in gain or coherence in 0.05 Hz or 0.10 Hz during HIIT or MICT.
Burma et al, June 2020	Secondary Analysis of the above Small Randomized Cross-Over Trial	II	Same as above	Same as above	TCD measured: Cerebrovascular reactivity to hypercapnia Cerebrovascular reactivity to hypocapnia	Significantly decreased absolute and relative MCA reactivity to hypercapnia immediately following HIIT up to hour 2 ($p<0.018$) compared to MICT and control ($p<0.022$). Significantly decreased relative MCA reactivity to hypercapnia immediately following MICT up to hour 1 ($p<0.024$). No significant differences in MCA reactivity to hypocapnia between conditions ($p>0.31$).
Coetsee et al, 2017	Small Randomized Controlled Trial	II	67 Inactive Adults HIIT (age 64.5 ± 6.3 years old) MCT (age 61.6 ± 5.8 years old) CON (age 62.5 ± 5.6 years old)	16-week intervention Treadmill 30 min, 3x/week 4 groups: • High intensity Interval training (HIIT, 4 min interval at 90–95% HRmax with 3 min active recovery 70% HRmax) • Moderate continuous training (MCT, 70–75% HRmax) • No-exercise control (CON).	Near-Infrared Spectroscopy measured during Cognitive Stroop test: • Oxygenated Hemoglobin • Deoxygenated Hemoglobin • Total Hemoglobin Index	No significant differences in oxygenated (effect size = .45, p = .3), deoxygenated (effect size = 0.67, p = .14), or total hemoglobin (effect size $< .6$, $p>.18$) after HIIT. Significant decrease in deoxygenated hemoglobin (effect size = 1.14, p = .01) and total hemoglobin index (effect size = 1.49, $p < .01$) after MCT. Significant increase in oxygenated hemoglobin in CON (effect size = .76, p = .03).
Drapeau et al, 2019	Small Randomized Clinical Trial	II	17 Endurance Trained Males HIIT$_{85}$ (age 26 ± 6 years old) HIIT$_{115}$ (age 28± 6 years old)	6-week intervention.Cycled until exhaustion, 3x/week2 groups: • HIIT$_{85}$ (1–7 min interval at 85% of maximal aerobic power, with active recovery of 50% of maximal aerobic power) • HIIT$_{115}$ (30sec– 1min interval at 115% of maximal aerobic power, with active recovery of 50% of maximal aerobic power).	TCD measured: Resting MCAv Resting CVCi Resting CVRi dCA via Transfer Function Analysis during forced MAP oscillations (repeated squat-stand maneuver)	Significant decrease in phase at 0.10 Hz in HIIT$_{85}$ and HIIT$_{115}$ (p = .048) with no differences between intensity groups. No significant difference in power spectral density ($p > .39$), gain ($p > .05$), or coherence ($p>.05$) between time or intensity. No significant differences in MCAv (p = .4), CVCi (p = .87), or CVRi (p = .87).
Northey et al, 2019	Small Randomized Controlled Trial	II	17 Female Breast Cancer Survivors HIIT (age 60.3 ± 8.1 years old) MOD (age 67.8 ± 7.0 years old) CON (61.5 ±7.8 years old)	12-week intervention Cycled 20–30 min 3x/week 3 groups: • High intensity interval training (HIIT, 30 sec intervals at ~90% maximal heart rate or ~105% peak power with 2 min active recovery) • Moderate intensity continuous power) • No-exercise control (CON)	TCD measured: Resting MCAv, Cerebrovascular Reactivity to CO$_2$	No significant differences in resting MCAv (p = .24) or cerebrovascular reactivity (p = .54) after HIIT compared to MOD. No significant differences in resting MCAv (p = .86) or cerebrovascular reactivity (p = .72) after HIIT compared to CON.

(*Continued*)

Table 1. (Continued)

Study	Design	Level of Evidence	Subjects	Intervention	Outcome measures	Results
Tallon et al, 2019	Small Randomized Cross-over Trial	II	8 Prepubertal Children (age 10 ± 1.9 years old)	2 Cycling conditions: • High intensity interval exercise (HIIE, 1 min interval at 90%max watt with 1 min active recovery at 20%max watt for 6 intervals) • Moderate-intensity steady-state exercise (MISS, 15 min at 44%max watt)	TCD measured: Exercise MCAv during each interval Immediate post-exercise MCAv 30-minutes post-exercise MCAv	Significant decrease in exercise MCAv during the 6th interval of HIIE compared to baseline (10.7%, p = .004). Significant decrease in exercise MCAv during the 3rd and 4th intervals of HIIE compared to MISS (p = .001). Significant decrease in MCAv immediately post-exercise following HIIE and MISS (p < .001). No significant difference in MCAv at 30-minutes post-exercise following HIIE and MISS compared to baseline (p>.05). Significant increase in exercise MCAv during the 2nd minute of MISS compared to baseline (5.8%, p = .004).
Monroe et al, 2016	Nonrandomized Cross-Over Trial	III	15 Recreationally Active Adults (age 21.3 ± 2.4 years old)	2 cycling conditions: • Sprint Interval Cycling (SIC, 30 second all-out sprint interval with 4 min active recovery for 4 intervals) • Constant Resistance Cycling (CRC, 18 min at 70rpm with resistance set by matching total work performed during SIC)	Near-Infrared Spectroscopy measured: Oxygenated Hemoglobin (HbO2) Deoxygenated Hemoglobin (HHb)	Significant increase in average HbO2(effect size = .536, p = .001), minimum HbO2 during recovery (effect size = .392, p < .001) and maximum HbO2 during recovery (effect size = .588, p = .001) in SIC compared to CRC. Significant increase in average HHb during SIC compared to CRC (effect size = .386, p = .003).

MCAv = middle cerebral artery blood velocity, dCA = dynamic cerebral autoregulation, min = minute, HRmax = maximum heart rate, CVCi = cerebrovascular conductance index, CVRi = cerebrovascular resistance index, CO_2 = carbon dioxide.

involved healthy individuals, although some studies only included men (n = 1) [45], women (n = 1) [46], or children (n = 1) [47]. Prior activity levels of participants ranged from inactive [50], recreationally active [51] and endurance trained [45].

High intensity interval protocols

Methods of prescribing HIIE varied greatly and made comparisons between studies difficult. HIIE protocols included 6- to 16-week exercise interventions (n = 3) [45, 46, 50] or one single bout of exercise(n = 4) [44, 47, 51, 53]. By examining 6- to 16-weeks of HIIE, the long-term or chronic effects of this intervention were studied. By examining a single bout of HIIE, the immediate or acute effects of the exercise were reported. In addition to the duration variability, we found that the mode of HIIE also differed across the included studies. One study used a treadmill as the mode of exercise with 4-minute intervals of 90–95% maximal heart rate for 30 minutes [50]. The remaining six studies used cycling as the mode of exercise but differed in parameters ranging from 30 seconds [46, 51] to 7-minute intervals [45] at 85% to 115% of maximal watts [45, 47, 51] or ~ 85% to 90% maximal heart rate [44, 46, 50, 53]. A constant between all studies included an active (rather than passive) recovery interval between sprints. However, the intensity and duration of recovery intervals differed greatly.

Cerebrovascular outcome measures

The results of this review can be operationalized based on the outcome variables measured during HIIE such as MCAv (n = 4) [44–47], dCA (n = 2) [44, 45], cerebral de/oxygenated hemoglobin (n = 2) [50, 51], cerebrovascular reactivity to CO_2 (n = 2) [46, 53] and cerebrovascular conductance/resistance index (n = 1) [45]. Table 2 describes whether HIIE increased, decreased or had no influence on the operationalized cerebrovascular measures. A meta-analysis was not performed due to low number of studies ($\leq$ 2) reporting each operationalized cerebrovascular measure.

MCAv. Of the studies reporting MCAv outcomes, resting MCAv (n = 2) [45, 46], exercise MCAv (n = 2) [44, 47] and MCAv immediately post exercise (n = 1) [47] were used. No significant differences were found for resting MCAv after 6- or 12-weeks of HIIE when compared to moderate continuous exercise or control [45, 46]. During an acute bout of HIIE, exercise MCAv was significantly decreased compared to moderate continuous exercise [44, 47]. Conflicting results were found between two studies comparing exercise MCAv to rest. Burma et al. [44] reported no significant difference between average exercise MCAv and rest in adults. However, rather than reporting average exercise MCAv of the entire HIIE bout, Tallon et al. [47] reported exercise MCAv for each 1-minute sprint interval of HIIE. During the 6th sprint interval of HIIE, Tallon et al. [47] reported significantly decreased exercise MCAv compared to rest which remained immediately following exercise [47].

dCA. Transfer function analysis of dCA was reported in the very low and low frequency bands (n = 2) [44, 45]. Drapeau et al. [45] conducted a 6-week intervention of HIIE and reported a significant decrease in phase compared to rest with no significant change in coherence or gain. Burma et al. [44] conducted a single bout of HIIE and reported decreased MCAv systolic phase immediately following exercise that extended up to four hours later.

De/oxygenated hemoglobin. Oxygenated and deoxygenated hemoglobin were reported during a single bout of HIIE (n = 1) [51] and during a 16-week HIIE intervention (n = 1) [50]. Monroe et al. [51] conducted a single bout of HIIE and reported an increase in oxygenated

Table 2. Summary of the effects of HIIE on operationalized cerebrovascular measures.

	Resting MCAv	Exercise MCAv	Post-Exercise MCAv	dCA phase	dCA Gain	dCA Coherence	De/Oxygenated Hemoglobin	Cerebrovascular Conductance Resistance Index	Cerebrovascular Reactivity to CO_2
Burma et al, March 2020		↓ To moderate		↓	#	#			
Burma et al, June 2020									↓ To moderate and control
Coetsee et al, 2017							# During cortical activation		
Drapeau et al, 2019	#			↓	#	#		#	
Northey et al, 2019	#								#
Tallon et al, 2019		↓ To moderate and rest	↓ To rest						
Monroe et al, 2016							↑ During HIIE		

↓ = Decreased effect, ↑ = Increased effect, # = no effect and deoxygenated hemoglobin during HIIE compared to moderate continuous exercise. Coetsee et al. [50] conducted a 16-week intervention of HIIE and reported no significant lasting changes in oxygenated or deoxygenated hemoglobin during cortical activation.

Cerebrovascular reactivity. Cerebrovascular reactivity to CO_2 were reported during a single bout of HIIE (n = 1) [53] and during a 12-week HIIE intervention (n = 1) [46]. After a single bout of HIIE, cerebrovascular reactivity to higher CO_2, or hypercapnia, was significantly decreased by 37% and remained an hour later [53]. The reduced cerebrovascular reactivity to hypercapnia was also significantly different than moderate intensity and control. Cerebrovascular reactivity to lower CO_2, or hypocapnia, was not significantly different following a single bout of HIIE [53]. Cerebrovascular reactivity to CO_2 was also not significantly different following 12 weeks of HIIE [46].

Cerebrovascular conductance and resistance. Cerebrovascular conductance index and cerebrovascular resistance index were only reported in a single study [45]. A 6-week HIIE intervention reported no significant changes in cerebrovascular conductance index or cerebrovascular resistance index [45].

Quality review

The quality review of each study is presented in Table 3. Out of seven total quality criteria, 2 studies reported five quality criteria [44, 53], one study reported four quality criteria [45] and the remaining four studies reported three quality criteria [46, 47, 50, 51]. Therefore, the overall quality criteria results were moderately poor. All studies accounted for subjects and monitored the HIIE protocol parameters. No studies reported avoidance of contamination or co-intervention. No studies reported blinding of the outcome assessments. Only Burma et al. [44, 53] and Monroe et al. [51] reported their reliability via coefficient of reproducibility and intraclass coefficients of their measures. And only Burma et al. [44, 53] and Drapeau et al. [45] reported validity of their respective cerebrovascular outcomes.

Discussion

This review met the objective of reporting the results of various HIIE studies and the effects on operationalized cerebrovascular function in healthy individuals. This review is the first to report the effects HIIE on cerebrovascular function compared to moderate continuous

Table 3. Summary of quality review.

	Avoided Contamination and Co-Intervention	Random Assignment to Conditions	Blinded Assessment	Monitored Intervention	Accounted for All Subjects	Reported Reliability of Measures Used	Reported Validity of Measures Used	Total Number of Criteria Met
Burma et al, March 2020	No	Yes	No	Yes	Yes	Yes	Yes	5
Burma et al, June 2020	No	Yes	No	Yes	Yes	Yes	Yes	5
Coetsee et al, 2017	No	Yes	No	Yes	Yes	No	No	3
Drapeau et al, 2019	No	Yes	No	Yes	Yes	No	Yes	4
Northey et al, 2019	No	Yes						
Tallon et al, 2019	No	Yes	No	Yes	Yes	No	No	3
Monroe et al, 2016	No	No	No	Yes	Yes	Yes	No	3

exercise and rest in healthy individuals. In general, we found that the acute and chronic effects of HIIE on cerebrovascular function vary largely depending on the methods and outcomes measured.

MCAv

In these studies, 6- to 12-week HIIE interventions had no effect on resting MCAv in healthy individuals. No significant change in resting MCAv may be due to the HIIE intervention duration being too short. Also a ceiling effect may be observed for young, healthy individuals and could explain no changes in resting MCAv [19]. During a single bout of HIIE, hyperventilation and downstream arteriole vasoconstriction may explain the acute decreases in exercise MCAv compared to moderate continuous exercise [15, 31, 73, 74]. Vasoconstriction may play a protective role during HIIE due to heightened peripheral blood pressure potentially causing hyper-perfusion [75] or damage to the blood brain barrier [36]. During a single bout of HIIE, there is contradictory evidence comparing exercise MCAv to rest. One study reported no change in average exercise MCAv compared to resting [44]. Another study reported decreased exercise MCAv after six sprint intervals of HIIE and remained decreased compared to rest immediately following HIIE [47]. The differences reported in exercise MCAv compared to rest could be due to age [19] (adults versus prepubertal children) or due to the analysis of MCAv during HIIE (average over entire exercise versus separate sprint intervals). Decreases in exercise MCAv compared to rest may only occur in the late intervals of HIIE, during hyperventilation [76]. Therefore, exercise MCAv should be reported for each interval of HIIE rather than an average of the entire exercise bout.

dCA

After a 6-week intervention and single bout of HIIE, dCA phase was decreased compared to rest. The chronic effects of HIIE on dCA phase may be due to elevated cardiorespiratory fitness in endurance trained individuals being associated with attenuated dCA [45, 48]. In healthy individuals, increased frequency within MCAv and MAP waveforms (that can occur with HIIE) may cause a reduction in phase due to dCA being a high-pass filter [77, 78]. Burma et al. [44] also suggests that systolic phase may reveal greater changes in dCA than both diastolic and mean phase. After a single bout of HIIE, reduction in systolic phase extended up to 4 hours and therefore the common approach of abstaining from exercise 12 hours before research studies [79–81] may be too conservative [44].

Although not included in this review due to the observational study design, contradictory evidence of sustained dCA during HIIE has been reported [63]. Differences in exercise parameters between HIIE may be the cause to contradictory findings due to exhaustive exercise showing decreased dCA [34, 82]. More studies are needed to confirm the acute and chronic decreases in dCA following HIIE.

De/oxygenated hemoglobin

After a 16-week HIIE intervention, oxygenated and deoxygenated hemoglobin during cortical activation did not change [50]. However, the 16-week HIIE intervention decreased reaction time during cortical activation and therefore may have increased efficiency of cortical oxygen use [50]. During a single bout of HIIE oxygenated and deoxygenated hemoglobin increased compared to moderate continuous exercise [51]. As suggested by Coetsee et al, increased oxygenated hemoglobin during neuronal activation may suggest engaging additional regions of the brain [50]; while decreased oxygenated hemoglobin may indicate reduced neuronal activity due to task-efficiency [50]. The acute and chronic effects of HIIE on oxygenated and deoxygenated hemoglobin still needs further investigation due to each only being reported in a single study.

Cerebrovascular reactivity

A 12-week HIIE intervention did not significantly change cerebrovascular reactivity which could be due to vascular desensitization from chronic exposure to CO_2 during HIIE [36, 83]. Following a single bout of HIIE, cerebrovascular reactivity to hypercapnia was decreased showing the inability of the cerebrovascular system to maximally vasodilate. The maximal capacity for vasodilation after HIIE may be reduced following HIIE due to prolonged cerebrovascular vasoconstriction that occurs with hyperventilation during HIIE [36, 53]. Cerebrovascular reactivity to hypocapnia was not changed following a single bout of HIIE due to the ability of the vessels to vasoconstrict remaining intact [53]. The reduction in cerebrovascular reactivity to higher CO_2 remains an hour after HIIE. Therefore, the authors conclude again that the common approach of abstaining from exercise 12 hours before research studies [54, 80, 84] may be too conservative [53].

Cerebrovascular conductance and resistance

Cerebrovascular conductance and resistance were not significantly changed following a 6-week HIIE intervention. While a 6-week HIIE intervention significantly improved peripheral arterial conductance and resistance [4], this change in the peripheral arteries may not be demonstrated in the cerebrovascular arterial conductance or resistance [41, 45]. However, due to the cerebrovascular conductance or resistance index being reported in only a single study, no conclusive effects of HIIE can be determined.

Future research

We recommend future research on the effects of HIIE on cerebrovascular function should include: 1) examining the cerebrovascular response during HIIE before and after an intervention of HIIE, 2) analyzing cerebrovascular outcomes during each separate interval of HIIE rather than an average of the entire bout, 3) simultaneously measuring MCAv, blood pressure, heart rate, and CO_2 during HIIE, 4) measuring cerebrovascular outcomes during HIIE, immediately following HIIE and at a follow up 30 minutes to 4 hours post exercise.

Limitations

The authors acknowledge a risk of publication bias by only including peer-reviewed articles written in English and did not include grey literature. The cerebrovascular function measures included within this review vary greatly and have vast heterogeneity. The overall quality of studies is moderately poor due to the lack of avoiding contamination, not blinding the assessment, and scarce reporting of the reliability and validity of the outcomes measured. These studies report the effects of HIIE on cerebrovascular function in healthy young individuals which limits generalizability and cannot be translated to clinical populations with altered cerebrovascular function at baseline, such as stroke [37, 39, 85, 86].

While HIIE is not a new mode of exercise, studying cerebrovascular measures during HIIE is novel. There are potential limitations to using TCD during HIIE and MCAv may be underestimated [87]. Cerebral oxygenation may also be underestimated due to the two-channel near infrared spectrometer not measuring the motor, occipital, or parietal cortex [50, 51]. Authors could only identify seven small studies with the oldest article dating back to 2015. The primary outcome of MCAv (n = 4) and dCA (n = 2) were reported in few studies with low power.

Therefore, a meta-analysis could not be performed due to insufficient mathematical combination.

Conclusion

This review has provided preliminary information studying the effects of HIIE on cerebrovascular function. Currently, there are a sparse number of research studies with moderately poor quality criteria that have reported the acute and chronic effects of HIIE on cerebrovascular function. An increased amount of studies and greater quality of research avoiding contamination, blinding the assessments, and reporting reliability and validity is needed. Randomized controlled trials with large sample sizes are needed to conduct a meta-analysis to combine and statistically analyze the summary results of HIIE on cerebrovascular function. Additionally, more studies are needed to determine the optimal interval parameters of HIIE to provide a consistent exercise dose between studies.

With increased interest in healthy brain aging and implementing interventions to maintain or improve brain health [88], studying the effects of HIIE interventions are critically needed [41, 42]. While this review only included healthy individuals, we provide an early reference to understanding "normal" physiological effects of HIIE on cerebrovascular function and the need to compare to clinical populations. Researchers should make further efforts to investigate and report the effects of HIIE on diverse measures of cerebrovascular function. To do so, it is imperative that researchers implement high quality criteria within the planning of future studies.

Supporting information

S1 Checklist. PRISMA 2009 checklist.
(DOC)

S1 Fig. PRISMA 2009 flow diagram.
(DOC)

S1 Table. Study exclusion from systematic review.
(DOCX)

Author Contributions

Conceptualization: Alicen A. Whitaker, Sandra A. Billinger.

Data curation: Alicen A. Whitaker, Sandra A. Billinger.

Formal analysis: Alicen A. Whitaker, Mohammed Alwatban, Andrea Freemyer, Jaime Perales-Puchalt.

Investigation: Alicen A. Whitaker.

Methodology: Alicen A. Whitaker, Sandra A. Billinger.

Writing – original draft: Alicen A. Whitaker, Mohammed Alwatban, Andrea Freemyer, Jaime Perales-Puchalt, Sandra A. Billinger.

Writing – review & editing: Alicen A. Whitaker, Mohammed Alwatban, Andrea Freemyer, Jaime Perales-Puchalt, Sandra A. Billinger.

References

1. Batacan RB Jr., Duncan MJ, Dalbo VJ, Tucker PS, Fenning AS. Effects of high-intensity interval training on cardiometabolic health: a systematic review and meta-analysis of intervention studies. Br J Sports Med. 2017; 51(6):494–503. Epub 2016/11/01. https://doi.org/10.1136/bjsports-2015-095841 PMID: 27797726.
2. Costa EC, Hay JL, Kehler DS, Boreskie KF, Arora RC, Umpierre D, et al. Effects of High-Intensity Interval Training Versus Moderate-Intensity Continuous Training On Blood Pressure in Adults with Pre- to Established Hypertension: A Systematic Review and Meta-Analysis of Randomized Trials. Sports Med. 2018; 48(9):2127–42. Epub 2018/06/28. https://doi.org/10.1007/s40279-018-0944-y PMID: 29949110.
3. Ramos JS, Dalleck LC, Tjonna AE, Beetham KS, Coombes JS. The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. Sports Med. 2015; 45(5):679–92. Epub 2015/03/17. https://doi.org/10.1007/s40279-015-0321-z PMID: 25771785.
4. Rakobowchuk M, Tanguay S, Burgomaster KA, Howarth KR, Gibala MJ, MacDonald MJ. Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow-mediated dilation in healthy humans. Am J Physiol Regul Integr Comp Physiol. 2008; 295(1):R236–42. Epub 2008/04/25. https://doi.org/10.1152/ajpregu.00069.2008 PMID: 18434437; PubMed Central PMCID: PMC2494806.
5. Tabata I, Nishimura K, Kouzaki M, Hirai Y, Ogita F, Miyachi M, et al. Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO2max. Med Sci Sports Exerc. 1996; 28(10):1327–30. Epub 1996/10/01. https://doi.org/10.1097/00005768-199610000-00018 PMID: 8897392.
6. Cunningham DA, McCrimmon D, Vlach LF. Cardiovascular response to interval and continuous training in women. Eur J Appl Physiol Occup Physiol. 1979; 41(3):187–97. Epub 1979/07/02. https://doi.org/10.1007/BF00430011 PMID: 488084.
7. Edge J, Bishop D, Goodman C. The effects of training intensity on muscle buffer capacity in females. Eur J Appl Physiol. 2006; 96(1):97–105. Epub 2005/11/12. https://doi.org/10.1007/s00421-005-0068-6 PMID: 16283370.
8. Ainslie PN, Cotter JD, George KP, Lucas S, Murrell C, Shave R, et al. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. J Physiol. 2008; 586(16):4005–10. Epub 2008/07/19. https://doi.org/10.1113/jphysiol.2008.158279 PMID: 18635643; PubMed Central PMCID: PMC2538930.
9. Prestgaard E, Mariampillai J, Engeseth K, Erikssen J, Bodegard J, Liestol K, et al. Change in Cardiorespiratory Fitness and Risk of Stroke and Death. Stroke. 2018:STROKEAHA118021798. Epub 2018/12/26. https://doi.org/10.1161/STROKEAHA.118.021798 PMID: 30580727.
10. Tari AR, Nauman J, Zisko N, Skjellegrind HK, Bosnes I, Bergh S, et al. Temporal changes in cardiorespiratory fitness and risk of dementia incidence and mortality: a population-based prospective cohort study. Lancet Public Health. 2019; 4(11):e565–e74. Epub 2019/11/05. https://doi.org/10.1016/S2468-2667(19)30183-5 PMID: 31677775.
11. Bayati M, Farzad B, Gharakhanlou R, Agha-Alinejad H. A practical model of low-volume high-intensity interval training induces performance and metabolic adaptations that resemble 'all-out' sprint interval training. J Sports Sci Med. 2011; 10(3):571–6. Epub 2011/01/01. PMID: 24150635; PubMed Central PMCID: PMC3737829.
12. Hsieh SS, Chueh TY, Huang CJ, Kao SC, Hillman CH, Chang YK, et al. Systematic review of the acute and chronic effects of high-intensity interval training on executive function across the lifespan. J Sports Sci. 2020:1–13. Epub 2020/08/12. https://doi.org/10.1080/02640414.2020.1803630 PMID: 32780634.
13. Aaslid R, Lindegaard KF, Sorteberg W, Nornes H. Cerebral autoregulation dynamics in humans. Stroke. 1989; 20(1):45–52. Epub 1989/01/01. https://doi.org/10.1161/01.str.20.1.45 PMID: 2492126.
14. Li Z, Zhang M, Xin Q, Luo S, Zhou W, Cui R, et al. Assessment of cerebral oxygenation oscillations in subjects with hypertension. Microvasc Res. 2013; 88:32–41. Epub 2013/04/16. https://doi.org/10.1016/j.mvr.2013.04.003 PMID: 23583904.
15. Smith KJ, Ainslie PN. Regulation of cerebral blood flow and metabolism during exercise. Exp Physiol. 2017; 102(11):1356–71. Epub 2017/08/09. https://doi.org/10.1113/EP086249 PMID: 28786150.
16. Brassard P, Ainslie PN, Secher NH. Cerebral oxygenation in health and disease. Front Physiol. 2014; 5:458. Epub 2014/12/17. https://doi.org/10.3389/fphys.2014.00458 PMID: 25505422; PubMed Central PMCID: PMC4241837.
17. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. Stroke. 2000; 31(7):1672–8. Epub 2000/07/08. https://doi.org/10.1161/01.str.31.7.1672 PMID: 10884472.

18. Witte E, Liu Y, Ward JL, Kempf KS, Whitaker A, Vidoni ED, et al. Exercise intensity and middle cerebral artery dynamics in humans. Respir Physiol Neurobiol. 2019; 262:32–9. Epub 2019/02/03. https://doi.org/10.1016/j.resp.2019.01.013 PMID: 30710650; PubMed Central PMCID: PMC6393201.

19. Ward JL, Craig JC, Liu Y, Vidoni ED, Maletsky R, Poole DC, et al. Effect of healthy aging and sex on middle cerebral artery blood velocity dynamics during moderate-intensity exercise. Am J Physiol Heart Circ Physiol. 2018; 315(3):H492–H501. Epub 2018/05/19. https://doi.org/10.1152/ajpheart.00129.2018 PMID: 29775407; PubMed Central PMCID: PMC6172645.

20. Brugniaux JV, Marley CJ, Hodson DA, New KJ, Bailey DM. Acute exercise stress reveals cerebrovascular benefits associated with moderate gains in cardiorespiratory fitness. J Cereb Blood Flow Metab. 2014; 34(12):1873–6. Epub 2014/10/02. https://doi.org/10.1038/jcbfm.2014.142 PMID: 25269518; PubMed Central PMCID: PMC4269737.

21. Davenport MH, Hogan DB, Eskes GA, Longman RS, Poulin MJ. Cerebrovascular reserve: the link between fitness and cognitive function? Exerc Sport Sci Rev. 2012; 40(3):153–8. Epub 2012/04/17. https://doi.org/10.1097/JES.0b013e3182553430 PMID: 22504726.

22. Fisher JP, Hartwich D, Seifert T, Olesen ND, McNulty CL, Nielsen HB, et al. Cerebral perfusion, oxygenation and metabolism during exercise in young and elderly individuals. J Physiol. 2013; 591(7):1859–70. Epub 2012/12/12. https://doi.org/10.1113/jphysiol.2012.244905 PMID: 23230234; PubMed Central PMCID: PMC3624856.

23. Brys M, Brown CM, Marthol H, Franta R, Hilz MJ. Dynamic cerebral autoregulation remains stable during physical challenge in healthy persons. Am J Physiol Heart Circ Physiol. 2003; 285(3):H1048–54. Epub 2003/08/14. https://doi.org/10.1152/ajpheart.00062.2003 PMID: 12915389.

24. Ogoh S, Fadel PJ, Zhang R, Selmer C, Jans O, Secher NH, et al. Middle cerebral artery flow velocity and pulse pressure during dynamic exercise in humans. Am J Physiol Heart Circ Physiol. 2005; 288(4): H1526–31. Epub 2004/12/14. https://doi.org/10.1152/ajpheart.00979.2004 PMID: 15591094.

25. Steventon JJ, Hansen AB, Whittaker JR, Wildfong KW, Nowak-Fluck D, Tymko MM, et al. Cerebrovascular Function in the Large Arteries Is Maintained Following Moderate Intensity Exercise. Front Physiol. 2018; 9:1657. Epub 2018/12/07. https://doi.org/10.3389/fphys.2018.01657 PMID: 30519192; PubMed Central PMCID: PMC6258791.

26. Herholz K, Buskies W, Rist M, Pawlik G, Hollmann W, Heiss WD. Regional cerebral blood flow in man at rest and during exercise. J Neurol. 1987; 234(1):9–13. Epub 1987/01/01. https://doi.org/10.1007/BF00314001 PMID: 3102697.

27. Jorgensen LG, Perko M, Hanel B, Schroeder TV, Secher NH. Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. J Appl Physiol (1985). 1992; 72(3):1123–32. Epub 1992/03/01. https://doi.org/10.1152/jappl.1992.72.3.1123 PMID: 1568967.

28. Thomas SN, Schroeder T, Secher NH, Mitchell JH. Cerebral blood flow during submaximal and maximal dynamic exercise in humans. J Appl Physiol (1985). 1989; 67(2):744–8. Epub 1989/08/01. https://doi.org/10.1152/jappl.1989.67.2.744 PMID: 2507500.

29. Hellstrom G, Wahlgren NG. Physical exercise increases middle cerebral artery blood flow velocity. Neurosurg Rev. 1993; 16(2):151–6. Epub 1993/01/01. https://doi.org/10.1007/BF00258249 PMID: 8345909.

30. Moraine JJ, Lamotte M, Berre J, Niset G, Leduc A, Naeije R. Relationship of middle cerebral artery blood flow velocity to intensity during dynamic exercise in normal subjects. Eur J Appl Physiol Occup Physiol. 1993; 67(1):35–8. Epub 1993/01/01. https://doi.org/10.1007/BF00377701 PMID: 8375362.

31. Ogoh S, Ainslie PN. Regulatory mechanisms of cerebral blood flow during exercise: new concepts. Exerc Sport Sci Rev. 2009; 37(3):123–9. Epub 2009/06/25. https://doi.org/10.1097/JES.0b013e3181aa64d7 PMID: 19550203.

32. Maeda H, Matsumoto M, Handa N, Hougaku H, Ogawa S, Itoh T, et al. Reactivity of cerebral blood flow to carbon dioxide in various types of ischemic cerebrovascular disease: evaluation by the transcranial Doppler method. Stroke. 1993; 24(5):670–5. Epub 1993/05/01. https://doi.org/10.1161/01.str.24.5.670 PMID: 8488521.

33. Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, et al. Regional brain blood flow in man during acute changes in arterial blood gases. J Physiol. 2012; 590(14):3261–75. Epub 2012/04/13. https://doi.org/10.1113/jphysiol.2012.228551 PMID: 22495584; PubMed Central PMCID:

34. Bailey DM, Evans KA, McEneny J, Young IS, Hullin DA, James PE, et al. Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. Exp Physiol. 2011; 96(11):1196–207. Epub 2011/08/16. https://doi.org/10.1113/expphysiol.2011.060178 PMID: 21841038.

35. Phillips AA, Matin N, Jia M, Squair JW, Monga A, Zheng MMZ, et al. Transient Hypertension after Spinal Cord Injury Leads to Cerebrovascular Endothelial Dysfunction and Fibrosis. J Neurotrauma. 2018; 35

(3):573–81. Epub 2017/11/17. https://doi.org/10.1089/neu.2017.5188 PMID: 29141501; PubMed Central PMCID: PMC6421994.

36. Lucas SJ, Cotter JD, Brassard P, Bailey DM. High-intensity interval exercise and cerebrovascular health: curiosity, cause, and consequence. J Cereb Blood Flow Metab. 2015; 35(6):902–11. Epub 2015/04/03. https://doi.org/10.1038/jcbfm.2015.49 PMID: 25833341; PubMed Central PMCID: PMC4640257.

37. Aries MJ, Elting JW, De Keyser J, Kremer BP, Vroomen PC. Cerebral autoregulation in stroke: a review of transcranial Doppler studies. Stroke. 2010; 41(11):2697–704. Epub 2010/10/12. https://doi.org/10.1161/STROKEAHA.110.594168 PMID: 20930158.

38. Castro P, Azevedo E, Sorond F. Cerebral Autoregulation in Stroke. Curr Atheroscler Rep. 2018; 20(8):37. Epub 2018/05/23. https://doi.org/10.1007/s11883-018-0739-5 PMID: 29785667.

39. Kempf KS, Whitaker AA, Lui Y, Witte E, Perdomo SJ, Ward JL, et al. The Effect of Stroke on Middle Cerebral Artery Blood Flow Velocity Dynamics During Exercise. J Neurol Phys Ther. 2019; 43(4):212–9. Epub 2019/08/27. https://doi.org/10.1097/NPT.0000000000000289 PMID: 31449179; PubMed Central PMCID: PMC6744289.

40. Franklin BA, Thompson PD, Al-Zaiti SS, Albert CM, Hivert MF, Levine BD, et al. Exercise-Related Acute Cardiovascular Events and Potential Deleterious Adaptations Following Long-Term Exercise Training: Placing the Risks Into Perspective-An Update: A Scientific Statement From the American Heart Association. Circulation. 2020; 141(13):e705–e36. Epub 2020/02/27. https://doi.org/10.1161/CIR.0000000000000749 PMID: 32100573.

41. Lucas SJE, Cotter JD, Brassard P, Bailey DM. High-intensity interval exercise and cerebrovascular health: curiosity, cause, and consequence. J Cerebr Blood F Met. 2015; 35(6):902–11. https://doi.org/10.1038/jcbfm.2015.49 WOS:000355575600003. PMID: 25833341

42. Calverley TA, Ogoh S, Marley CJ, Steggall M, Marchi N, Brassard P, et al. HIITing the brain with exercise: mechanisms, consequences and practical recommendations. J Physiol. 2020; 598(13):2513–30. Epub 2020/04/30. https://doi.org/10.1113/JP275021 PMID: 32347544.

43. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009; 339:b2535. Epub 2009/07/23. https://doi.org/10.1136/bmj.b2535 PMID: 19622551; PubMed Central PMCID: PMC2714657.

44. Burma JS, Copeland P, Macaulay A, Khatra O, Wright AD, Smirl JD. Dynamic cerebral autoregulation across the cardiac cycle during 8 hr of recovery from acute exercise. Physiol Rep. 2020; 8(5):e14367. Epub 2020/03/13. https://doi.org/10.14814/phy2.14367 PMID: 32163235; PubMed Central PMCID: PMC7066871.

45. Drapeau A, Labrecque L, Imhoff S, Paquette M, Le Blanc O, Malenfant S, et al. Six weeks of high-intensity interval training to exhaustion attenuates dynamic cerebral autoregulation without influencing resting cerebral blood velocity in young fit men. Physiol Rep. 2019; 7(15):e14185. Epub 2019/08/03. https://doi.org/10.14814/phy2.14185 PMID: 31373166; PubMed Central PMCID: PMC6675921.

46. Northey JM, Pumpa KL, Quinlan C, Ikin A, Toohey K, Smee DJ, et al. Cognition in breast cancer survivors: A pilot study of interval and continuous exercise. J Sci Med Sport. 2019; 22(5):580–5. Epub 2018/12/18. https://doi.org/10.1016/j.jsams.2018.11.026 PMID: 30554923.

47. Tallon CM, Simair RG, Koziol AV, Ainslie PN, McManus AM. Intracranial Vascular Responses to High-Intensity Interval Exercise and Moderate-Intensity Steady-State Exercise in Children. Pediatr Exerc Sci. 2019; 31(3):290–5. Epub 2019/03/06. https://doi.org/10.1123/pes.2018-0234 PMID: 30832540.

48. Labrecque L, Rahimaly K, Imhoff S, Paquette M, Le Blanc O, Malenfant S, et al. Diminished dynamic cerebral autoregulatory capacity with forced oscillations in mean arterial pressure with elevated cardiorespiratory fitness. Physiol Rep. 2017; 5(21). Epub 2017/11/11. https://doi.org/10.14814/phy2.13486 PMID: 29122957; PubMed Central PMCID: PMC5688778.

49. Fantini S, Sassaroli A, Tgavalekos KT, Kornbluth J. Cerebral blood flow and autoregulation: current measurement techniques and prospects for noninvasive optical methods. Neurophotonics. 2016; 3(3):031411. Epub 2016/07/13. https://doi.org/10.1117/1.NPh.3.3.031411 PMID: 27403447; PubMed Central PMCID: PMC4914489.

50. Coetsee C, Terblanche E. Cerebral oxygenation during cortical activation: the differential influence of three exercise training modalities. A randomized controlled trial. Eur J Appl Physiol. 2017; 117(8):1617– .

51. Monroe DC, Gist NH, Freese EC, O'Connor PJ, McCully KK, Dishman RK. Effects of Sprint Interval Cycling on Fatigue, Energy, and Cerebral Oxygenation. Med Sci Sports Exerc. 2016; 48(4):615–24. Epub 2015/11/13. https://doi.org/10.1249/MSS.0000000000000809 PMID: 26559448.

52. Auger H, Bherer L, Boucher E, Hoge R, Lesage F, Dehaes M. Quantification of extra-cerebral and cerebral hemoglobin concentrations during physical exercise using time-domain near infrared spectroscopy.

Biomed Opt Express. 2016; 7(10):3826–42. Epub 2016/11/22. https://doi.org/10.1364/BOE.7.003826 PMID: 27867696; PubMed Central PMCID: PMC5102543.

53. Burma JS, Macaulay A, Copeland P, Khatra O, Bouliane KJ, Smirl JD. Comparison of cerebrovascular reactivity recovery following high-intensity interval training and moderate-intensity continuous training. Physiol Rep. 2020; 8(11):e14467. Epub 2020/06/09. https://doi.org/10.14814/phy2.14467 PMID: 32506845; PubMed Central PMCID: PMC7276190.

54. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. J Appl Physiol (1985). 2007; 102(3):870–7. Epub 2006/11/18. https://doi.org/10.1152/japplphysiol.00906.2006 PMID: 17110510.

55. Scheeren TW, Schober P, Schwarte LA. Monitoring tissue oxygenation by near infrared spectroscopy (NIRS): background and current applications. J Clin Monit Comput. 2012; 26(4):279–87. Epub 2012/04/03. https://doi.org/10.1007/s10877-012-9348-y PMID: 22467064; PubMed Central PMCID: PMC3391360.

56. Duffin J, Sobczyk O, McKetton L, Crawley A, Poublanc J, Venkatraghavan L, et al. Cerebrovascular Resistance: The Basis of Cerebrovascular Reactivity. Front Neurosci. 2018; 12:409. Epub 2018/07/06. https://doi.org/10.3389/fnins.2018.00409 PMID: 29973862; PubMed Central PMCID: PMC6020782.

57. Fierstra J, Sobczyk O, Battisti-Charbonney A, Mandell DM, Poublanc J, Crawley AP, et al. Measuring cerebrovascular reactivity: what stimulus to use? J Physiol. 2013; 591(23):5809–21. Epub 2013/10/02. https://doi.org/10.1113/jphysiol.2013.259150 PMID: 24081155; PubMed Central PMCID: PMC3872753.

58. Sackett DL. How to read clinical journals. Can Med Assoc J. 1982; 126(12):1373. Epub 1982/06/15. PMID: 20313761; PubMed Central PMCID: PMC1863136.

59. Sackett DL. Rules of evidence and clinical recommendations for the management of patients. Can J Cardiol. 1993; 9(6):487–9. Epub 1993/07/01. PMID: 8221340.

60. Dupuy O, Tremblay J. Impact of Carbohydrate Ingestion on Cognitive Flexibility and Cerebral Oxygenation during High-Intensity Intermittent Exercise: A Comparison between Maple Products and Usual Carbohydrate Solutions. Nutrients. 2019; 11(9). Epub 2019/08/31. https://doi.org/10.3390/nu11092019 PMID: 31466305; PubMed Central PMCID: PMC6770040.

61. Willis SJ, Borrani F, Millet GP. Leg- vs arm-cycling repeated sprints with blood flow restriction and systemic hypoxia. Eur J Appl Physiol. 2019; 119(8):1819–28. Epub 2019/06/13. https://doi.org/10.1007/s00421-019-04171-0 PMID: 31187281.

62. Klein T, Bailey TG, Abeln V, Schneider S, Askew CD. Cerebral Blood Flow during Interval and Continuous Exercise in Young and Old Men. Med Sci Sports Exerc. 2019; 51(7):1523–31. Epub 2019/02/16. https://doi.org/10.1249/MSS.0000000000001924 PMID: 30768552.

63. Tsukamoto H, Hashimoto T, Olesen ND, Petersen LG, Sorensen H, Nielsen HB, et al. Dynamic Cerebral Autoregulation Is Maintained during High-Intensity Interval Exercise. Med Sci Sports Exerc. 2019; 51(2):372–8. Epub 2018/09/27. https://doi.org/10.1249/MSS.0000000000001792 PMID: 30252775.

64. Hanssen H, Minghetti A, Magon S, Rossmeissl A, Rasenack M, Papadopoulou A, et al. Effects of different endurance exercise modalities on migraine days and cerebrovascular health in episodic migraineurs: A randomized controlled trial. Scand J Med Sci Sports. 2018; 28(3):1103–12. Epub 2017/11/22. https://doi.org/10.1111/sms.13023 PMID: 29161767.

65. Kirkham AA, Shave RE, Bland KA, Bovard JM, Eves ND, Gelmon KA, et al. Protective effects of acute exercise prior to doxorubicin on cardiac function of breast cancer patients: A proof-of-concept RCT. Int J Cardiol. 2017; 245:263–70. Epub 2017/07/25. https://doi.org/10.1016/j.ijcard.2017.07.037 PMID: 28735755.

66. Gjellesvik TI, Becker F, Tjonna AE, Indredavik B, Nilsen H, Brurok B, et al. Effects of High-Intensity Interval Training after Stroke (The HIIT-Stroke study)—A Multicenter Randomized Controlled Trial. Arch Phys Med Rehabil. 2020. Epub 2020/03/08. https://doi.org/10.1016/j.apmr.2020.02.006 PMID: 32145280.

67. Scott JM, Tucker WJ, Martin D, Crowell JB, Goetchius E, Ozgur O, et al. Association of Exercise and Swimming Goggles With Modulation of Cerebro-ocular Hemodynamics and Pressures in a Model of Spaceflight-Associated Neuro-ocular Syndrome. JAMA Ophthalmol. 2019; 137(6):652–9. Epub 2019/04/19. https://doi.org/10.1001/jamaophthalmol.2019.0459 PMID: 30998818; PubMed Central PMCID:

68. Saoi M, Percival M, Nemr C, Li A, Gibala M, Britz-McKibbin P. Characterization of the Human Skeletal Muscle Metabolome for Elucidating the Mechanisms of Bicarbonate Ingestion on Strenuous Interval Exercise. Anal Chem. 2019; 91(7):4709–18. Epub 2019/03/06. https://doi.org/10.1021/acs.analchem.9b00149 PMID: 30835436.

69. Labrecque L, Drapeau A, Rahimaly K, Imhoff S, Billaut F, Brassard P. Comparable blood velocity changes in middle and posterior cerebral arteries during and following acute high-intensity exercise in young fit women. Physiol Rep. 2020; 8(9):e14430. Epub 2020/04/29. https://doi.org/10.14814/phy2.14430 PMID: 32342622; PubMed Central PMCID: PMC7186567.

70. Peyravi A, Yazdanpanahi N, Nayeri H, Hosseini SA. The effect of endurance training with crocin consumption on the levels of MFN2 and DRP1 gene expression and glucose and insulin indices in the muscle tissue of diabetic rats. J Food Biochem. 2020; 44(2):e13125. Epub 2019/12/19. https://doi.org/10.1111/jfbc.13125 PMID: 31849103.

71. Komiyama T, Tanoue Y, Sudo M, Costello JT, Uehara Y, Higaki Y, et al. Cognitive Impairment during High-Intensity Exercise: Influence of Cerebral Blood Flow. Med Sci Sports Exerc. 2020; 52(3):561–8. Epub 2019/10/15. https://doi.org/10.1249/MSS.0000000000002183 PMID: 31609297.

72. Hansen RK, Nielsen PS, Schelske MW, Secher NH, Volianitis S. CO2 supplementation dissociates cerebral oxygenation and middle cerebral artery blood velocity during maximal cycling. Scand J Med Sci Sports. 2020; 30(3):399–407. Epub 2019/10/28. https://doi.org/10.1111/sms.13582 PMID: 31650627.

73. Gonzalez-Alonso J, Dalsgaard MK, Osada T, Volianitis S, Dawson EA, Yoshiga CC, et al. Brain and central haemodynamics and oxygenation during maximal exercise in humans. J Physiol. 2004; 557(Pt 1):331–42. Epub 2004/03/09. https://doi.org/10.1113/jphysiol.2004.060574 PMID: 15004212; PubMed Central PMCID: PMC1665053.

74. Linkis P, Jorgensen LG, Olesen HL, Madsen PL, Lassen NA, Secher NH. Dynamic exercise enhances regional cerebral artery mean flow velocity. J Appl Physiol (1985). 1995; 78(1):12–6. Epub 1995/01/01. https://doi.org/10.1152/jappl.1995.78.1.12 PMID: 7713801.

75. Brassard P, Kim YS, van Lieshout J, Secher NH, Rosenmeier JB. Endotoxemia reduces cerebral perfusion but enhances dynamic cerebrovascular autoregulation at reduced arterial carbon dioxide tension. Crit Care Med. 2012; 40(6):1873–8. Epub 2012/05/23. https://doi.org/10.1097/CCM.0b013e3182474ca7 PMID: 22610190.

76. Ogoh S, Ainslie PN. Cerebral blood flow during exercise: mechanisms of regulation. J Appl Physiol (1985). 2009; 107(5):1370–80. Epub 2009/09/05. https://doi.org/10.1152/japplphysiol.00573.2009 PMID: 19729591.

77. van Beek AH, Claassen JA, Rikkert MG, Jansen RW. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. J Cereb Blood Flow Metab. 2008; 28 (6):1071–85. Epub 2008/03/20. https://doi.org/10.1038/jcbfm.2008.13 PMID: 18349877.

78. Hamner JW, Cohen MA, Mukai S, Lipsitz LA, Taylor JA. Spectral indices of human cerebral blood flow control: responses to augmented blood pressure oscillations. J Physiol. 2004; 559(Pt 3):965–73. Epub 2004/07/16. https://doi.org/10.1113/jphysiol.2004.066969 PMID: 15254153; PubMed Central PMCID: PMC1665190.

79. Ainslie PN, Barach A, Murrell C, Hamlin M, Hellemans J, Ogoh S. Alterations in cerebral autoregulation and cerebral blood flow velocity during acute hypoxia: rest and exercise. Am J Physiol Heart Circ Physiol. 2007; 292(2):H976–83. Epub 2006/10/03. https://doi.org/10.1152/ajpheart.00639.2006 PMID: 17012355.

80. Ainslie PN, Hamlin M, Hellemans J, Rasmussen P, Ogoh S. Cerebral hypoperfusion during hypoxic exercise following two different hypoxic exposures: independence from changes in dynamic autoregulation and reactivity. Am J Physiol Regul Integr Comp Physiol. 2008; 295(5):R1613–22. Epub 2008/09/05. https://doi.org/10.1152/ajpregu.90420.2008 PMID: 18768767.

81. Smirl JD, Hoffman K, Tzeng YC, Hansen A, Ainslie PN. Relationship between blood pressure and cerebral blood flow during supine cycling: influence of aging. J Appl Physiol (1985). 2016; 120(5):552–63. Epub 2015/11/21. https://doi.org/10.1152/japplphysiol.00667.2015 PMID: 26586907; PubMed Central PMCID: PMC4773644.

82. Ogoh S, Dalsgaard MK, Yoshiga CC, Dawson EA, Keller DM, Raven PB, et al. Dynamic cerebral autoregulation during exhaustive exercise in humans. Am J Physiol Heart Circ Physiol. 2005; 288(3): H1461–7. Epub 2004/10/23. https://doi.org/10.1152/ajpheart.00948.2004 PMID: 15498819.

83. Thomas BP, Yezhuvath US, Tseng BY, Liu P, Levine BD, Zhang R, et al. Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO2. J Magn Reson Imaging. 2013; 38(5):1177–83. Epub 2013/03/26. https://doi.org/10.1002/jmri.24090 PMID: 23526811; PubMed Central PMCID: PMC3695025.

Physiol. 2011; 589(Pt 12):3039–48. Epub 2011/04/28. https://doi.org/10.1113/jphysiol.2011.206052 PMID: 21521758; PubMed Central PMCID: PMC3139085.

85. Alexandrov AV, Bladin CF, Norris JW. Intracranial blood flow velocities in acute ischemic stroke. Stroke. 1994; 25(7):1378–83. Epub 1994/07/01. https://doi.org/10.1161/01.str.25.7.1378 PMID: 7912863.

86. Aoi MC, Hu K, Lo MT, Selim M, Olufsen MS, Novak V. Impaired cerebral autoregulation is associated with brain atrophy and worse functional status in chronic ischemic stroke. PLoS One. 2012; 7(10):

e46794. Epub 2012/10/17. https://doi.org/10.1371/journal.pone.0046794 PMID: 23071639; PubMed Central PMCID: PMC3469603.

87. Coverdale NS, Gati JS, Opalevych O, Perrotta A, Shoemaker JK. Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. J Appl Physiol (1985). 2014; 117(10):1090–6. Epub 2014/07/12. https://doi.org/10.1152/japplphysiol.00285.2014 PMID: 25012027.

88. Gorelick PB, Furie KL, Iadecola C, Smith EE, Waddy SP, Lloyd-Jones DM, et al. Defining Optimal Brain Health in Adults: A Presidential Advisory From the American Heart Association/American Stroke Association. Stroke. 2017; 48(10):e284–e303. Epub 2017/09/09. https://doi.org/10.1161/STR.0000000000000148 PMID: 28883125; PubMed Central PMCID: PMC5654545.

16

Reproducibility of isokinetic knee testing using the novel isokinetic SMM iMoment dynamometer

Tim Kambič[1,2]*, **Mitja Lainščak**[3,4], **Vedran Hadžić**[2]

1 Department of Research and Education, General Hospital Murska Sobota, Murska Sobota, Slovenia, **2** Laboratory of Sports and Medical Diagnostics, Faculty of Sport, University of Ljubljana, Ljubljana, Slovenia, **3** Division of Cardiology, General Hospital Murska Sobota, Murska Sobota, Slovenia, **4** Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

* tim.kambic@gmail.com

Abstract

Isokinetic dynamometry is the gold standard for testing maximal strength in elite sport and rehabilitation settings. To be clinically useful, such tests should be valid and reliable. Despite some evidence regarding the relative test vs retest reliability of knee dynamometry, there is still a paucity of research regarding the absolute reliability parameters. The purpose of this study was to assess the absolute and relative intra-device reproducibility of isokinetic knee flexion and extension using the novel SMM iMoment dynamometer. A total of 19 participants (13 males and 6 females, aged 24 (2) years, height 178 (9) cm and weight 76 (11) kg) performed two identical knee isokinetic tests with at least a week of rest between measurements. Peak torque of knee extension and flexion were determined at 60˚/s. Moderate (0.892) to excellent (0.988) relative reliability using the intraclass correlation coefficient (ICC) was obtained for peak knee torque. Absolute reliability assessed with a standard error of measurement (SEM %) was low, ranging from 2.54% to 6.93%, whereas the smallest real difference (SRD %) was moderate, ranging from 7.04% to 19.22%. Furthermore, there were no significant correlations between means and differences of two measurements, and Bland-Altman plots also showed no signs of heteroscedasticity. Our measurement protocol established the moderate to excellent reliability of the novel SMM iMoment isokinetic dynamometer. Therefore, this dynamometer can be applied in sport rehabilitation settings to measure maximal knee strength.

Editor: Yumeng Li, Texas State University, UNITED STATES

Introduction

Voluntary muscle contraction is vital for human physical functioning [1], as muscles generate joint forces necessary for movement, joint stabilization, and posture maintenance [2]. Thus, the accurate assessment of individual's muscular capacities is important to identify possible weakness related to disease or ageing [2], and later appropriately prescribe and monitor the progress of the athletic or rehabilitation exercise program [3].

Competing interests: The authors have declared that no competing interests exist.

Muscle strength can be expressed in numerous ways, including maximum weight lifted on an exercise device, maximum isometric and maximum isokinetic torque with angle specific or nonspecific assessment [1]. Since the early introduction of isokinetic dynamometry in 1967 [4], the method has become the gold standard in the evaluation of muscle performance and pathology in research, elite sport, and clinical practice [5,6]. An isokinetic dynamometer assesses joint-related muscle maximal concentric and eccentric strength under constant velocities throughout the range of motion [7]. To be clinically significant, such tests should be valid and reliable. The test-retest reliability was previously assessed using different isokinetic machines, such as Biodex [8–10], Cybex [6,11–14], Kin Com [5,15], Merac [16], Lido [17], iSam 9000 [18], and Technogym's REV9000 [7,19].

The majority of the older studies evaluated test-retest reliability of knee isokinetic torque only using the intraclass correlation coefficient (ICC) as an indicator of relative reliability. Some older studies using the ICC showed excellent reliability (>0.92) [8,11,13,20,21], while others, mainly more recent studies all demonstrated good (0.89) to excellent (0.98) test-retest reliability of knee flexors and extensors at a velocity 60 ˚/s [5,7,9,14,22,23]. In contrast, only a few studies have also examined the absolute reliability using the standard error of measurement (SEM) and/or the smallest real difference (SRD) for knee peak torque and work [5,7,14,19,21]. The SEM and SRD varied between 3.5–6.7% and 9.7–19.47%, respectively [5,7,14,21].

Furthermore, there is a paucity of research regarding the SRD during eccentric knee flexion, with only three studies published [5,7,14]. In addition, most of the previous dynamometers settings were controlled manually, from the repositioning of the dynamometer axis to changes of seat settings and starting position angle of the arm or leg [5,7,13,14,16,18,19,21,22]. To date, no device has used software manoeuvred robotic dynamometer position adjustments, which would have the potential to improve the reliability of the measurement of isokinetic maximal knee torque further. Therefore, this study aimed to determine the test-retest absolute and relative reliability of knee peak torque flexion and extension on the novel iMoment dynamometer, and to encourage its possible use in clinical and research settings.

Methods

Study design

This study was designed as a reproducibility study in a test-retest fashion, with at least one week break between both tests, as advised by the previous studies on the reliability of isokinetic testing [5]. Both tests were conducted during the same time of the day to reduce the effect of diurnal subject-linked variability [5]. To additionally optimize the accuracy of the measurements, all tests were conducted by the same researcher (TK). The leg testing order was randomly selected (e.g., leg tested first on the test, was tested second on a retest) in order to minimize the possible learning effect. The absolute and relative reliability was assessed with SEM (%), SRD (%) and ICC, respectively.

Subjects

Out of 24 healthy recreational subjects initially enrolled in the study, 19 completed both isokinetic tests (13 males and 6 females, aged 24(3) years, height 178 (9) cm, weight 76 (11) kg, all were left leg dominant (100%)) with 8 (3) days break between measurements. The dominant leg was defined as the leg used to kick a ball [24]. Four participants were excluded due to health problems (pain during or after the trial repetitions and/or test) and one participant left the study due to personal reasons.

No adverse cardiovascular or musculoskeletal problems were reported during the data collection. All subjects were advised to continue with their normal physical activity regimen, with the exception of vigorous intensity aerobic activities and sports, and lower limb strength training during the study. In addition, subjects were advised to avoid any moderate to vigorous physical activities at least two days prior to measurements and verbally recalled all recent physical activities to the researcher to ensure similar pre-test conditions.

Prior to inclusion into the study, all participants were informed about the methods and procedures, as well as possible risks during the isokinetic testing. Written consent was signed prior to inclusion into the study. The study was conducted according to the Declaration of Helsinki guidelines for the use of human participants. The study protocol was approved by the Board of Ethics in Sport, held at the Faculty of Sport, University of Ljubljana (identifier: 15/2018).

Study protocol

Measurements were performed on an isokinetic dynamometer SMM iMoment (SMM production systems, Ltd., Maribor, Slovenia) using a standard leg attachment. This is a novel self-constructed dynamometer in co-operation between SMM d.o.o., Faculty of Sport in Ljubljana and Faculty of Mechanical Engineering in Ljubljana (Fig 1). The device is a robotic dynamometer that is operated through software in all aspects, including dynamometer height, dynamometer position, chair position, seat length, seat backrest inclination as well as the rotation of the chair. Prior to each testing day, the machine was calibrated using a standard calibration weight (31.3 Nm), and before each test, the participant's leg was weighted for gravitational error torque (GET).

The warm-up consisted out of 8 minutes of cycling at 100 to 120 W with a cadence of 60 to 70 cycles per minute. Later all subjects performed a short dynamic stretching of lower limb and 10 repetitions of squat and hip thrust exercise. The test was performed with the participants in the sitting position. Participants were strapped with belts across the chest, pelvis and test leg thigh to minimize body movement and compensations of other muscles. Furthermore, the dynamometer axis of rotation was aligned with knee's joint axis of rotations using lateral epicondyle as an anatomic mark. The range of motion was 60˚ (from 90˚ to 30˚ of knee flexion, with full knee extension being 0˚).

After the general warm-up, each subject performed 10 submaximal concentric contractions of knee extension and flexion and 5 submaximal eccentric contractions in flexion at 60 ˚/s as part of a special warm-up and familiarization with the test. Intensity in the general warm up sets was progressed during each repetition from 50% to 80% of individual‘s perceived maximal strength of knee extensors and flexors. During the testing, subjects performed 5 maximal concentric contractions of knee extension and flexion in the first set followed by 5 maximal eccentric contractions of knee flexion in the second set. In the first set, each concentric contraction of knee extensors was followed by concentric contraction of knee flexors; in the second set, eccentric contraction was followed by concentric contraction of knee flexors. There was a 2-minute break between both sets. Participants were verbally encouraged by the investigator to give their maximal effort, and visual feedback was provided throughout the test on the dynamometer‘s monitor (Fig 1a).

Statistical analysis

The iMoment's software evaluation report provided data for each mode of contraction and muscle for the left and right legs. The highest peak contraction torque of each set of both tests was used in the reliability analysis. All data were calculated using the IBM SPSS Software for

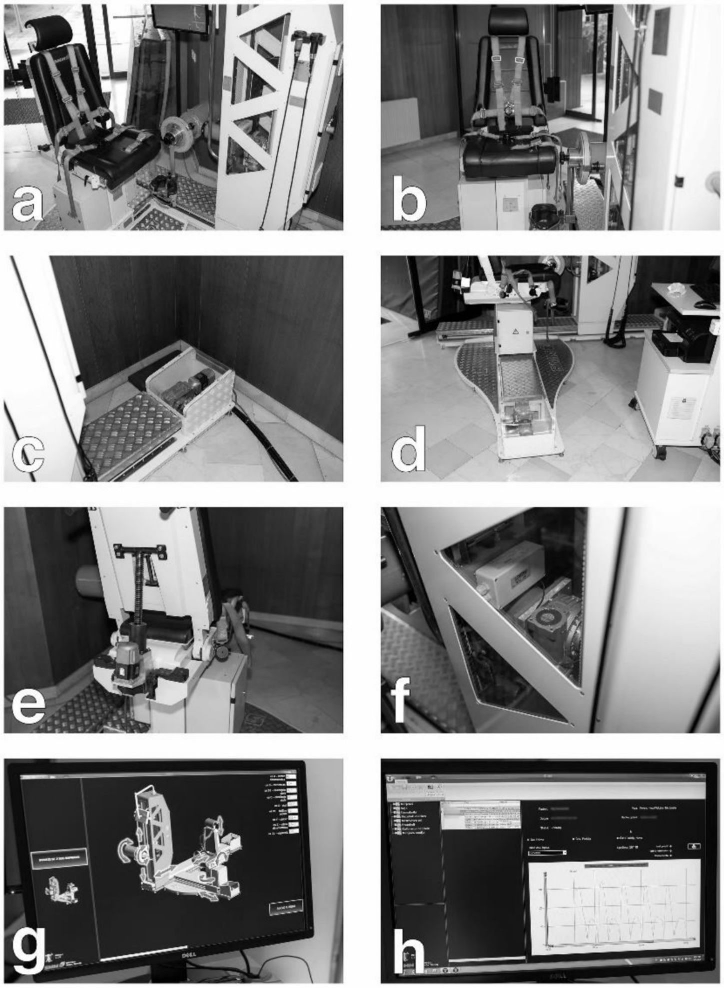

Fig 1. The novel SMM isokinetic dynamometer.

Windows (version 21, SPSS Inc., Armonk, New York, USA) and MedCalc (MedCalc Software, Seoul, Republic of Korea).

Categorical variables are displayed as numbers and percentages, and numeric variables are presented as means and standard deviations. All numeric variables were first checked for normality of distribution with Shapiro-Wilk's test. The differences between test and retest were assessed with repeated measure analysis of variance (ANOVA) for normally distributed variables and with Friedman's test in case of asymmetrically distributed variables.

The agreement between measurements was assessed with the intraclass correlation coefficient (ICC), and with the 95% confidence interval (95% CI) for ICC. Values of the ICC are interpreted according to recent guidelines [25]. Absolute and relative measurement error were assessed with the standard error of measurement ($\mathrm{SEM} = \mathrm{SD} \times \sqrt{1 - ICC}$) and with the SEM %, respectively [5]. The latter represents the limit for the smallest change that indicates a real improvement for a group of participants following a given intervention (e.g., exercise training). To calculate the smallest change that indicates a real improvement for a single participant, the absolute value and the percentage (%) of the smallest real difference (SRD) was used [26]. In contrast to SEM, the SEM % and SRD % are independent of the units of measurement.

Table 1. Peak torque on test and retest.

		Mean (SD)	95% CI for Mean	% difference test vs re-test	F	p
	Concentric extension at 60˚/s					
Left leg	Test	282.44 (64.00)	251.59, 313.29	4%	2.579	0.167*
	Re-test	271.60 (55.02)	245.08, 298.12			
Right leg	Test	243.82 (55.02)	217.30, 270.34	1%	0.761	0.395
	Re-test	241.31 (58.06)	213.33, 269.30			
	Concentric flexion at 60˚/s					
Left leg	Test	141.86 (30.18)	127.31, 156.40	1%	0.288	0.598
	Re-test	140.64 (32.63)	124.91, 156.37			
Right leg	Test	145.87 (32.99)	129.97, 161.77	-1%**	0.337	0.569
	Re-test	147.63 (31.13)	132.63, 162.64			
	Eccentric flexion at 60˚/s					
Left leg	Test	168.72 (38.84)	150.00, 187.45	1%	0.183	0.674
	Re-test	167.10 (37.77)	148.90, 185.30			
Right leg	Test	180.76 (36.30)	163.26, 198.25	4%	1.316	0.359*
	Re-test	172.88 (42.06)	152.61, 193.16			

*—Friedman test,

**—minus sign indicates better performance on retest, SD-standard deviation, CI-confidence interval, F-test statistic

The qualitative assessment of systematic changes between test and retest means was performed using Bland-Altman plots. These graphs can illustrate the possible issue of heteroscedasticity, which occurs when the test-retest difference increases as the mean of the value of both test decreases [27]. Additionally, the quantitative assessment of heteroscedasticity was calculated with the Pearson‘s correlation coefficient or Spearman's rank correlation coefficient for normally or asymmetrically distributed variables, respectively. The significance level was set at p-values <0.05.

Results

Table 1 summarizes test and retest findings. There were no statistically significant differences between test and retest in all measured isokinetic torque parameters (Table 1).

Table 2 presents the ICC, and the absolute and relative reliability statistics. The mean values of ICC for maximal peak concentric torque of the left and right quadriceps, the left and right hamstring, and the peak eccentric torque of the left hamstring showed excellent reliability,

Table 2. Reproducibility measures of the isokinetic concentric and eccentric knee flexion and extension.

	ICC	95% CI for ICC	p	d (Nm)	CVSD	SEM (Nm)	SEM %	SRD	SRD %
Concentric left quadriceps at 60˚/s (Nm)	0.967	[0.914, 0.987]	<0.001	10.84	1.7%	10.67	3.85%	29.57	10.67%
Concentric right quadriceps at 60˚/s (Nm)	0.988	[0.967, 0.995]	<0.001	2.51	1.5%	6.16	2.54%	17.07	7.04%
Concentric left hamstring at 60˚/s (Nm)	0.975	[0.935, 0.990]	<0.001	1.21	2.2%	4.91	3.47%	13.60	9.63%
Concentric right hamstring at 60˚/s (Nm)	0.955	[0.884, 0.983]	<0.001	-1.76	2.5%	6.66	4.54%	18.45	12.58%
Eccentric left hamstring at 60˚/s (Nm)	0.951	[0.873, 0.981]	<0.001	1.62	2.4%	8.28	4.93%	22.95	13.67%
Eccentric right hamstring at 60˚/s (Nm)	0.892	[0.718, 0.958]	<0.001	7.87	2.8%	12.26	6.93%	33.99	19.22%

ICC-intraclass correlation coefficient, d- mean difference between test and retest, CI-confidence interval, CVSD-coefficient of variation of standard deviation, SEM-standard error of measurement, SRD-smallest real difference while the ICC for peak eccentric torque of the right hamstring showed only good reliability. Based on the 95% CI for ICC, the reliability was excellent for the peak concentric torque of the left and right quadriceps, and the left hamstring. Moreover, the 95% CI for ICC showed good to excellent reliability for peak concentric torque of the right hamstring and peak eccentric torque for the left hamstring. Additionally, the reliability according to 95% CI for ICC was moderate to excellent for peak eccentric torque of the right hamstring. All ICC were significant ($p<0.001$).

The values of CVSD and SEM were low. The lowest CVSD was obtained for the peak concentric torque of the left quadriceps (1.7%), as the peak eccentric torque of the right hamstring had the highest CVSD (2.8%). Similarly, the low SEM % ranged from 2.54% to 6.93% for peak concentric torque of right quadriceps and peak eccentric torque for right hamstring, respectively.

Quantitative assessment of the systematic change showed no significant correlation between test-retest means and test-retest difference for all variables. All correlations values were low and ranged from -0.322 to +0.252. Furthermore, the qualitative results via the Bland-Altman plots showed good agreement between measurements and homoscedasticity for concentric torque of Quadriceps at 60°/s (-28.2; 41.6 Nm), concentric torque of the hamstring at 60°/s (-23.0;22.5 Nm) and eccentric torque of the hamstring at 60°/s (-36.2;45.7 Nm). We identified one outlier in concentric torque of the left quadriceps and the right hamstrings at 60°/s, and two outliers in the eccentric torque of the right hamstring (Fig 2a–2c).

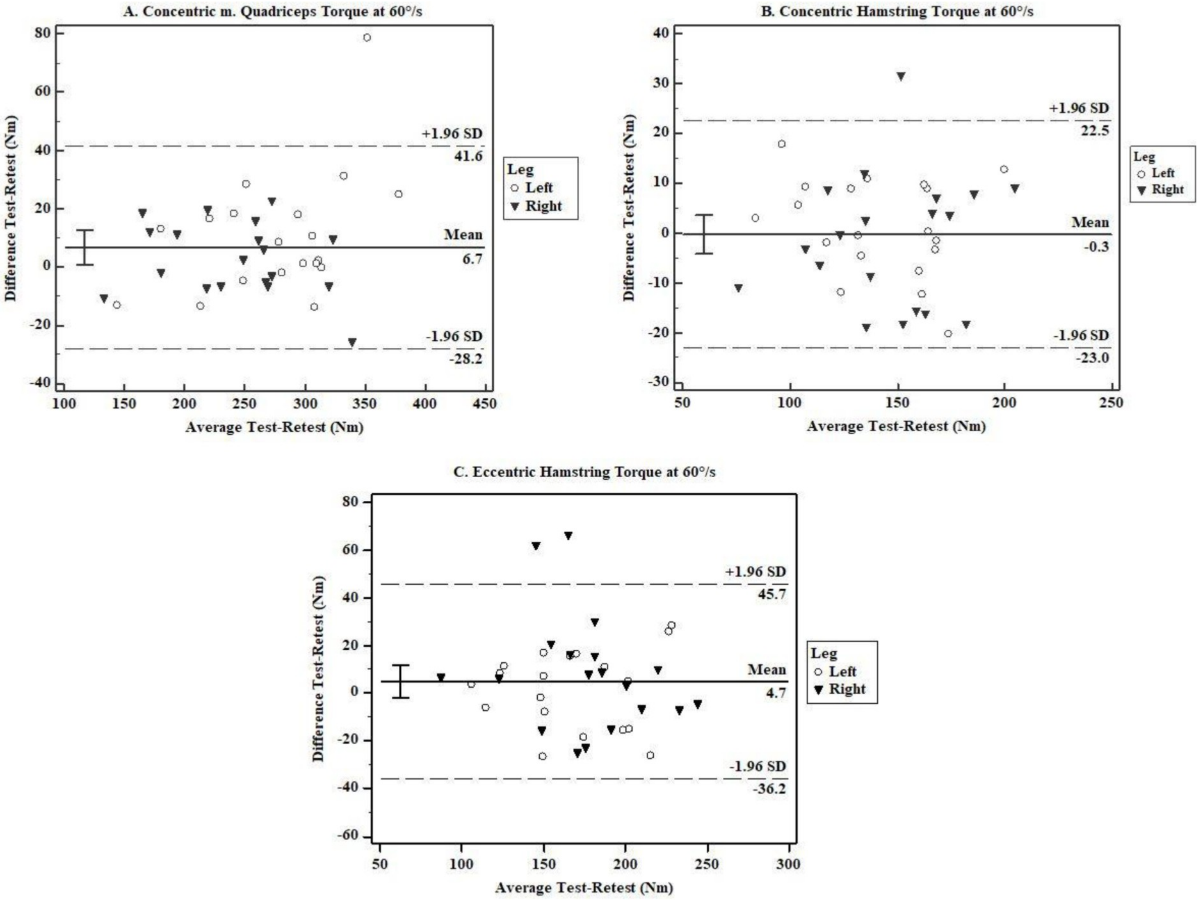

Fig 2. Test-retest qualitative agreement of concentric torque of Quadriceps (a.) and Hamstrings (b.), and eccentric torque of Hamstrings (c.).

Discussion

The results have demonstrated an excellent test-retest reliability for concentric peak torque in knee extension and flexion, except for moderate reproducibility for the eccentric torque of the right hamstring. There were small test-retest differences and SRD was acceptable, indicating the potential for the clinical use of novel SMM iMoment dynamometer for the concentric and eccentric evaluation of knee flexion and extension.

The ICC values obtained in our study ranged from 0.892 (0.718–0.958) for the eccentric right hamstring peak torque to 0.988 (0.967–0.995) for concentric right quadriceps peak torque. This is in line with previously published data, which showed excellent reliability of knee extensors and flexors in a concentric and eccentric manner using both older [8,11,21] and the latest types of isokinetic dynamometers [5,14,22,23]. The absolute percentage difference between test and retest was low and varied between 1% and 4%. In addition, we have also performed a subsequent analysis to control for leg dominance and the leg tested first to eliminate any potential learning effects or effects of central fatigue, and the analysis have shown that the choice of first tested leg had no impact on the results.

Small measurement error and smallest real difference are important for clinical implication of the measurement protocol [5]. However, only a few previous studies reported the SEM, SEM %, and SRD values as indicators of absolute reliability [7,14,21] or real test-retest improvement of the sample [28] (Table 3). Our SEM values range (6.66–12.26 Nm) are in line with previous reports (5.57–13.00 Nm) using the Technogym's REV 9000 dynamometer [19], the Kincom 500H dynamometer [5] or the IsoMed 2000 dynamometer [22].

Compared to other reports, we obtained lower SEM % values [5,7,14,19]. Our SEM % values were lowest for the right quadriceps peak concentric torque (2.54%) and the left hamstring concentric torque (3.47%), and the highest for hamstring eccentric peak torque (4.93–6.93%). Slightly higher SEM % values, ranging from 3.50% to 10.71%, were reported by previous studies [5,7,14,19]. Interestingly, we noted an intra-dynamometer reliability differences using short (30°) or full ROM (70°) on the REV 9000 dynamometer [7,19]. The full ROM demonstrated higher absolute reliability (3.5–5.1%) [7] compared to short ROM (8.52–10.71%) [19]. In this case, the number of maximal repetitions in the measurement might play a possible role, as five maximal repetitions [7] using full ROM may induce higher reliability compared to only two maximal efforts using short ROM protocol [28]. Additionally, our results also showed low to moderate error (SRD %) to detect a real test-retest change, which is supported by previous studies. The SRD % in other studies was very similar [5,7,14], ranging from 9.7% [7] to 19.47% [5]. One possible reason for such minor discrepancies may lie in the protocol design, which consisted of five maximal repetitions with longer rest duration (2 minutes) between sets, while others mainly used two to three maximal effort separated by 60 seconds break [5,14]. Shorter breaks between testing sets may lead to a higher rate of fatigue accumulation and subsequent performance decrement in the later stages of the given protocol. Moreover, there were no differences between longer test-retest break (>7 days) in our case compared with shorter test-retest breaks (96 h) reported in other studies [7,14]. For example, Sole et al. (2007) conducted a second measurement after 7 days and reported the highest values of SRD % to date using the Kincom 500H dynamometer. Also, there was no heteroscedasticity observed in either of the measurement via Bland-Altman plots or correlation, which is comparable with previous studies [5,19].

Lastly, we must also emphasize some mechanical characteristics of this new dynamometer that could additionally influence good-to-excellent reproducibility parameters. First, the chair has a very stable backrest and the movement of the chair was motorized, allowing us to align dynamometer and knee joint axes very precisely. The majority of other commercially available

Table 3. Overview of current studies examining absolute and relative reliability using different isokinetic dynamometers.

Device, year, reference	N	Type of contraction at 60˚/s	ICC	SEM	SEM %	SRD	SRD %
Biodex, 1990, [8]	19	Concentric extension	0.95	NR	NR	NR	NR
		Concentric flexion	0.98	NR	NR	NR	NR
Cybex 6000, 1993, [11]	20	Concentric extension	0.94	NR	NR	NR	NR
		Concentric flexion	0.97	NR	NR	NR	NR
Cybex 6000 DYN, 1996, [13]	18	Concentric extension	0.84	NR	NR	NR	NR
		Concentric flexion	0.83	NR	NR	NR	NR
		Eccentric flexion	0.84	NR	NR	NR	NR
Biodex System 2, 1997, [21]	21	Concentric extension*	0.97	NR	4.8	NR	NR
		Concentric flexion*	0.97	NR	4.9	NR	NR
Biodex System 3 Pro, 2005, [9]	13	Concentric extension	0.98	NR	NR	NR	NR
		Concentric flexion	0.97	NR	NR	NR	NR
Tecnogym REV9000, 2006, [19]	16	Concentric right extension	0.89	10.68	8.52	NR	NR
		Concentric left extension	0.81	13.00	10.71	NR	NR
		Concentric right flexion	0.92	6.74	7.22	NR	NR
		Concentric left flexion	0.92	6.47	7.16	NR	NR
KinCom 500H, 2007, [5]	18	Concentric extension	0.93	8.21	6.48	22.75	17.95
		Concentric flexion	0.93	5.57	7.02	15.45	19.47
		Eccentric flexion	0.94	6.48	6.88	17.97	19.07
Cybex NORM, 2008, [14]	18	Concentric right extension	0.98	NR	4.3	NR	12.0
		Concentric left extension	0.95	NR	4.7	NR	13.0
		Concentric right flexion	0.95	NR	5.2	NR	14.5
		Concentric left flexion	0.93	NR	6.7	NR	18.6
		Eccentric right flexion	0.94	NR	6.5	NR	18.0
		Eccentric left flexion	0.97	NR	5.2	NR	14.5
IsoMed 2000, 2012, [22]	35	Concentric right extension	0.96	8.7	NR	NR	NR
Cybex II, 2013, [6]	16	Concentric extension	0.95	NR	NR	NR	NR
		Concentric flexion	0.89	NR	NR	NR	NR
Technogym REV9000, 2013, [7]	24	Concentric right extension	0.93	NR	3.6	NR	9.9
		Concentric left extension	0.96	NR	3.8	NR	10.5
		Concentric right flexion	0.89	NR	4.9	NR	13.5
		Concentric left flexion	0.96	NR	4.8	NR	13.3
		Eccentric right flexion	0.91	NR	5.1	NR	14.1
		Eccentric left flexion	0.98	NR	3.5	NR	9.7
Biodex System 3, 2018 [23]	26	Concentric extension	0.99	NR	NR	NR	NR
		Concentric flexion	0.97	NR	NR	NR	NR
		Eccentric flexion	0.96	NR	NR	NR	NR
SMM iMoment, 2020	19	Concentric right extension	0.99	6.16	2.54	17.07	7.04
		Concentric left extension	0.97	10.67	3.85	29.57	10.67
		Concentric right flexion	0.96	6.66	4.54	18.45	12.58
		Concentric left flexion	0.98	4.91	3.47	13.60	9.63
		Eccentric right flexion	0.89	12.26	6.93	33.99	19.22
		Eccentric left flexion	0.95	8.28	4.93	22.95	13.67

ICC-intraclass correlation coefficient; SEM-standard error of measurement; SRD-smallest real difference; NR-not reported dynamometers have manual adjustment of the chair and increments for adjustments are not as smooth as in the present case. Other dynamometer characteristics, such as maximal torque on the rotational axis with maximal angular velocity were comparable to other devices.

During the course of our study we have identified few limitations. Firstly, we have included only young, physically active adults, which means that our findings may not be valid for different age groups or in groups of patients. Thus, it would be interesting to extend our research to include such specific groups. Secondly, our study would benefit from cross confirmation of our strength findings with commercially available dynamometers (e.g., Biodex or CSMI Norm) [2,9], however, this comparison was not possible at the time of the study. Finally, future reliability studies should implement the same leg testing order at test and rest for each participant to minimize potential onset of central fatigue [29], which was not observed in our study.

In conclusion, our study has established moderate to excellent reliability and reproducibility with low measurement error for knee flexors and extensor using a novel self-constructed SMM iMoment isokinetic dynamometer. Therefore, we believe that our results indicate the potential applicability of the SMM iMoment isokinetic dynamometer in research, sport rehabilitation and exercise settings in order to monitor athlete progress during training. Nevertheless, future research is needed to assess the reliability of unilateral and bilateral imbalance muscle ratios and the reliability of SMM iMoment dynamometer in clinical settings.

Supporting information

S1 Data.
(SAV)

Acknowledgments

The authors would like to thank all participants for their effort during the study. We would like to thank SMM Production Systems for the technical support of their mechanical engineers and Prof. Zeevi Dvir, who has provided professional counselling and inspiration for the development of this dynamometer. The work is dedicated to the late Prof. Marjan Prebil from the Faculty of Mechanical Engineering in Ljubljana.

Author Contributions

Conceptualization: Tim Kambič, Vedran Hadžić.

Data curation: Tim Kambič.

Formal analysis: Tim Kambič.

Funding acquisition: Mitja Lainščak, Vedran Hadžić.

Investigation: Tim Kambič.

Methodology: Tim Kambič, Mitja Lainščak, Vedran Hadžić.

Project administration: Tim Kambič, Vedran Hadžić.

Supervision: Mitja Lainščak, Vedran Hadžić.

Writing – original draft: Tim Kambič.

Writing – review & editing: Tim Kambič, Mitja Lainščak, Vedran Hadžić.

References

1. Anderson DE, Madigan ML, Nussbaum MA. Maximum voluntary joint torque as a function of joint angle and angular velocity: Model development and application to the lower limb. J Biomech. 2007; 40 (4):3105–13.
2. Breno de Araujo Ribeiro Alvares J, Rodrigues R, de Azevedo Franke R, Gonçalves B, Pinto RS, Aurélio M, et al. Inter-machine reliability of the Biodex and Cybex isokinetic dynamometers for knee flexor/ extensor isometric, concentric and eccentric tests. Phys Ther Sport. 2015; 16(1):59–65. https://doi.org/10.1016/j.ptsp.2014.04.004 PMID: 24913915
3. Brown LE, Weir JP. ASEP Procedures Recommendation I: Accurate Assessment of Muscular Strength and Power. J Exerc Physiol. 2001; 4(3):1–21.
4. Hislop HJ, Perrine J. The Isokinetic Concept of Exercise. Phys Ther. 1967; 47(1):114–7.
5. Sole G, Hamrén J, Milosavljevic S, Nicholson H, Sullivan SJ. Test-Retest Reliability of Isokinetic Knee Extension and Flexion. Arch Phys Med Rehabil. 2007; 88(5):626–31. https://doi.org/10.1016/j.apmr.2007.02.006 PMID: 17466732
6. Toonstra J, Mattacola CG. Test–Retest Reliability and Validity of Isometric Knee-Flexion and -Extension Measurement Using 3 Methods of Assessing Muscle Strength. J Sport Rehabil. 2013; 7:1–5.
7. Pereira de Carvalho Froufe Andrade AC, Caserotti P, Manuel C, De Carvalho P. Reliability of Concentric, Eccentric and Isometric Knee Extension and Flexion when using the REV9000 Isokinetic Dynamometer by. J Hum Kinet. 2013; 37(1):47–53.
8. Feiring DC, Ellenbecker TS, Derscheid GL. Test-Retest Reliability of the Biodex lsokinetic Dynamometer. J Orthop Sport Phys. 1990; 11(7):298–300.
9. Lund H, Søndergaard K, Zachariassen T, Christensen R, Bulow P, Henriksen M, et al. Learning effect of isokinetic measurements in healthy subjects, and reliability and comparability of Biodex and Lido dynamometers. Clin Physiol Funct Imaging. 2005; 25:75–82. https://doi.org/10.1111/j.1475-097X.2004.00593.x PMID: 15725305
10. Ordway NR, Hand N, Briggs G, Ploutz-Snyder L I. Reliability of knee and ankle strength measures in an older adult population. J Strength Cond Res. 2006; 20(1):82–7. PMID: 16503696
11. Bandy WD, Mclaughlin S. Intramachine and Intermachine Reliability for Selected Dynamic Muscle Performance Tests. J Orthop Sport Phys. 1993; 18(5):609–13.
12. Blacker SD, Fallowfield JL, Bilzon JLJ, Willems MET. Within-day and between-days reproducibility of isokinetic parameters of knee , trunk and shoulder movements. Isokinet Exerc Sci. 2010; 18(1):45–55.
13. Che Tin Li R, Wu Y, Maffulli N, Ming Chan K, Chan JLC. Eccentric and concentric isokinetic knee flexion and extension: a reliability study using the Cybex 6000 dynamometer. Br J Sports Med. 1996; 30:156–60. https://doi.org/10.1136/bjsm.30.2.156 PMID: 8799603
14. Impellizzeri FM, Bizzini M, Rampinini E, Cereda F, Maffiuletti NA. Reliability of isokinetic strength imbalance ratios measured using the Cybex NORM dynamometer. Clin Physiol Funct Imaging. 2008; 28:113–9. https://doi.org/10.1111/j.1475-097X.2007.00786.x PMID: 18070123
15. Tredinnick TJ, Duncan PW. Reliability of Measurements of Concentric and Eccentric Isokinetic Loading. Phys Ther. 1998; 68(5):656–9.
16. Capranica L, Battenti M, Demarie S, Figura F. Reliability of isokinetic knee extension and flexion strength testing in elderly women. J Sports Med Phys Fitness. 1998; 38(2):169–76. PMID: 9763804
17. Iga J, George K, Lees A, Reilly T. Reliability of Assessing Indices of Isokinetic Leg Strength in Pubertal Soccer Players. Pediatr Exerc Sci. 2006; 18:436–45.
18. Orri JC, Darden GF. Reliability and validity of the iSAM 9000 isokinetic dynamometer. J Strength Cond Res. 2008; 22(1):310–7. https://doi.org/10.1519/JSC.0b013e31815fa2c8 PMID: 18296991
19. Dervisevic E, Hadžić V, Radjo I. The influence of different ranges of motion testing on the isokinetic strength of the quadriceps and hamstrings. Isokinet Exerc Sci. 2006; 14(1):269–78.
20. Perrin DH. Reliability of Isokinetic Measures. J Ahtletic Train. 1986; 21:319–21.
21. Pincivero DM, Lephart S. M., Karunakara RA. Reliability and precision of isokinetc strength and muscular endurance for the quadriceps and hamstrings.pdf. Int J Sports Med. 1997; 18(2):113–7. https://doi.org/10.1055/s-2007-972605 PMID: 9081267
22. Dirnberger J, Kosters A, Muller E. Concentric and eccentric isokinetic knee extension: A reproducibility study using the IsoMed 2000-dynamometer. Isokinet Exerc Sci. 2012; 20(1):31–5.
23. Duarte JP, Valente-Dos-Santos J, Coelho-E-Silva MJ, Couto P, Costa D, Martinho D, et al. Reproducibility of isokinetic strength assessment of knee muscle actions in adult athletes: Torques and antagonist-agonist ratios derived at the same angle position. PLoS One. 2018; 13(8):e0202261–e0202261. https://doi.org/10.1371/journal.pone.0202261 PMID: 30110385

24. van Melick N, Meddeler BM, Hoogeboom TJ, Nijhuis-van der Sanden MWG, van Cingel REH. How to determine leg dominance: The agreement between self-reported and observed performance in healthy adults. PLoS One. 2017; 12(12):e0189876. https://doi.org/10.1371/journal.pone.0189876 PMID: 29287067

25. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med. 2016; 15(2):155–63. https://doi.org/10.1016/j.jcm.2016.02.012 PMID: 27330520

26. Beckerman H, Roebroeck ME, Lankhorst GJ, Becher JG, Bezemer PD, Verbeek ALM. Smallest real difference , a link between reproducibility and responsiveness. Qual Life Res. 2001; 10(7):571–8. https://doi.org/10.1023/a:1013138911638 PMID: 11822790

27. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986; 327:307–10.

28. Hadzic V, Ursej E, Kalc M, Dervisevic E. Reproducibility of shoulder short range of motion in isokinetic and isometric strength testing. J Exerc Sci Fit. 2012; 10(2):83–9.

29. Gandevia SC, Allen GM, McKenzie DK. Central Fatigue. In: Gandevia SC, Enoka RM, McComas AJ, Stuart DG, Thomas CK, Pierce PA, editors. Fatique: Neural and Muscular Mechanisms. Boston, MA: Springer US; 1995. p. 281–94.