Science and Practice of Strength Training

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1 Modeling record scores in the snatch and its variations in the long-term training of young weightlifters

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Abstract

The primary aim of the current study was to determine the time curves of changes in the record scores in the snatch and its variations during a two-year training cycle in young weightlifters. This study also aimed at assessing the ratios between these scores and at predicting the snatch record scores at the end of the subsequent annual training macrocycle. The final purpose was to compare the record scores with the isometric peak torgue values of the trunk and knee extensors. The study involved 16 weightlifters who were tested seven times at three-month intervals. The overall mean ratios of the record scores in the hang snatch to those in the snatch and the record scores in the hang power snatch to those in the snatch were approximately constant and amounted to 0.95 and 0.79, respectively. The overall mean ratio between the scores in the power snatch to those in the snatch was approximately 0.88. Statistically significant differences (p < 0.05) between the individual time trajectories of record scores in the snatch and its derivatives were identified in two consecutive annual training macrocycles. The error in predicting record results at the end of the following annual training macrocycle was 6.7 ± 4.7% or 8.1 ± 3.4% depending on the way the measurement data were modeled. The results of the study also indicate that the measurements of the isometric peak torque of the trunk extensors performed in laboratory conditions can be useful in diagnosing the strength capacity of young weightlifters.

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Introduction

Elite weightlifters typically perform 20,000–25,000 lifts per year. Most of the lifts are done with a load equal to 80–90% of maximal capacity, and 4–7% are executed at more than 90% of 1 repetition maximum (1 RM) [1,2]. Drechsler [3] reports that in weightlifting training, 10% of the total time is dedicated to warm-ups, 45% to competitions and specific exercise, 40% to complementary strength exercise, 3% to supplementary exercise, and 2% to other sport- and training-related activities. The heavy use of specific exercise in training programs indicates that it is expected to have a direct influence on improving weightlifters' performance.

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

The use of weightlifting exercises has previously been shown to enhance strength, power, and speed, hence its popularity within performance training programs in different sport disciplines [4–12]. In a survey presented in the work of Waryasz et al. [13], 92% out of 167 crossfit trainers used weightlifting exercise in their training programs, and a study by Smith et al. [14] showed that 94.2% applied the snatch and/or its variations.

The basic variations of the snatch (S) are the power snatch (PS), the hang power snatch (HPS), and the hang snatch (HS). A characteristic feature of the power snatch is that the bar is only lifted for a short time, since the lifter does not drop into a full catch position [15–16]. Moreover, smaller loads are used in this exercise, thanks to which the barbell can attain higher vertical velocity. Taking into account the two above-mentioned aspects of the power snatch, it is presumed that incorporating this exercise in the training causes beneficial neuromuscular adaptations, which increase the power generated by the lifter's muscles [7,9,17]. Power is regarded as a valuable predictor of weightlifters' performance [18]. It can be defined as a product of the weight of the barbell and its vertical velocity (the power of the barbell) and can be used in assessing exercise intensity [15].

Hang snatches are variations of the snatch where the starting position of the barbell is above the lifter's knees. This initial configuration of the body helps the lifter achieve triple extension, that is extension in the hip, knee, and ankle joints [11,17,19–20]. A smaller difference between the starting and finishing position of the barbell makes it easier to master the technique of performing the hang snatch and the hang power snatch. Owing to the fact that these exercises do not include the technically difficult elements that are characteristic of the snatch, they are recommended for athletes with shorter training experience, and their use increases the strength and power capacities of the muscles [15,20].

Snatch derivatives are used mainly as supplementary exercises aimed at improving the technique and outcomes of the snatch in weightlifters [2,21]. These exercises have also been found to be beneficial for enhancing motor capacity. Training incorporating the hang snatch was observed to produce improvements in vertical jump height, 1-RM back squats, and 40-yard sprint in female collegiate students [19]. A study by Canavan et al. [22] showed that this exercise was useful in developing the power of the lower extremities in football players and track field athletes. Other authors have noted a strong correlation between power snatch and shot put/weight throw results in well-trained collegiate throwers [23] as well as between the results in the hang snatch (and hang power snatch) and isometric knee extensor torque in young weightlifters [24].

Despite the extensive use of the snatch and its derivatives in training programs in different sport disciplines, there is no research available identifying time trends in the snatch record scores and its derivatives over several years of training. Neither has it been investigated whether there is a similarity between increases in lifters' record scores in the snatch and increases in the peak torque of the extensors of the lower extremities responsible for the triple extension. One way to examine these issues is by long-term modeling of empirical data from measurements conducted at regular intervals.

In light of the above, the primary aim of the study was to determine the time curves of changes in the record scores in the snatch and its variations during a two-year training cycle in young weightlifters. This study also aimed at assessing the ratios between these scores and at predicting the snatch record scores at the end of the subsequent annual training macrocycle. The final purpose was to investigate the increases in the peak torque of the trunk and knee extensors and verify whether these increases corresponded with those in the record scores in the snatch in the period analyzed.

Materials and methods

Subjects

The study involved 16 weightlifters, who were selected from a training group of 25 persons. The criterion for participation in the research was at least one year of training experience and age below 20 years. One year of specific weightlifting training meant participation in 2-hour training sessions at least three times a week, during which the techniques of the snatch, the clean and jerk, and specific exercises were taught and improved. After a one-year training cycle, the weightlifter was able to correctly perform the basic lifts and exercises, including those whose performance was analyzed in this article. When the research began, the lifters were 16.5 ± 3.63 years old and had a body mass of 73.38 ± 18.91 kg. The participants included medalists in Polish championships in particular age categories and members of Polish national teams in European and World Championships. The study was carried out over a two-year period of specialized weightlifting training. The type of training and the percentage contribution of specific exercises to the training loads were similar to those described in our previous work [24]. During this period, the lifters prepared to participate in two main competitions (Youth European Weightlifting Championships in September 2016 and September 2017), and during the preparatory phases, they took part in regional and national tournaments. Eight subjects continued their training in the following year, which made it possible to conduct an additional measurement session at the end of the following training macrocycle in September 2018. Five of the eight weightlifters who did not continue their participation in the research changed their place of residence and club membership because they started their studies, while three withdrew for health or personal reasons. The subjects were provided with information on the research procedure and on the possible risks and benefits related to participating in the study. All subjects and their parents signed informed consent forms. The investigations were performed in accordance with the ethical standards of the Helsinki Declaration, and the study was approved by the University Research Ethics Committee.

Measurements

The record scores in the snatch and its derivatives as well as isometric peak torque values were measured every three months in the first week of the months given in Table 1. All of these assessments were made on Mondays because Sunday was a day without training regardless of the phase of the macrocycle. That way the weightlifters were not tired before the measurement sessions. Over the following four days, the lifters' maximal performance in the snatch and its three derivatives was tested. The tests were performed in a random order. There are 24 permutations of 4 measurement sessions; seven permutations were randomly selected by the computer without repetitions. Each test was preceded with a warm-up, during which loads no greater than 90% of the lifters' existing personal best scores were used. Afterwards, the lifters performed 3 maximal lifts with progressive increases in load, starting from a load equal to or greater than 90% of 1 RM. The mean increase in the loads between consecutive repetitions was 2 or 3 kg. The repetition with the highest load (1 RM) was recorded as the final result of the

Table 1.	Mean body	mass of weightlifters	during the study	v in kg (± SD).
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Measurement	M ₁	M ₂	M ₃	M ₄	M5	M ₆	M ₇
Date	March	June	September	December	March	June	September
	2016	2016	2016	2016	2017	2017	2017
Body mass	73.38	74.28	75.75	76.51	77.34	78.91	79.41
(SD)	(18.91)	(18.89)	(17.86)	(18.41)	(20.22)	(20.15)	(19.96)

test. Owing to the differences in body mass between subjects and its within-subject variations over the course of the study, the lifters' body mass changes were recorded regularly (<u>Table 1</u>), and the results achieved in particular lifts were converted into Sinclair points. Sinclair coefficients were calculated according to the formula valid from 1 January 2017 to 31 December 2020.

During the study, the training loads that were implemented were also continuously monitored (Fig 1). The values of loads at measuring points M_I - M_7 are the sums of loads from the quarter preceding the measurement and were standardized in relation to the subjects' body mass (BM).

The peak torque values of the muscles of the lower extremities and trunk were measured in isometric conditions [9,25–26]. Considering the decisive influence of the trunk and knee extensors on performance in weightlifting, only the torque of these two muscle groups was measured. The measurements were carried out on a LR2-P (JBA Zb. Staniak, Poland) measuring station [27]. The subjects adopted a standard position on a chair and were stabilized with back, thigh, and ankle pads, as shown in Fig 2. The angle in the hip and knee joints was 90 degrees, similarly as in the above mentioned papers. During the test, the lifters completed three repetitions of extension for no longer than 3 seconds. The current torque values were displayed and recorded by a dynamometer, and the best score was used in the analysis.

Statistical analysis

The empirical data were tested for normality of distribution using the Shapiro-Wilk test. Statistical significance was set at p < 0.05. The results of the test confirmed that the data were normally distributed. The data were then represented in a univariate form. The time of the first measurement session was set to 0. Due to the fact that the measurements were carried out quarterly, the time of subsequent sessions increased by 0.25. By coding the first time period as 0, we directly attributed the intercept to the value of the dependent variable in the first measurement.

In the analysis of our data, we used an individual growth approach [28–31]. This method is based on hierarchical modeling, where repeated observations from a single subject represent the level-1 variables, whereas the between-subject variables are defined at level-2. According to the generally accepted practice in hierarchical modeling, we built several models in order to answer particular research questions.

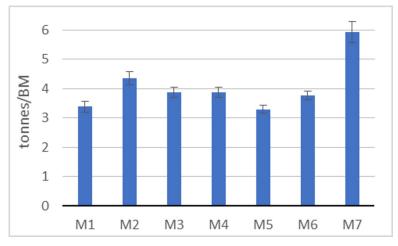


Fig 1. Relative training volume during the period analyzed.



Fig 2. Measurement of peak torque of trunk extensors (left) and knee extensors (right) in isometric conditions.

One usually begins with an unconditional random intercept model to estimate the intraclass correlation coefficient (ICC). The unconditional means model was defined as follows:

$$snatch_{ij} = \beta_{0j} + r_{ij} \tag{1}$$

$$\beta_{0j} = \gamma_{00} + u_{0j}, \tag{2}$$

where *snatch*_{*ij*} represents the snatch score at time *i* for subject *j*, β_{0j} is the subject-specific intercept (the snatch score at *time* = 0), r_{ij} stands for residual error, γ_{00} represents the overall mean for snatch record scores, and u_{0j} is the random deviation from the overall mean. Eqs 1 and 2 represent the level-1 and level-2 models, respectively.

In the second stage of the modeling process, we defined the fixed relationship between the record snatch scores and time at the level-1 as

$$snatch_{ij} = \beta_{0j} + \beta_{1j}time_{ij} + \beta_{2j}time_{ij}^2 + r_{ij}, \qquad (3)$$

where β_{1j} is the subject-specific slope and β_{2j} is the subject-specific quadratic term for snatch scores over time.

The coefficients in Eq 1 can be broken down into two components at level-2:

$$\beta_{0j} = \gamma_{00} + u_{0j} \tag{4}$$

$$\beta_{1j} = \gamma_{10} + u_{1j} \tag{5}$$

$$\beta_{2j} = \gamma_{20} + u_{2j}.$$
 (6)

Components γ_{00} , γ_{10} , and γ_{20} represent the mean intercept, slope, and quadratic term across all subjects, whereas u_{0j} , u_{1j} , and u_{2j} represent random deviations from these means for subject *j*. The random aspect of these variables indicates that they have variances and covariances, and the random intercepts, slopes, and quadratic terms may be correlated. The intercept variance was defined as τ_{00} , the slope variance as τ_{10} , and the quadratic term variance as τ_{20} .

After inserting Eqs 4-6 into Eq 3, the unconditional relationship between snatch record scores and time was expressed in a compact form as

$$snatch_{ij} = (\gamma_{00} + \gamma_{10}time_{ij} + \gamma_{20}time_{ij}^2) + (u_{0j} + u_{1j}time_{ij} + u_{2j}time_{ij}^2 + r_{ij}),$$
(7)

where the elements in the left bracket define the fixed part of the model, whereas the elements in the right bracket define the random part of the model.

A brief analysis of training loads (Fig 1) shows that they changed in all subjects in a similar way. It was therefore reasonable to calculate the average load for each subject and to treat it as a time-invariant covariate. Eq 4 of the final model was thus modified as follows

$$\beta_{0j} = \gamma_{00} + \gamma_{01} \overline{load}_j + u_{0j}, \tag{8}$$

with \overline{load}_i denoting subject j's mean load.

Defining the final model in the above way, we assumed that mean load was treated as a predictor of the intercept. The mean loads of the subjects were also grand-mean centered in order to simplify the interpretation of the results of the computations.

We performed the statistical analysis in the R environment (R Foundation for Statistical Computing, Austria) using the *lmerTest* [32] package, which overloads the basic *lmer* function from the *lme4* package [30]. We chose the *lmerTest* package because it has several useful features, such as reporting *p*-values for *anova* and *summary* tables, testing the reduction of random-effect terms to simpler structures (*ranova* method), and performing automatic backward model selection of fixed and random parts of the linear mixed model (*step* method). The models described above were coded in R as follows:

$$model1 = lmer(snatch \sim 1 + (1|subject), data, REML = FALSE)$$
 (9)

$$model2 = lmer(snatch \sim time + I(time2) + (time + I(time2)|subject), + + data, REML = FALSE)$$
(10)

$$model3 = lmer(snatch \sim time + I(time^{2}) + \overline{load} + (time + I(time^{2})|subject), + + data, REML = FALSE).$$
(11)

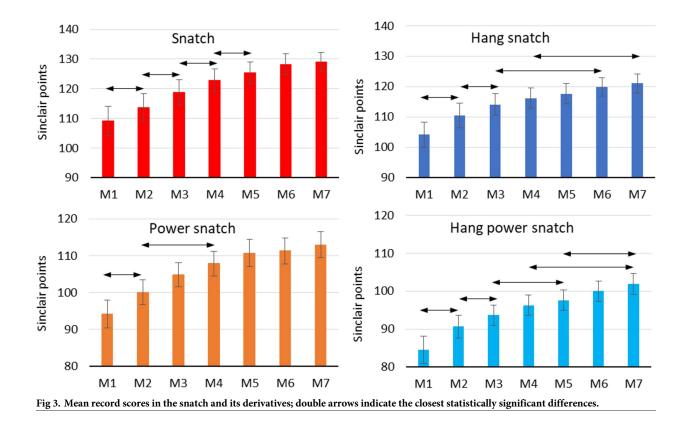
The terms in inner brackets denote the random parts of the models, *data* is the file in a univariate (long) format, and *REML* = *FALSE* means that, instead of the default restricted maximum likelihood (*REML*), maximum likelihood (*ML*) estimation was used for computations. Applying the *ML* method allows for a direct comparison between the two models nested in each other using the *anova* method [30].

The *lmerTest* package was chosen since it was assumed that subject record scores were independent and homoscedastic over time.

Significant differences in scores between time points were identified using the *gls* function (with the corAR(1) argument) from the *nlme* package. The use of this argument makes it possible to perform calculations for correlated measurements.

Results

An important element of longitudinal analyses is a preliminary examination of measurement data, which are presented in Fig 3. During the two-year training period, the lifters' mean scores in the snatch improved by 19.78 ± 15.63 Sinclair points. As far as particular variations of the snatch are concerned, the greatest increases were found in the power snatch (18.78 ± 10.71)



points), followed by the hang power snatch $(17.39 \pm 9.82 \text{ points})$ and the hang snatch $(16.86 \pm 11.32 \text{ points})$. As mentioned earlier, the subjects had at least one year's training experience. Thus, it was reasonable to assume that the ratios of snatch derivatives to snatch record scores were approximately constant, and the overall means of these ratios were calculated. The overall mean values of the PS/S, HS/S, and HPS/S ratios were 0.88 ± 0.07 , 0.95 ± 0.06 , and 0.79 ± 0.07 , respectively. The calculation of these ratios for the measurements $(M_1 \dots M_7)$ confirmed the above assumption. Visual inspection of Fig 3 also reveals a non-linear time trend for the record scores in the snatch and its derivatives and a considerable decrease in these scores in the second training macrocycle.

Table 2 presents the results of statistical analysis for the models. The ICC for model 1 was 204.81/(204.81 + 90.13) = 0.69, suggesting that about 69% of the total variation in the snatch record scores was due to between-subject differences. It was evident that the relationship between snatch record scores and time could be identified using an individual growth curves approach.

The results of model 2 show that the average snatch record score at M_I was 108.85 Sinclair points, the average linear slope was 23.10 (Sinclair points)/quarter, and the quadratic term was -6.34 (Sinclair points)/quarter². All coefficients were significant (p < 0.001) indicating between-subject differences in the initial snatch record scores and in the values of linear and quadratic coefficients of time trajectories. As expected, the *anova* method applied to models 1 and 2 returned a very large value of the χ^2 statistic at the level of 159.5. The corresponding pvalue of about 10⁻¹⁶ proved that model 2 fit the data considerably better. It also turned out that the value of pseudo R^2 , (204.81 – 11.98)/204.81 = 0.94, explained almost 94% of within-subject variability.

Parameter	Model 1	Model 2	Model 3	
	Coefficient (SE)			
Fixed effects				
Intercept (γ_{00} for β_{oj})	121.02*** (3.69)	108.85*** (4.68)	108.84*** (4.96)	
Γime (γ_{10} for β_{1j})		23.10*** (2.79)	23.10*** (2.81)	
Γime2 (γ20 for β2j)		-6.34*** (1.60)	-6.34*** (1.62)	
Load $(\gamma_{01} \text{ for } \beta_{0j})$			9.37* (3.96)	
		Variance (SD)		
Random effects				
Level-1 residual (<i>r_{ij}</i>)	204.81 (14.31)	11.98 (3.46)	11.92 (3.46)	
Level-2 residuals				
ntercept (u_{00})	90.13 (9.49)	340.95 (18.47)	384.56 (19.61)	
Slope (u_{10})		35.95 (5.99)	37.76 (6.15)	
Quadratic term (u_{20})		4.66 (2.16)	5.48 (2.34)	
-2LL	867.2	707.7	703.6	

Table 2. Estimation of model parameters.

* p < 0.05

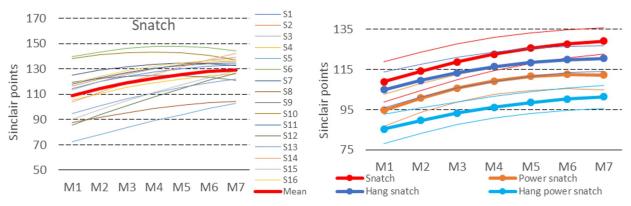
*** *p* < 0.001

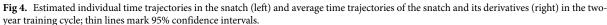
LL–log likelihood

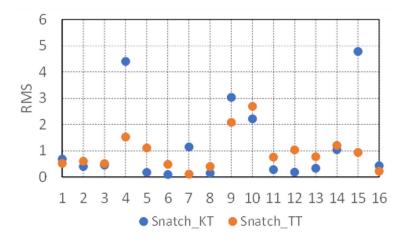
The results of model 3 revealed significant between-subject differences in the subjects' intercept, slope, quadratic term (p < 0.001), and average load (p < 0.05) values. The *anova* method applied to models 2 and 3 returned a χ^2 value of 4.13 (p < 0.05), confirming that model 3 fit the data significantly better. It is no surprise that the average trajectory for model 3 had the same coefficients as the trajectory for model 2. After subject loads were grand-mean centered, the γ_{01} coefficient did not affect the average trajectory for model 3 but influenced the intercepts of the individual trajectories.

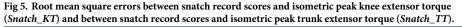
Fig 4 (left) shows the time trajectories of the snatch record scores fitted with model 2. The differences between the individual trajectories and the differences between these trajectories and the average trajectory (red thick line) are clearly visible. It can also be noted that the snatch record scores of some subjects (e.g., S_6 , and S_{10}) decreased in the two-year training cycle.

Derivatives of the snatch are an important component of training loads. For this reason, the trajectories of these targeted exercises were obtained using the unconditional model 2. A









comparison of the average curves for the snatch and its derivatives is presented in Fig 4 (right). A similar pattern of the snatch, hang snatch, and hang power snatch curves can be easily recognized, as can a non-monotonous pattern of the power snatch curve. The values of γ_{20} coefficients for the hang snatch, the hang power snatch, and the power snatch were -6.44, -5.08, and -10.21, whereas the values of γ_{10} were 20.09, 18.32, and 27.19, respectively.

The closest significant statistical differences between the successive measurements are presented in Fig 3. The highest values of Cohen's coefficient for correlated measurements were 1.09 for the snatch, 1.24 for the power snatch, 0.96 for the hang snatch, and 0.93 for the hang power snatch, and they occurred between the first (M_1) and second measurement (M_2) . This indicates a large effect size in each case.

Eight trajectories were extrapolated over time without being constrained by the results at the end of the third training macrocycle. The mean relative percentage error between the actual and estimated values was $8.1 \pm 3.4\%$ for model 2, $6.7 \pm 4.7\%$ for model 3, and 9.4 ± 4.8 for ordinary least squares approximation in Excel. No statistically significant differences were found between these errors.

As for relationships between the snatch record scores and the isometric peak torques of the knee and trunk extensors, discrete sets of empirical data for these variables were first normalized (i.e., the mean of each variable was zero, and the standard deviation was equal to 1) in order to make it possible to compare them. The root mean square errors between the snatch record scores and isometric peak knee extensor torque and those between the snatch record scores and isometric peak trunk extensor torque were then calculated for each subject. The results of these calculations are shown in Fig 5. It is visible that lower values of root mean square error occurred between the snatch record scores and trunk extensor torque (*Snatch_TT*). The difference between *Snatch_TT* and knee extensor torque (*Snatch_KT*) was also statistically significant (p < 0.05).

Discussion

The primary aim of the study was to identify the time trajectories of the record scores achieved in the snatch and its different variations during a two-year training cycle in young weightlifters. The calculations were made using an individual growth approach, and, in line with generally accepted practices in the field [28–30], several models were constructed. The results of the calculations showed that the method had been chosen adequately since there were statistically significant differences between individual subject trajectories, and second-degree polynomial approximation was sufficient.

The second aim of the study was to predict record scores in the snatch at the end of the following annual training macrocycle. Considering the fact that the models explained approximately 94% of within-subject variability, an absolute relative percentage error between actual and predicted values of 6.7% and 8.1% can be regarded as satisfactory. This article has described in detail the results for two models, but it is worth mentioning that several other models were tested when making the calculations. For example, one of the models used the time-varying covariate *load* instead of time as a predictor, according to the algorithm described by other authors [33-34] (-2LL = 730, error = 6.1%), and another model was created by combining this model with model 2 (-2LL = 689.3, error = 9.3%). These results and those given in Table 2 suggest that a better fit of the model to the measurement data (smaller -2LL) had a minor negative impact on its predictive value.

The power snatch is one of the fundamental special exercises which make it possible to improve the performance of the snatch. During the power snatch, a lifter does not need to perform a full squat when receiving the bar [6,15,16], which makes it easier to execute than the snatch. For this reason, the power snatch is often used in the training of young weightlifters [24] and non-professional weightlifters [35–36]. Higher values of coefficients γ_{10} and γ_{20} compared to those for the snatch indicate that mean increases in record scores in the power snatch occurred at a faster rate, and the trajectory of these records was more curved. Starting from measurement M_6 , there was a decrease in the values of the record scores despite a considerable increase in the training loads applied during this period. The results confirm the reports of other authors that further improvement of the results in this exercise requires an increase in lifters' speed capacity [37] or the adequate use of other snatch variations in the training program [16].

An exercise that is often used in Olympic weightlifting training [1,15] and in crossfit training [13] is the hang snatch, in which the starting position for the barbell is at the knees, and the finishing position is the same as the one used in the snatch. An important element of this exercise is an adequate level of the flexibility angle in the ankle joint and a correct balance of the body with respect to the barbell. Thus, the hang snatch is classified as an exercise which is difficult to perform from a technical point of view. Comparable values of the γ_{20} coefficients for the snatch and the hang snatch indicate a similar curvature of the trajectory, while the somewhat higher value of the γ_{10} coefficient for the snatch evidences a higher increase in the record scores in the snatch compared to the hang snatch. The similarities between the curves also indirectly indicate that the subjects, who had minimum one-year training experience, had mastered the adequate technique of performing this exercise. This observation is additionally confirmed by the fact that the ratio of the record scores in the hang snatch to those in the snatch remained at an approximately constant level of 0.95.

Another snatch variation which is frequently implemented in strength training in different sport disciplines is the hang power snatch [19,22]. This exercise is similar to the power snatch, but the starting position of the barbell is at the knees. In specific Olympic weightlifting training, the hang power snatch is used to improve the speed of performing the second pull during the snatch. In this exercise, similarly as in the power snatch, an adequate flexibility angle in the ankle joint and a correct balance of the lifter's body with respect to the barbell are less important. Therefore, mastering the appropriate technique of performing the hang power snatch is not difficult [37], and that is why it is used in the training of novice weightlifters. The study showed that the trajectories of the record scores in the hang power snatch were similar to

those in the hang snatch and the snatch. The ratio of the record scores in the hang power snatch to those in the snatch remained at an approximately constant level of 0.79, which is an additional reason to use the hang power snatch in the training of young weightlifters and other athletes.

The largest statistically significant differences for the snatch and its derivatives were found at the beginning of the specialized weightlifting training. The statistically significant increase in record scores in the snatch was observed every 3 months until the beginning of the second training macrocycle (M_5). This confirms the efficacy of the applied training program focused on the improvement of record scores in this lift. The significant increase in record scores in the snatch derivatives was not so regular, especially in the case of the power snatch.

An unquestionable advantage of using the snatch and its variations in the training of weightlifters and athletes practicing other disciplines is that it helps them master the habit of triple extension [1,17, 19–20]. Since at the beginning of hang snatches, the muscles of the lower limbs and trunk work in isometric conditions [38], the strength of these muscles was assessed using the isometric peak torque of these muscles. The isometric peak relative torque values of the knee extensors ranged from 4.98 ± 0.76 Nm/kg (M_1) to 5.55 ± 1.08 Nm/kg (M_7) and approximately corresponded with the mean values of this torque (4.86 Nm/kg) measured for the joint angle of 96° in recreational weightlifters aged 39.2 years [4]. The current values were, on the other hand, higher than those achieved by young weightlifters (4.18 Nm/kg) in the study of Jaszczuk et al. [25]. The values of trunk extensor torque changed between 6.37 ± 1.11 Nm/kg (M_1) and 9.91 ± 2.03 Nm/kg (M_7). Starting from M_2 (8.29 ± 1.41 Nm/kg), they were similar to those obtained by the young weightlifters (9.19 Nm/kg) examined by Jaszczuk et al. [25].

The changes in the torque of the trunk extensors approximately corresponded with the changes in the record scores in the snatch. This would mean that the training undergone by weightlifters in the initial period of their careers leads to a synchronous increase in the strength of the trunk extensors. This observation is supported by the calculations made by Bartonietz [15], which showed that during the performance of the snatch the power generated in the hip joint is more than 3 times greater than that generated in the knee joints. Similar results were reported by Lee et al. [35], who investigated the power snatch in non-professional weightlifters.

When discussing the findings of the current study, one should emphasize the importance of at least three factors that can limit the strength of the conclusions drawn from the research. The first factor is the accuracy of determining the values of 1 RM in the snatch and its variations. The measurement resolution that was used (2.5 kg) produced errors amounting to at least 5% in the values of the record scores in the hang snatch and the hang power snatch in some of the weightlifters in M_1 and M_2 . The second factor which made it difficult to interpret the results obtained was the use of new equipment to perform the isometric measurements of the torque of the trunk and knee extensors, for which there are no referential data for young weightlifters. Finally, we assumed subject record scores were independent and homoscedastic over time. In order to verify this assumption, we performed additional computations using the *lme* function from the *nlme* package [30], which makes it possible to check models with various forms of heteroscedasticity and autocorrelation. The computations did not show statistically significant differences (p < 0.082) between model 2 and its extended version containing autoregressive (corAR1()) and heterogeneity terms or between model 3 and its extended version (p < 0.074). However, low *p*-values suggest that some of the variability of the measurement data may be explained when subject record scores are non-independent and heteroscedastic over time.

Conclusions

The results of the study revealed that the highest level of correspondence between performances in the snatch and the hang snatch as well as the snatch and the hang power snatch justifies implementing these exercises at the beginning of the careers of young weightlifters with the aim of helping them perfect the Olympic-style snatch. These exercises are also useful in other sport disciplines where learning the complicated technique of the snatch is not a priority in the training.

We believe that knowing the ratios between the record scores for the snatch and its variations may be important both for the coaches of young weightlifters and crossfit trainers. Young weightlifters make the greatest progress at the beginning of their sport career, and an adequate selection of special exercises as well as of their volume and intensity is one of the key elements of planning athletic training. Crossfit trainers, on the other hand, work with persons who have various levels of motor capacity, for whom the selection of an appropriate load is difficult, and the performance of 1 RM may be dangerous.

Tracking record scores in the snatch at regular intervals makes it possible to predict future results, and the trend in these scores is not linear in young weightlifters.

The changes in the isometric peak torque values of the trunk in the two-year training macrocycle corresponded with the changes in the record scores in the snatch, which justifies using the measurement of these torques in diagnosing the strength capacities of young weightlifters on an ongoing basis.

Supporting information

S1 Dataset. Empirical data coded in a long format; loadBM_mean appears in the text as *load*. (XLSX)

Acknowledgments

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Methodology: Adam Czaplicki, Janusz Jaszczuk.

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2 Post-activation potentiation effect of eccentric overload and traditional weightlifting exercise on jumping and sprinting performance in male athletes

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Abstract

The aim of this study was to evaluate the post-activation potentiation (PAP) effects following eccentric overload (EOL) and traditional weightlifting (TW) exercise on standing long jump (SLJ), countermovement jump (CMJ), and 5 m sprint acceleration performance. Ten male athletes were involved in a randomized, crossover study. The subjects performed 3 sets of 6 repetitions of EOL or TW half squat exercise followed by SLJ, CMJ, and 5 m sprint tests at 1 min, 3 min and 7 min, in separate sessions using a randomized order. Bayes factor (BF₁₀) was reported to show the strength of the evidence. Differences were found using EOL for SLJ distance at 3 min (BF₁₀ = 7.24, +8%), and 7 min (BF₁₀ = 19.5, +7%), for CMJ at 3 min (BF₁₀ = 3.25, +9%), and 7 min (BF₁₀ = 4.12, +10.5%). Differences were found using TW exercise for SLJ at 3 min ($BF_{10} = 3.88, +9\%$), and 7 min ($BF_{10} = 12.4, +9\%$), CMJ at 3 min (BF₁₀ = 7.42, +9.5%), and 7 min (BF₁₀ = 12.4, +12%). No meaningful differences were found between EOL and TW exercises for SLJ ($BF_{10} = 0.33$), CMJ ($BF_{10} = 0.27$), and 5 m sprint (BF₁₀ = 0.22). In conclusion, EOL and TW exercises acutely increase SLJ and CMJ, but not 5 m sprint performance. The PAP time window was found between 3 min and 7 min using both protocols. This study did not find differences between EOL and TW exercises, and so both methodologies can be used to stimulate a PAP response.

Introduction

Post-activation potentiation (PAP) is a physiological phenomenon associated with an acute improvement in muscular performance after a resistance training protocol [1,2]. Neuromuscular, mechanical and biochemical changes may induce these temporary improvements in performance but the exact underlying mechanisms are still not fully understood [1,3]. The most strongly supported explanation for the effects of PAP relates to a greater rate of cross-bridge

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attachment as a result of phosphorylation of myosin regulatory light chains during muscle contraction [4]. Furthermore, PAP is proposed to result from increased sensitivity of contractile proteins to calcium (Ca^{2+}) released from the sarcoplasmic reticulum, resulting in a cascade of events leading to an enhanced muscular response [3–5].

PAP may be induced through the use of resistance training exercises prior to the main sport-specific activity, leading to an increase in performance [6]. Generally, following a preload exercise, a temporary fatigue-induced decrement in performance is observed, which is subsequently replaced by a PAP response [3,7]. Traditional weightlifting (TW) is one of the modalities used by coaches to elicit a PAP response for subsequent competitive activities [3]. The majority of research investigating TW and its PAP response has reported a positive effect on reducing short distance sprint time and improving countermovement jump (CMJ) performance [3,8]. Both heavy and moderate load back squat (90% and 60% 1RM, respectively) have been shown to potentiate the sprinting and jumping performance of male professional rugby players [9]. Some previous studies on acute lower limb performance have found positive improvements after traditional pre-load strategies, while others have failed to confirm such results [3]. Such discrepancies may be a result of differences in the interventions relating to protocol characteristics including exercise modality, volume, intensity, muscle action, and duration of rest between the pre-load exercise and the subsequent sport-specific task, all of which have been identified as key variables determining the magnitude of a PAP response [6,10].

Flywheel ergometers are commonly used in sports training to chronically improve elite soccer players' jump and sprint performances [1,11,12]. Such devices are capable of stimulating an eccentric overload (EOL), in which the generated eccentric muscular force exceeds the maximal concentric force [13,14]. The user rotationally accelerates the flywheel with maximal velocity during the concentric phase of the movement (*e.g.* extension phase of a squat), resulting in a flywheel inertial torque that imparts high linear resistance during the subsequent eccentric phase of the movement (*e.g.* flexion phase of a squat) [1,11,12]. The main advantage of this exercise methodology is related to the high mechanical overload of the eccentric phase, which may enable strength and conditioning practitioners to improve athletes' performances both chronically and acutely [7,12]. Indeed, the greater eccentric load may recruit higher order motor units or fast-twitch muscle fibers at a greater extent and therefore likely facilitate a greater PAP response in subsequent sport-specific performance [4]. Moreover, eccentric load generated by a flywheel device may contribute to acutely improving stretch-shortening cycle performance and transfer effects on the explosive athletic tasks such as vertical jumps, horizontal jumps and sprinting [7,14,15].

Very few studies have evaluated the acute PAP induced improvement in lower limb performance following flywheel exercise [16]. Recently, acute sprint (20 m) and CMJ performance improvements have been found after EOL exercise [1]. Similar EOL-induced PAP improvements were reported in quadriceps concentric peak torque, hamstring concentric and eccentric peak torque during an isokinetic test ($60^{\circ} \cdot s^{-1}$) [7]. Moreover, augmented CMJ height, impulse, peak power and peak force were observed following the same EOL exercise protocol [7]. This study reported that PAP improves lower limb performance after 3 minutes of recovery following a flywheel squat exercise, with optimal time windows from 3 to 9 min. Previous studies using TW exercises have revealed inconsistent findings since several confounding factors may affect PAP response [4]. Indeed, PAP response may be affected by subjects' resistance training experience and competitive level [3,8]. It is not currently well established whether EOL is a more beneficial methodology to increase PAP and consequent lower-limb performance than TW, or *vice versa*. Such a comparison may have several practical applications in strength and conditioning in sport as well as for warm-up strategies before some competitions. EOL and TW may be valid strategies to elicit acute PAP mediated improvements in lower limb power and therefore may play a functional role in sports performance training. Standing long jump (SLJ), CMJ and sprinting are well established tests to assess the lower-limb capacities. The aims of this study were: firstly, to study the acute effect of EOL and TW exercise on such sport-specific tasks; and secondly, to compare the magnitude of such acute effects between EOL and TW exercises. Such knowledge may be relevant for practitioners in order to generate PAP strategies prior to competition and training. Considering the greater peak power generated during the eccentric phase of the squat exercise, it could be supposed that EOL exercise may produce a higher PAP response in the subsequent sport-specific tasks than TW. However, authors hypothesize that both protocols should stimulate a positive PAP response in jumping and sprinting performance.

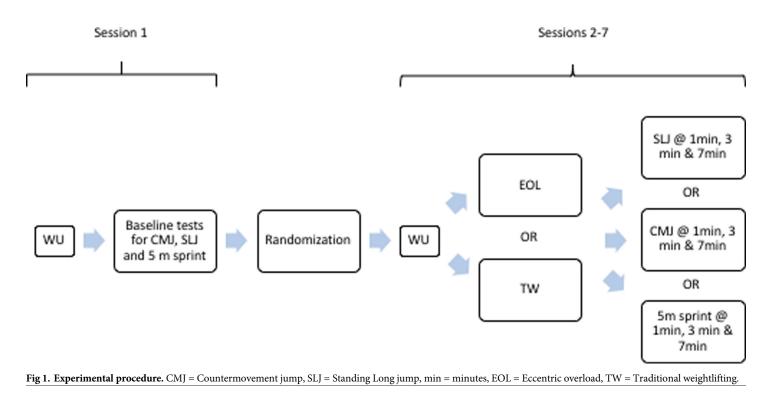
Materials and methods

Subjects

Ten male amateur athletes were enrolled in this study (mean \pm SD: age 22 \pm 2 years; body mass 73.2 \pm 8.0 kg; height 1.79 \pm 0.05 m) with \geq 4 years experience with heavyweight training at a regional level. Inclusive criteria for participation were the absence of any injury or illness and regular participation in training activities (a minimum of 3 training sessions per week) as used in previous research [7]. A Bayesian adaptive sample size approach was used in this study to estimate the number of subjects [17] based on previous research of the same group [14]. Subjects were familiar with TW and EOL exercises and test procedures. All subjects were informed about the potential risks and benefits of the current procedures and gave their written informed consent. The Ethics Committee of the School of Science, Technology, and Engineering, University of Suffolk (UK) approved this study. All procedures were conducted according to the Declaration of Helsinki for studies involving human subjects.

Experimental overview

The acute effects of EOL vs TW exercise on SLJ, CMJ, and 5 m sprint performance were investigated in the present randomized, cross-over study design. Each subject attended the laboratory on seven separate occasions. This was necessary to remove a possible transient fatigue effect. The sessions were separated by 48 h of recovery to allow an adequate recovery period. Researchers required subjects to maintain their normal nutritional intake during the experimental period. Alcohol and caffeine were not permitted prior to the experimental sessions but hydration was allowed during the sessions. In the first session subjects performed the baseline condition and familiarization to EOL and TW [14]. During the baseline conditions athletes performed the same warm-up protocol utilized during the experimental condition but without any pre-load exercise (neither EOL or TW). In each of the following sessions (sessions were performed in a randomized order using Randomization.com, in order to remove any possible learning effect), the subjects performed the warm-up procedure utilized during the baseline condition followed by one of the two exercise modalities (EOL and TW). At 1 min, 3 min and 7 min after completion of the final EOL or TW set, one of the three performance tests (SLJ, CMJ, or 5 m linear sprint acceleration) were performed to evaluate the PAP effect (procedure reported in Fig 1). The authors considered that use of this protocol limited the confounding effect of repeated jumps as previously reported [7,14]. These time windows were used to observe PAP optimization, as used with success in previous studies [3,7].



Procedures

Body mass and height were recorded by Stadiometer (Seca 286dp, Hamberg, Germany). A standardized warm-up was conducted each session, including 10 min of cycling at a constant power (1 W per kg of subject's body mass) on an ergometer (Sport Excalibur lode, Groningen, Netherland). Dynamic mobilization was performed in both the baseline and experimental conditions. Mobilization was performed immediately after the cycling warm-up for a duration of 3 minutes and consisted of dynamic movements mimicking the exercise (*e.g.* half squat) and dynamic hip, knee, and ankle movements. Such procedure was utilized prior to baseline tests as previously utilized by the same research group [14].

A SLJ was utilized to test the explosive horizontal power capabilities of the lower limb musculature, as previously reported [18]. Subjects performed one maximal bilateral anterior jump with arm swing. Jump distance was measured from the starting line to the point at which the heel contacted the ground on landing [19]. The validity of this test was previously reported in the literature involving a sample of physical education students [20]. An *excellent* (ICC = 0.90) baseline test-retest intrasession reliability was found in the current study. The smallest worth-while change (SWC) was 5 cm for SLJ.

CMJ height was investigated using an infrared device (OptoJump, Microgate, Bolzano, Italy). The subjects were instructed to stand, lower themselves to a self-selected depth and immediately jump. Arms were placed on the hips to minimize the confounding effects of arm swing and the subjects were instructed to minimize knee flexion before landing. An *excellent* (ICC = 0.92) baseline test-retest intrasession reliability was found in the current study. The SWC was 1.2 cm for CMJ.

Five-meter sprints were performed to evaluate improvements in acceleration ability. Infrared timing gates (Microgate, Bolzano, Italy) were placed at the start and end of a measured 5 m distance. On the "Go" command, the subjects were instructed to sprint through the timing gates positioned as previously reported in literature [21]. No countermovement before the sprint was permitted. A *good* (ICC = 0.86) test-retest intrasession reliability was found in the current study. The SWC was 0.03 s for 5 m sprint performance.

Intervention

EOL half squat exercise was performed using a flywheel ergometer (D11 Full, Desmotec, Biella, Italy). The protocol consisted of 3 sets x 6 repetitions of half squats, interspersed by 2 min of passive recovery [7]. The subjects were instructed to perform the concentric phase with maximal velocity and to control the eccentric phase until the knees where flexed to approximately 90° [14]. The following load was used for each subject: one Pro disc (diameter = 0.285 m; mass = 6.0 kg; moment of inertia = 0.06 kg·m²) based on previous published research [14]. The moment of inertia of the ergometer was estimated as 0.0011 kg·m². Power was calculated for each repetition using an integrated rotary position transducer.

TW was performed as a half squat exercise using an Olympic bar. The PAP protocol consisted of 3 sets x 6 repetitions of half squats, interspersed by 2 min of passive recovery [3]. The subjects were instructed to perform the concentric phase with maximal velocity and to control the eccentric phase until the knees were flexed to approximately 90° [22]. During the familiarization session, the TW squat loads were adjusted in order to match the peak concentric power production between TW and EOL. This was achieved by increasing the barbell load by 5 kg until the concentric peak power was within 10% of that of the EOL. The mean load was 57.7 ± 10.1 kg. Lower limb power was assessed during TW exercise by a linear position transducer (Cronojump, Barcelona, Spain).

The EOL (1097 ± 341 W, 14.98W/Kg) and TW (1030 ± 298 W, 14.07 W/Kg) concentric peak power during load matching were not meaningfully different between the conditions: Bayes factor (BF₁₀) = 0.88 (*anecdotal*; effect size = 0.51; 95% credible interval [CI]: -0.20, 1.35). The EOL (1138 ± 263 W) and TW (798 ± 286 W) eccentric power were meaningfully different: BF₁₀ = 44.42 (*very strong*; effect size = 1.88; 95% CI: 0.61, 3.05).

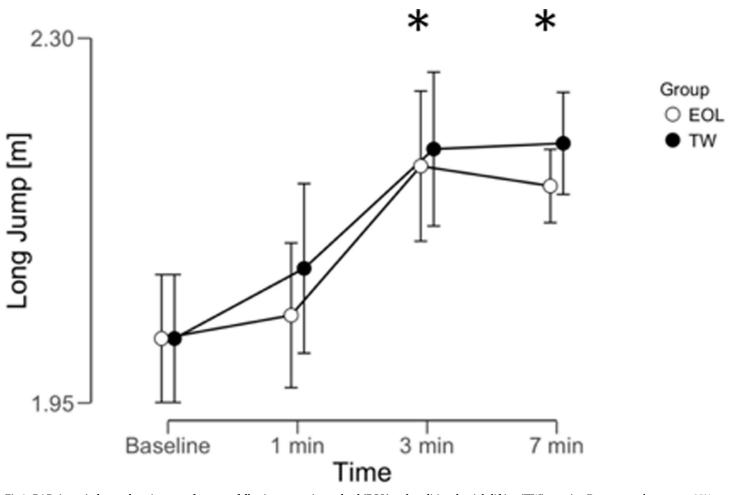
Statistical analysis

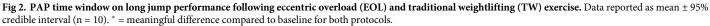
Data were presented as mean \pm SD. The test-retest intrasession reliability (during baseline session) was assessed using an intraclass correlation coefficient (ICC) and interpreted as follows: $ICC \ge 0.9 = excellent; 0.9 > ICC \ge 0.8 = good; 0.8 > ICC \ge 0.7 = acceptable; 0.7 > ICC \ge 0.6$ = questionable; $0.6 > ICC \ge 0.5 = poor$; ICC < 0.5 = unacceptable [23]. A fully Bayesian statistical approach to provide probabilistic statements was used in this study [24]. Each analysis was conducted with a "noninformative" prior (Cauchy, 0.707). Bayesian repeated measures ANOVA was used to evaluate the effects of time (within; baseline, 1 min, 3 min, 7 min) and exercise modality (between; EOL vs TW) on each of SLJ, CMJ, and 5 m sprint performance. If a meaningful BF10 was found, a Bayesian post-hoc was performed [25]. Markov Chain Monte Carlo with Gibbs sampling was used to make inferences (10000 samples) [26]. Estimates of median standardized effect size and 95% credible interval were calculated. Evidence for the alternative hypothesis (H₁) was set as $BF_{10} > 3$ and evidence for null hypothesis was set as BF_{10} < 1/3 [27]. BF₁₀ was reported to indicate the strength of the evidence for each analysis (between and within). The BF₁₀ was interpreted using the following evidence categories: $1 < BF_{10} < 3 = anecdotal$ evidence for H_1 ; $BF_{10} \ge 3 = moderate$; $BF_{10} \ge 10 = strong$; $BF_{10} \ge 30$ = very strong; BF₁₀ \ge 100 = extreme [27]. SWC was calculated as 0.2 x SD for SLJ, CMJ and 5 m sprint performance. Statistical analyses were performed within JASP (Amsterdam, Netherland) software Version 0.9.1.

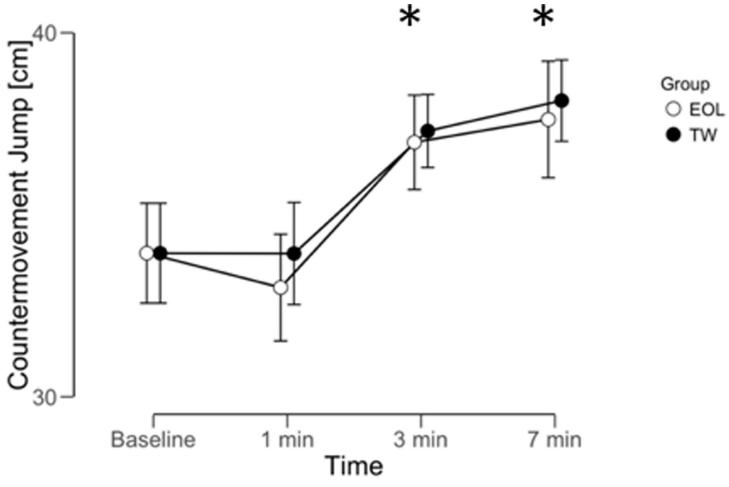
Results

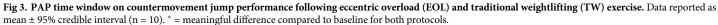
The repeated ANOVA reported differences within (time) using EOL exercise for SLJ ($BF_{10} = 354.2$, *extreme*), CMJ height ($BF_{10} = 698.3$, *extreme*), but not in 5 m sprint ($BF_{10} = 0.61$, *anec-dotal*). The repeated ANOVA reported differences within (time) using TW exercise for SLJ ($BF_{10} = 193.1$, *extreme*), CMJ height ($BF_{10} = 6967.3$, *extreme*), but not in 5 m sprint ($BF_{10} = 0.37$, *anecdotal*). A graphical representation of time effect on SLJ, CMJ and 5 m sprint was reported in Figs 2–4. No meaningful time x condition interactions were reported for any parameter analyzed: SLJ ($BF_{10} = 0.182$, *moderate* in favor of H_0); CMJ ($BF_{10} = 0.159$, *moderate* in favor of H_0); 5 m sprint ($BF_{10} = 0.049$, *moderate* in favor of H_0). The repeated ANOVA did not report meaningful differences between (conditions) EOL and TW exercise for SLJ ($BF_{10} = 0.33$, *moderate* in favor of H_0), CMJ ($BF_{10} = 0.27$, *moderate* in favor of H_0), or 5 m sprint ($BF_{10} = 0.218$, *moderate* in favor of H_0).

Bayesian post-hoc analysis comparing baseline values and time following EOL was reported for the following parameters: SLJ at 1 min (BF₁₀ = 0.165, *moderate* in favor of H₀), 3 min (BF₁₀ = 7.24, *moderate*, +8%), and 7 min (BF₁₀ = 19.5, *strong*, +7%); CMJ at 1 min (BF₁₀ = 0.19, *moderate* in favor of H₀), 3 min (BF₁₀ = 3.25, *moderate*, +9%), and 7 min (BF₁₀ = 4.12, *moderate*, +10.5%). Bayesian post-hoc analysis comparing baseline values and time following TW was reported for









the following parameters: SLJ at 1 min (BF₁₀ = 0.22, *moderate* in favor of H₀), 3 min (BF₁₀ = 3.88, *moderate*, +9%), and 7 min (BF₁₀ = 12.4, *moderate*, +9%); CMJ at 1 min (BF₁₀ = 0.12, *moderate* in favor of H₀), 3 min (BF₁₀ = 7.42, *moderate*, +9.5%), and 7 min (BF₁₀ = 12.4, *strong*, +12%). Post-hoc analysis regarding 5 m sprint was not performed since no time effect was reported.

Discussion

To the best of the authors' knowledge, this study is the first to investigate the PAP response following EOL and TW exercises on SLJ, CMJ and 5 m sprint tasks. This study compares, also for the first time, PAP magnitude between EOL and TW exercises on functional lower limb tests, matching concentric peak power between the exercises. The present study showed that a meaningful positive PAP response can be observed after 3 min of recovery (and persists until at least 7 min) following both EOL and TW exercises on SLJ and CMJ performance but not on 5 m sprint performance in male amateur athletes. Furthermore, meaningful evidence (in favor of H_0) revealed no differences in PAP response for each performance variable analyzed between EOL and TW protocols, therefore both protocols exhibited similar PAP responses. These findings may have an important impact on practitioners' strength training strategies in order to develop PAP and enhance its magnitude and time window.

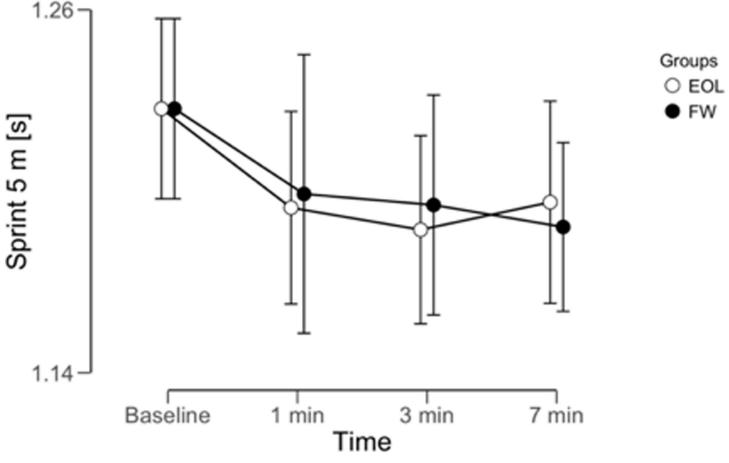


Fig 4. PAP time window on sprint 5 m performance following eccentric overload (EOL) and traditional weightlifting (TW) exercise. Data reported as mean \pm 95% credible interval (n = 10). * = meaningful difference compared to baseline for both protocols.

PAP is a physiological phenomenon that can be observed following a pre-load strategy and has previously been identified as a strength-power-potentiation complex [8]. Previous studies reported that PAP effects on CMJ performance (e.g. jump height, peak power, and impulse) and short sprinting tasks may be obtained after an EOL exercise [1,7]. Similarly, the literature supports such positive improvements (e.g. horizontal and vertical jump performance) following TW exercises [28]. For example, TW back squat, using different intensities (e.g. moderate or heavy), may induce a positive PAP response on jumping activities following a recovery period [3]. The current study supports the general knowledge that PAP is observed following a recovery period [4]. Therefore, a passive recovery of around 3 min seems to be sufficient between the pre-load strategy (e.g. EOL or TW) and the following sport-specific task (e.g. SLJ, CMJ) in order to observe performance benefits. Previous evidence reported that PAP time windows may be altered using different pre-load strategies [8]. However, the current study finds similar PAP time windows between EOL and TW exercises [4,6,7]. Future investigations, however, are needed to better clarify the optimal onset of PAP (e.g. lower volume protocols may be beneficial for PAP without stimulating as much acute fatigue) since the current study used 3 sets of 6 repetitions. This study reports that following an acute improvement in sportspecific tasks at 3 min, such positive effects were confirmed at 7 min using both EOL and TW exercises. Such a result is in line with the major body of evidence in the literature reporting an

optimal time window between 3 and 10 min [7,29,30]. A recent meta-analysis found that the recovery time affects PAP magnitude, and that the optimal time window should be between 4 to 8 minutes, while a shorter recovery time (*e.g.* 1 min) may reduce the PAP effect on sport specific tasks [8].

The findings reported in the current study support the previous knowledge on performance improvements following a pre-load exercise except for 5 m sprint, for which a PAP effect was not found following either EOL or TW exercise. This result may be attributable to the following observations: 5 m may not be a suitable sprint distance to assess PAP and a longer (*e.g.* 10 or 20 m) sprinting distance could be more suitable (reliability of the sprint test increases with distance) [21]; from a biomechanical perspective short accelerations may be affected by subjects' coordination, which could have impaired the 5 m sprint PAP response obtained with the present protocols; and finally, because both EOL and TW exercises were not biomechanically similar to the sprint, which may have limited the transfer to sprinting capacity. Indeed, the kinetic responses to a pre-load exercise may be related to its specific directional loading nature (*e.g.* vertical loading during a squat exercise) [31]. Therefore, a different exercise such as a barbell hip trust or a single step acceleration using a flywheel may be more effective for acute sprinting improvements due to the more horizontal nature of those exercises relative to the participant.

To the authors' knowledge, this is the first study that compares the PAP effects of an EOL squat with TW squat exercise matching the concentric peak power. Therefore, a comparison between the current study and the literature is not possible. EOL and TW reported no meaningful differences in concentric peak power production, while EOL reported a very strong difference in eccentric peak power compared to TW. These results support the validity of the protocol used to match EOL and TW concentric intensities and underline also the EOL induced by flywheel exercise compared to TW. This greater eccentric load is a *peculiaritas* of flywheel exercises, since during the positive (extension) phase of a squat, the subject executes a high velocity movement (generally maximal) while during the negative (flexion) phase of the squat, the subject has to break the load accumulated during the previous phase.[1] Therefore, the principal advantage of EOL is related to an enchained mechanical load that is not possible using TW exercises. Authors supposed that a high eccentric load may have better stimulated higher order motor units (which require the utilization of high load), which may have guaranteed a positive transfer in motor unit recruitment, force, and power production during the following tasks (e.g. SLJ and CMJ) [7,32]. Additionally, acute performance improvements in sport-specific tasks may be associated with increased motor unit recruitment, rate coding, and neuromuscular inhibition [7,33]. Despite this strong theoretical rationale, this study found *moderate* effect in favor of H_0 for PAP responses between the two pre-load exercises. Therefore, EOL and TW exercises, when matched for concentric peak power, reported equivalent PAP responses on SLJ, CMJ, and 5 m sprint performance. It is noteworthy that there is *moder*ate statistical evidence in favor of similarity between the two methods (evidence in favor of H_0). The results reported in the current study should be considered innovative since no previous studies have compared the PAP time windows following EOL and TW exercises. Furthermore, they may help strength and conditioning coaches to augment PAP strategies for athletes. Practitioners need to individualize recovery time and PAP onset obtained by EOL and TW exercises in order to enhance benefits from such strategies in competitions and complex training interventions [34]. Future studies on this argument are needed to confirm or contradict the findings of this study.

Existing literature reports that PAP effect magnitudes are related to the pre-load modality adopted. For instance, plyometric exercises seems to be more effective than both moderate and high intensity TW exercises, while maximal isometric contractions do not seem beneficial [8].

However, many factors may affect PAP such as the subject's resistance training background (experienced vs inexperienced), as well as fitness level, where stronger individuals generally exhibit a larger PAP effect than weaker [8]. Moreover, PAP time window and magnitude may be related to the subjects' muscle properties such as percentage of fast fibers [4,35]. Those factors should be further studied to understand the possible PAP differences between EOL and TW. Furthermore, the magnitude of the PAP effect could be different if an experienced (in strength training) cohort was enrolled. Such speculation may be supported by Dello Iacono et al. [31] that showed a PAP response (in acceleration tasks) following both moderate and intensive barbell hip trust exercises but that the effects differed according to the subject's strength level. Authors may speculate that subjects' device-specific resistance training back-ground should be considered when selecting an exercise modality. For example, it is not known whether previous TW experience can be easily transferred to EOL exercise PAP response.

The current study is not without limitation. Firstly, this research has the assumption that PAP is the main explanation for the observed findings of improved performance but there is no explicit measurement of muscular activity and therefore direct evidence that PAP is the only mechanism underpinning such changes. Such limitation should be taken into consideration since the current research did not use a control group, but this design was utilized to reduce intrasession fatigue [7], furthermore because a possible placebo effect associated with the subjects' knowledge of PAP could explain some changes. Secondly, this study compared EOL and TW exercise matching the intensity using the peak concentric power output. However, during the eccentric phase of EOL exercise the peak power was meaningfully greater than the eccentric peak power of TW exercise. Therefore, the total power (concentric and eccentric) generated by subjects was greater during the EOL than the TW squat exercise. Furthermore, practitioners need to consider access to EOL equipment in their daily practice, which could be less common than TW equipment. Lastly, this study enrolled a sample of amateur male athletes, therefore wider generalization cannot be inferred to other samples with different characteristics such as female athletes and professional athletes who may exhibit different PAP time window and magnitude responses [8,36,37].

Conclusions

The present study suggests that both EOL and TW squat exercises acutely increase SLJ distance and CMJ height but not 5 m sprint performance in male amateur athletes. The onset of the PAP time window was found at 3 min following the protocol and the improvements in sportspecific tasks persisted at 7 min. This study did not find differences between EOL and TW exercises in PAP amplitude. Therefore, both exercise methodologies can be used to acutely stimulate PAP in a similar way before competitions and training sessions. Further research is needed to better clarify the similarities or differences in PAP time window and magnitude between EOL and TW squat exercise.

Practical applications

Practitioners may use either EOL or TW squat exercises to stimulate a PAP response in athletes. Such acute potentiation has a positive effect on horizontal and vertical jumping performance, however, both protocols seem not to be efficient in improving sprinting acceleration performance. Future studies should explore this topic before drawing final conclusions, as well as clarifying the differences between the protocols. To optimize the PAP effect using EOL and TW preload methodologies (3 x 6 repetitions, with concentric peak power outputs of 1097 W and 1030 W, respectively), it is necessary to wait for 3 minutes following pre-load before initiating sportspecific movements; such PAP effects remain at least 7 min after completion of either pre-load strategy. Therefore, practitioners should consider such PAP time windows in sport-specific tasks before competitions or during training sessions (*e.g.* complex training). Furthermore, authors suggest individualizing the PAP protocol on the basis of athletes' training experience, strength level, and morphological characteristics. This may help to optimize PAP as well as minimize acute fatigue and soreness. Authors suggest consideration of pre-load exercise (EOL or TW) on the basis of athletes' previous strength training experience with such protocols.

Author Contributions

Conceptualization: Marco Beato, Alexander E. J. Bigby, Giuseppe Coratella.

Formal analysis: Marco Beato.

Investigation: Giuseppe Coratella.

Methodology: Giuseppe Coratella, Stuart A. McErlain-Naylor.

Software: Stuart A. McErlain-Naylor.

- Supervision: Fabio Y. Nakamura.
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- Writing review & editing: Marco Beato, Kevin L. De Keijzer, Giuseppe Coratella, Stuart A. McErlain-Naylor.

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3 The effects of exercise variation in muscle thickness, maximal strength and motivation in resistance trained men

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Abstract

Background

The objective of the present study was to compare the effects of a traditional resistance training program (fixed exercises and repetition ranges) to a resistance training program where exercises and repetition ranges were randomized on a session-by-session basis on markers of muscular adaptations and intrinsic motivation.

Methods

Twenty-one resistance trained men were randomized to perform an 8-week resistance training program using either a fixed exercise selection (CON) or having exercises randomly varied each session via a computerized app. Both groups performed 3 sets of 6 exercises, with training carried out 4 times per week.

Results

Both conditions promoted large, statistically significant increases in the bench press and back-squat 1 repetition maximum without differences between groups. Muscle thickness (MT) measures for the individual quadriceps showed large, statistically significant increases in of the vastus lateralis and rectus femoris for both conditions, with no observed betweengroup differences. Although no between-group in MT were noted for the vastus intermedius, only the CON displayed significant increases from baseline. Participants in EXP showed a significant, moderate improvement in the intrinsic motivation to training, while participants in the CON group presented non-significant decreases in this variable.

Conclusions

Varying exercise selection had a positive effect on enhancing motivation to train in resistance-trained men, while eliciting similar improvements in muscular adaptations.

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

Introduction

Resistance training (RT) is well-established as an effective method to increase muscle mass, strength and overall health in different populations [1-5]. It has been proposed that proper manipulation of RT variables may help to optimize muscular adaptations [2,6]. Practitioners can manipulate a variety of RT variables to elicit desired muscular adaptations. These include both quantitative variables, such as training volume, frequency, rest intervals or cadence [1,6,7], and qualitative variables, such as exercise selection. For example, with respect to training load, it has been shown that, when volume is equated, both light (i.e., <50% 1-RM) and heavy (i.e., > 80%1-RM) loads can elicit similar hypertrophic responses [8], while heavy loading seems to elicit greater increases in maximal strength [9].

Gaining muscle mass and strength while maintaining or increasing motivation to exercise seem to be a relevant factor to improve adherence to exercise. In this sense, some popular exercise programs advocate frequent rotation of exercises as a means to optimize results and improve exercise motivation [10]. The term "muscle confusion" has been coined to describe the effects of constantly varying exercise selection as a means to provide a novel stimulus that enhances muscular adaptations [10]. However, research on the topic is limited. Fonseca et al. [11] showed that changing lower body exercises every two weeks may elicit greater regional-specific hypertrophy of the quadriceps muscle compared to just performing the squat. More recently, Rauch et al. [12], demonstrated that varying exercise selection via autoregulation produced modestly greater increases in lean mass and strength compared to a fixed exercise protocol. However, to our knowledge, no study to date has endeavored to investigate the effects of randomly undulating exercises may enhance results by continually providing a novel stimulus to muscles and/or bolstering motivation to train.

The objective of the present study was to compare the effects of a traditional training program (fixed exercises and repetition ranges) to a training program where exercises and repetition ranges were randomized on a session-by-session basis on markers of muscular adaptations and intrinsic motivation in resistance trained men. We hypothesized that the random routine would increase intrinsic motivation without hampering gains in muscle mass and strength.

Material & methods

Participants

Twenty-one healthy men (age = 23.4 ± 3.5 years; body-mass = 77.5 ± 11 kg; body-height = 1.78 ± 0.05 m; body-fat = $13.6 \pm 2.5\%$; lean body mass = $86.3 \pm 2.5\%$) with at least 2 years of experience with resistance training voluntarily joined this investigation. Participants were required to meet the following inclusion criteria: 1) men between the ages of 18-35; 2) no existing musculoskeletal disorders; 3) claimed to be free from consumption of anabolic steroids or any other illegal agents known to increase muscle size; 4) experienced with RT, defined as consistently lifting weights at least 3 times per week for a minimum of 2 years. A total of 19 participants completed the study; two participants dropped out prior to completion, for personal reasons. Written informed consent was obtained from each participant after a thorough explanation of the testing protocol, the possible risks involved, and the right to terminate participation at will. The study was approved by the Institutional Review Board of the University of the Basque Country, Spain (ref. 2018/099) and all procedures were in accordance with the declaration of Helsinki (2013).

Training interventions

Participants were randomly allocated to either an experimental group (EXP) or a control group (CON). Participants in the CON group carried out an 8-week resistance training program consisting of 3 sets of 6 exercises performed 4 times per week. On Monday and Thursday, participants performed an upper-body workout, while on Tuesday and Friday they performed a lower-body workout, for a total of 32 RT sessions. Upper body exercises in CON group included bench-press, pendlay row, shoulder press, latpull down, dumbbell fly and dumbbell pull-over, while the lower body exercises included back squat, deadlift, leg press, hip thrust, leg extension and leg curl. Training load was linearly periodized by reducing the number of repetitions per set every 2 weeks, from 12RM to 6RM. Thus, there were a total of 8 training sessions with each XRM. See Table 1 for more details.

Participants in EXP group carried out a resistance training program with the same duration and sessions per week as CON, but with exercises randomly chosen each session from a computerized database of 80 different exercises via an iPhone app (Ace Workout) specifically designed for the present study. The randomization algorithm was written to select 3 pulling (e.g., pull-up, lat-pull down and pull-over) and 3 pushing (e.g., bench-press, standing military press and dumbbell flies) exercises for the upper-body, with no exercise repeated within the same workout. For the lower-body, the algorithm chose 3 exercises with greater participation of the anterior chain (ex., back-squat, leg extension and leg press) and 3 for the posterior chain (e.g., deadlift, hip-thrust and leg curl). Both EXP and CON were afforded two minutes rest between sets. Total training volume (measured as total number of sets and repetitions) was equated between groups (see Table 1 for more details). All participants took part in at least 95% of the training sessions.

Psychological measures

One day before and one day after the training intervention, the intrinsic motivation and demotivation factors of the Situational Motivation Scale were measured using a validated Spanish version of this questionnaire [13]. A total of 15 out of 19 participants completed the questionnaire online using an ad-hoc form that was sent to them, while 4 participants did not complete it for unspecified reasons. The validity of the intrinsic motivation factor was confirmed using Cronbach's alpha ($\alpha > 0.8$).

Muscle thickness

Muscle thickness (MT) was measured using B-mode ultrasound imaging (GE LOGIQTM e, GE Healthcare, WI, USA) with a linear-array transducer (code 12L-RS, variable frequency band 4.2–13.0 Mhz). Measurements were performed with participants supine, with arms and

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control group*	12 RM	12 RM	10 RM	10 RM	8 RM	8 RM	6 RM	6 RM
Experimental group								
Day 1 (upper-body)	8 RM	6 RM	6 RM	10 RM	12 RM	12 RM	10 RM	12 RM
Day 2 (lower-body)	10 RM	12 RM	12 RM	10 RM	6 RM	8 RM	8 RM	10 RM
Day 3 (upper-body)	10 RM	8 RM	12 RM	6 RM	10 RM	8 RM	8 RM	6 RM
Day 4 (lower-body)	8 RM	6 RM	6 RM	12 RM	8 RM	12 RM	10 RM	6 RM

Table 1. Distribution of training load (number of repetitions to failure per) set through the intervention in both the control and experimental groups.

*Participants in the control group performed the same number of repetitions to failure per set each day of the week

legs extended and relaxed. Prior to testing, participants remained in this position for 10 minutes to allow for stabilization of normal body fluids. The technician then applied a water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission gel; Parker Laboratories Inc., Fairfield, NJ, USA) to each measurement site and a 5 MHz ultrasound probe was placed perpendicular to the tissue interface without depressing the skin. When the quality of the image was deemed as satisfactory, the technician saved the image to the hard drive and obtained MT dimensions of the vastus lateralis (VL) and rectus femoris (RF) by measuring the distance from the subcutaneous adipose tissue-muscle interface to the muscle-bone interface as detailed in previous research [14,15]. Measurements for the vastus intermedius (VI) were obtained at the widest distance between the bony surface of the femur and RF muscle interface [14]. Distances were measured using the straight-line function of ImageJ software. Measurements were taken on the right side of the body at two different sites: medial quadriceps femoris, and lateral quadriceps femoris. For the quadriceps femoris, measurements were taken 50% between the lateral condyle of the femur and greater trochanter for the medial (RF and VI) and lateral (VL) aspects of the thigh. Three images were taken at each site and the values were averaged to obtain a final measurement. In an effort to help ensure that swelling in the muscles from training did not obscure results, images were obtained 48-72 h before commencement of the study and after the final training session. This timeframe is consistent with research showing that acute increases in MT return to baseline within 48 h following a resistance training session [16].

Body composition

One day before starting and one day after ending the 8-week intervention, body composition was measured using anthropometric methods. Participants were weighed on a calibrated digital scale whilst wearing minimal clothing. Height was measured with a stadiometer attached to the scale with participants standing shoeless and head aligned in the horizontal Frankfurt plane. Body Mass Index (BMI) was calculated as follows: total body mass (in kg) stature (in m) -2. Seven-site skinfold measurements (in mm) were taken from the biceps, triceps, scapular, abdominal, suprailiac, thigh and medial calf sites according to standard procedures using a skin fold caliper (Harpenden1, Baty International, West Sussex, UK). All skinfolds were measured to the nearest 1 mm and the mean of 3 readings was recorded as the final value at each site. All body composition measurements were taken by the same investigator 24-48h before and 24-48h after completion of the training protocol. Body fat percentage was estimated using the equation proposed by Faulkner [17].

Maximal dynamic strength

Subjects reported to the laboratory having refrained from any exercise other than activities of daily living for at least 48 hours prior to baseline testing and at least 48 hours prior to testing at the conclusion of the study. Maximal dynamic strength on the free-weight barbell bench-press and back squat exercises were measured before and after the training intervention via the 1-repetition maximum (1-RM) test [6]. All participants were familiar with 1-RM testing, and prior to testing were asked for their previous 1-RM value for each exercise. They subsequently performed 2 repetitions at 60, 70 and 80% and 1 repetition at 90 and 100% 1-RM. If a participant failed an attempt, the load was reduced in a range of 2.5-5kg to determine their 1-RM with a high degree of precision. Three minutes of passive rest were afforded between each trial.

Dietary adherence

To avoid the potential for dietary confounding, subjects were instructed to maintain their usual and customary eating habits while consuming a minimum protein intake of 2g/kg and a

eucaloric diet or slight energy surplus. To assess nutritional adherence, subjects tracked their meals with a nutritional tracking app (http://www.myfitnesspal.com) at the beginning and end of the intervention, providing data related to total consumed energy, as well as proteins, fat and carbohydrate distribution. Data were tracked at the beginning and the end of the study to ensure dietary adherence. Subjects agreed not to consume any supplement that could interfere with the studied outcomes (such as creatine and whey protein) throughout the investigation period.

Statistical analyses

We tested all variables for normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Data are presented as mean with standard deviations. An independent samples T-test was carried out on pre-intervention muscle thickness data to check for potential differences between groups. An Analysis of Covariance (ANCOVA) was employed to determine the potential differences between groups on the post-intervention scores, with the preintervention scores used as a covariate. Cohen's *d* effect size (ES) with 95% CIs were calculated to analyze the magnitude of the potential pre-post intervention differences, both within and between groups. The following criteria were employed for interpreting the magnitude of the ES: trivial (<0.2), small (0.2–0.6), moderate (0.6–1.0) and large (>1.0). All calculations were performed using JASP 0.9.2 for Mac (University of Amsterdam, Netherlands). The level of significance was set as p < 0.05.

Results

Intrinsic motivation

Participants in the EXP group showed a significant, moderate improvement in the intrinsic motivation to training (p< 0.05, ES = 1.28, 95% CI = 0.30, 2.22), while participants in the CON group showed non-significant decreases in this variable (p > 0.05, ES = -0.75, 95% CI = -1.55, 0.12). A moderate, significant between-group difference was observed for this variable (p < 0.05, ES = 0.58). No group showed significant post-study changes in the demotivation scale (p > 0.05).

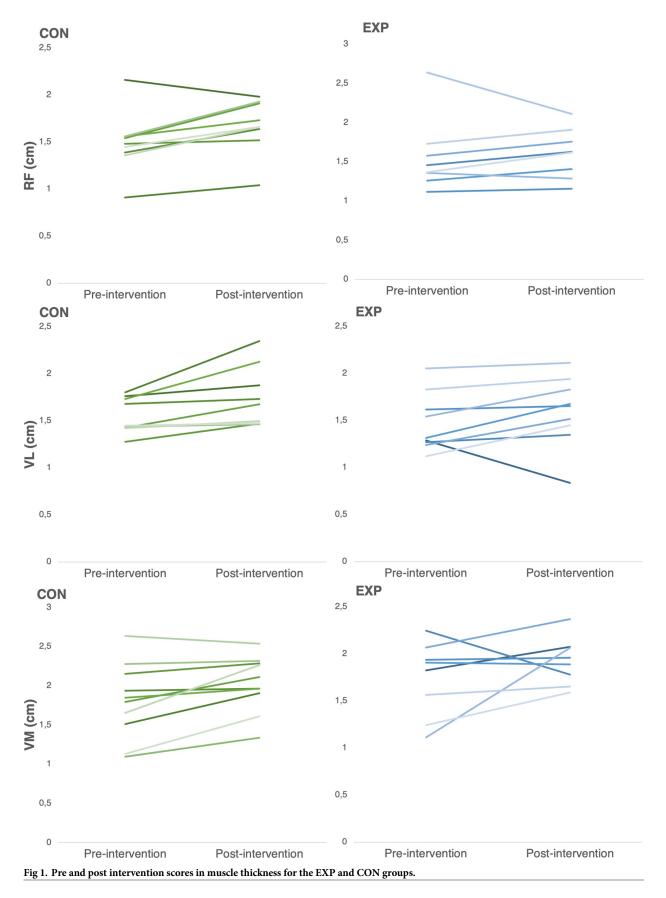
Muscle thickness

No significant differences were observed between groups in any of the MT variables analyzed at pre-intervention (p> 0.05). Also, normality of the distributions and homogeneity of the variances were confirmed for both groups at pre-intervention in those MT variables. Fig 1 illustrates changes in MT for the individual quadriceps' muscles. Both the EXP and CON group showed large, statistically significant increases in MT of the VL (EXP: p <0,05, ES = 1.43, 95% CI = 0.4, 2.42; CON: p <0,05, ES = 1.03, 95% CI = 0.19, 1.83). Trivial, non-statistically significant differences were observed between groups (p > 0.05, ES <0.2).

MT of the RF increased significantly in both EXP and CON (EXP: p<0.05, ES = 1.19, 95% CI = 0.17, 2.16; CON: p<0.05, ES = 1.05, 95%CI = 0.20, 1.86). No significant between-group differences were noted for change in this outcome (p > 0.05, ES = 0.30).

MT of the VI showed significant increases only in CON; (p <0,05, ES = 1.07, 95%CI = 0.30, 1.80); EXP showed absolute increases in this variable, but results were not statistically significant (p > 0.05, ES = 0.78, 95%CI = 0.17, 1.68). No significant between-group differences were noted for change in this outcome (p > 0.05, ES = 0.27).

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	Experimental			Control					
	Pre	Post	Mean Absolute change	Pre	Post	Mean Absolute change	Mean absolute difference between groups (95%CI)	Cohen's d	р
Ultrasound imaging									
VL (cm)	1.54 ± 0.31	1.69 ± 0.26	0.12 ± 0.24	1.57 ± 0.26	1.72 ± 0.35	0.18 ± 0.18	0.05 (-0.12, 0.21)	0.162	0.535
RF (cm)	1.54 ± 0.42	1.62 ± 0.31	0.05 ± 0.25	1.49 ± 0.30	1.67 ± 0.28	0.18 ± 0.17	0.09 (-0.08, 0.26)	0.306	0.275
VI (cm)	1.76 ± 0.43	1.90 ± 0.26	0.15 ± 0.40	1.84 ± 0.44	2.03 ± 0.33	0.22 ± 0.21	0.04 (-0.17, 0.26)	0.276	0.653
Anthropometry									
BMI (kg/m ²)	24.1 ± 2.6	23.9 ± 2.4	0.34 ± 0.49	24.7 ± 2.3	24.6 ± 2.5	0.44 ± 0.96	0.03 (-0.6, 0.7)	0.012	0.931
Body Fat (%)	13.9 ± 2.4	13.9 ± 2.6	0.47 ± 0.98	13.7 ± 2.7	12.7 ± 2.7	-0.4 ± 0.85	-0.8 (-1.8, 0.1)	-0.329	0.062
Strength									
Back squat 1-RM (kg)	120.0 ± 25.4	125.3 ± 23.7	10.2 ± 7.3	127.3 ± 37.7	135.5 ± 28.6	11.9 ± 6.8	1.6 (-5.5, 8.8)	0.063	0.636
Bench-press 1-RM (kg)	90.8 ± 20.9	91.5 ±21.8	4.0 ± 4.7	97.2 ± 17.8	101.8 ± 18.9	7.1 ± 4.2	2.5 (-1.7, 6.9)	0.125	0.229
Motivation scale									
Intrinsic motivation	4.6 ± 1.3	5.1 ± 1.6	0.5 ± 0.3	5.3 ± 0.8	5.1 ± 1.0	-0.1 ± 0.2	-0.8 (-1.0, -0.5)	-0.580	< 0.001
Demotivation	3.1 ± 0.7	3.5 ± 1.2	0.6 ± 0.7	2.7 ± 1.1	3.2 ± 1.2	0.4 ± 0.9	-0.1 (-1.2, 0.9)	-0.112	0.779

Table 2. Pre-post comparison between the experimental and control group in hypertrophy, anthropometrics, strength and motivation.

VL: vastus lateralis; RF: rectus femoris; VI: vastus intermedius

Body composition

Pre- and post-training values of percentage body fat and BMI are shown in <u>Table 2</u>. No significant differences in any measurement were noted from baseline to post study (p > 0.05). No significant difference was observed between groups (p > 0.05). The ES difference was trivial for BMI (ES<0.2), and small favoring CON for percentage body fat (ES = -0.32).

Maximal strength

Both the EXP and CON group showed large, statistically significant increases in the bench press (EXP: p <0.05, ES = 0.84, 95%CI = 0.09, 1.55; CON: p <0.05, ES = 1.68, 95% CI = 0.66, 2.7) and back-squat (EXP: p <0.05, ES = 1.40, 95% CI = 0.49, 2.27; CON: p <0.05, ES = 1.75, 95% CI = 0.66, 2.78). Trivial, non-statistically significant differences were observed between groups (p > 0.05, ES < 0.2). See Table 2 for details.

Discussion

The main goal of this study was to investigate the impact of random exercise selection and range of repetitions on MT, body composition, strength and intrinsic motivation. We hypothesized that random selection using a mobile app (AceWorkout) would enhance motivation levels without compromising improvements in study outcomes. Our hypothesis was confirmed, since the EXP group showed higher levels of motivation throughout the course of the training program and muscular outcomes were generally similar between conditions.

We attempted to isolate the effect of exercise selection by controlling for other RT variables. Both groups performed a total of 18 sets per muscle group per session for the lower body and 9 sets per session per movement pattern (pushing and pulling) for the upper body. Moreover, all participants trained in a range of 6 and 12 repetitions with total training volume equated and each set carried out to volitional muscle failure. These controls allowed us to more confidently draw causality as to how exercise selection impacted the studied outcomes.

A novel finding of our study was that only the EXP group significantly increased motivation levels from pre- to post-study; motivation levels in CON slightly declined. These findings suggest that varying exercise selection may be an important component for enhancing motivation to perform RT. This is an important finding, as evidence indicates that motivation is linked to exercise adherence in different populations [18,19]. Thus, developing strategies that increase motivation to resistance training might help in achieving long-term improvements in fitness and health and reduce the high drop-out ratio observed in fitness centers, that can be up to 80% after 24 weeks of training in some populations [19]. For example, it has been shown that increasing the levels of motivation to resistance training can significantly increase physical, psychological and social parameters in the elderly [20].

Research investigating the effects of exercise selection on muscle hypertrophy is scarce. The most pertinent study on the topic was carried out by Rauch et al. [12], who compared performing a predetermined list of exercises to self-selecting exercises based on individual preferences. Findings showed no between-group differences in LBM (as measured by DXA), although only the group that self-selected exercises showed significant increases from pre- to post-study. These results somewhat deviate from those in our study. The reasons for the discrepancy may be related to the fact that Rauch allowed subjects to choose exercises in an auto-regulated fashion whereas we randomly rotated exercises in the EXP group. Freedom of choice may allow subjects to select exercises that are more suited to their body type and liking, perhaps providing a greater stimulus for adaptation. It also should be noted that we employed more accurate site-specific measures of muscle growth (ultrasound) versus their use of DXA, which may help to further explain inconsistencies between findings.

In another study on the topic, Fonseca et al [11] reported greater increases in muscle cross sectional of the rectus femoris and vastus medialis (obtained by magnetic resonance imaging) when performing a variety of lower body exercises over the course of the study period compared to just the squat. Although these findings are intriguing and suggest a benefit to varying exercise selection, the study differed from ours in several ways. For one, the non-varied groups in Fonseca et al (2014) performed only a single exercise (squat) while our study involved multiple exercises for both conditions. Moreover, exercise variation in our study was random while Fonseca et al (2014) maintained a set schedule. Finally, our subjects were well-trained whereas theirs were untrained. Thus, it is difficult to compare and contrast findings between the two studies.

In regard to strength gains, both conditions showed significant pre- to post-study improvements in all three measurements with no statistical differences observed between groups; ES values were trivial in all of the studied strength-related outcomes. However, gross changes reveal a greater improvement in CON versus EXP for the 1RM bench press (4.7% VS 0.77%, respectively). This could be attributed to motor learning effects, since CON performed the bench press every session whereas EXP performed it with a lower frequency due to the random exercise prescription.

Our study had several limitations that should be acknowledged. For one, MT measurements were performed only on the quadriceps muscles; we therefore cannot generalize results to the upper body muscle groups. Moreover, we obtained MT measurements at a single site along each quadriceps head. There is evidence that the quadriceps hypertrophies in a regional manner [21]. Thus, it is possible that more proximal and/or distal sites may have experienced differential hypertrophy from the imposed alterations in exercise selection. In addition, while the sample was comprised of trained men (at least 2 years of RT experience), the particulars (consistency, technique, effort, etc) of their training approach varied from subject to subject. This

may increase standard deviation, thereby reducing statistical power and increasing the possibility of a type II error. In addition, although we attempted to control resistance training variables (e.g. repetition volume, intensity of load, rest interval), other variables such as total volume load per muscle group, volume progression, and muscle activation couldn't be controlled. These are inherent limitations that will arise when randomizing different exercises, therefore clouding the ability to determine whether results are attributed to varying exercise selection versus other confounding factors specific to the given routines. Finally, the relatively short intervention duration (eight weeks) may not have allowed sufficient time to realize meaningful differences in trained subjects.

Conclusions

Our study showed that randomization of exercise selection in trained men may enhance intrinsic motivation to exercise over an 8-week RT program. These results were obtained with relatively similar changes in muscular adaptations, although some outcomes appeared to be slightly attenuated from frequent rotation of exercises. The findings indicate that regularly changing exercise selection could help to enhance adherence to RT in those who lack motivation to train.

Practical applications

There may be a trade-off whereby too frequent rotation of exercises somewhat compromises muscle growth and strength; thus, those who wish to maximize these outcomes may wish to limit exercise variety. A possible solution is to keep more complex, free weight exercises (e.g. squats, deadlifts, rows, etc) in a regular rotation throughout a training cycle and vary movements that have limited degrees of freedom and thus do not require a high degree of motor learning (e.g. leg extensions, machine press, arm curls, etc). Finally and importantly, exercise rotation was carried out randomly, without attention to individual needs and abilities. It is possible that individualized programming whereby exercise selection is carefully manipulated to take into account biomechanical, physiological and anthropometric factors may further enhance muscular adaptations.

Author Contributions

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Supervision: Eneko Baz-Valle, Brad J. Schoenfeld, Carlos Balsalobre-Fernández.

Validation: Carlos Balsalobre-Fernández.

- Writing original draft: Eneko Baz-Valle, Brad J. Schoenfeld, Carlos Balsalobre-Fernández.
- Writing review & editing: Eneko Baz-Valle, Brad J. Schoenfeld, Jordan Santos-Concejero, Carlos Balsalobre-Fernández.

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4 Bilateral and unilateral load-velocity profiling in a machine-based, single-joint, lower body exercise

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Abstract

Background

To analyze the goodness of fit of the load-velocity relationship in a machine-based, singlejoint exercise performed both in a bilateral and unilateral manner, as well as to study its accuracy to estimate one repetition maximum (1-RM).

Methods

Fifteen resistance trained males performed an incremental test in the bilateral and unilateral leg extension exercise up to the 1-RM in two separate occasions. Mean vertical velocity of the weight plates in the leg extension machine was measured for every repetition using a smartphone application (My Lift).

Results

Linear regression analyses showed a high goodness of fit ($R^2 > 0.93$) and small standard errors of estimate (SEE < 5%1-RM) both in the bilateral and unilateral leg extension when individual load-velocity regressions for each participant were computed. Unilateral loadvelocity relationships showed significant differences in the intercept of the regression line with the Y-axis and the velocity at each percentage of the 1-RM (Cohen's d > 1.0, p < 0.05). Finally, non-significant differences were observed between actual and estimated 1-RM from the load-velocity relationships (r = 0.88.0-96, Cohen's d < 0.2, p > 0.05).

Conclusions

This proof of concept highlights that computing load-velocity relationships in a machinebased, single-joint, angular exercise can be appropriately performed by measuring the mean vertical velocity of the weight plates. These results could help strength and conditioning researchers and coaches who wish to analyze load-velocity relationship in other common machine-based exercises.

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Introduction

Measuring movement velocity during resistance training is known to be a non-invasive and accurate way to prescribe intensity and manage fatigue [1-4]. It has been extensively demonstrated that there is a nearly perfect association between movement velocity and the percentage of the 1-repetition maximum in different exercises, especially when individual load-velocity relationships are computed [1,3,5–7]. Therefore, movement velocity has been proposed as an alternative to traditional 1-repetition maximum (1-RM) testing, because it allows to estimate training intensity without conducting a maximal effort. Also, it has been observed that prescribing training loads based on velocity metrics, rather than using percentages of the 1-RM is more efficient to improve maximal strength and barbell kinematics [8,9]. The main problem of the so-called *velocity-based training* paradigm is that the load-velocity relationships are exercise-dependent [3,5], meaning that if movement velocity is to be used to prescribe training intensity in a certain exercise, its load-velocity relationship needs to be previously investigated. For example, it is known that lower-body exercises like the back-squat allow higher velocities at each percentage of the 1-RM in comparison with upper-body movements like the benchpress [5,6]. To date, the load-velocity relationships of several exercises like the bench-press, back squat, deadlift, hip-thrust or pull-up have been measured [1,5,10,11]. Despite most studies in the literature use barbell velocity to study the load-velocity relationships in different exercises, there is a number of investigations that analyzed the linear motion of the weight plates in machine-based exercises like the leg-press to calculate movement velocity [3,5]. For example, the load-velocity relationship in the lat-pull down and the seated cable row have been recently analyzed by recording the vertical ascent of the weight plates with a smartphone application [12], showing high levels of validity for the estimation of 1-RM [3]. Moreover, bilateral and unilateral force-velocity relationships of the leg extension exercise and its association with muscular performance have been recently analyzed by measuring the vertical ascent of the weight plates with a linear transducer [13]. However, the analysis of the load-velocity relationship in the leg extension exercise and its suitability to estimate 1-RM, both in a bilateral and unilateral manner, has not been previously investigated. This investigation aims to analyze the relationship between movement velocity and load (in terms of %1-RM) in the leg extension exercise, and to study its capacity to estimate the 1-RM. We hypothesize that there will be a very high association between velocity and load in the leg extension exercise performed in a bilateral and unilateral manner, and that there will be no statistically significant difference between actual and estimated 1-RM.

Materials & methods

Participants

Fifteen males with at least 2 years of experience in the bilateral and unilateral leg extension exercises took part in this study (N = 15; age = 33.6 ± 9.3 years). All subjects were instructed to avoid any strenuous exercise two days before each testing session. They were informed of the study procedures and signed a written informed consent form prior to initiating the study. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board at the European University of Madrid. The individuals in Fig 1 have given written informed consent to publish it in this study.

Experimental design

A correlational design was carried out to analyze the load-velocity relationship during the bilateral and unilateral leg extension exercise in fifteen resistance trained males by recording



Fig 1. Position of the smartphone to record mean velocity of the weight plates in the leg extension machine.

the weight plates of the machine with a slow-motion smartphone application. Load-velocity relationships were computed, and the goodness of fit of the load-velocity relationship was analyzed by means of linear regression models as extensively reported [6,14]. Finally, individual and general load-velocity relationships (i.e., the scores obtained when computing the load-velocity relationship of each individual *vs*. when using the whole dataset combined) obtained during the bilateral and unilateral leg extension exercise were compared.

Testing procedures

After conducting a 10-min warm-up consisting on 5 min of jogging and dynamic stretches, subjects' one repetition maximum (1-RM) in the bilateral leg extension exercise was measured using an incremental protocol as described elsewhere [6]. One repetition per set was performed, and 3-min of passive rest were allowed between each set. The leg extension machine was adjusted so each individual could start the movement at a knee flexion of 90°. Subjects were instructed to perform each repetition at maximum intended velocity and to complete the

full extension of the knee. A certified strength and conditioning coach supervised each 1-RM incremental test to ensure that subjects performed the bilateral leg extension exercise with proper technique (i.e., without any hip extension and achieving full leg extension). If a repetition did not meet the aforementioned criteria, the coach asked the participant to perform the repetition again after 1-min. of passive rest. The initial external load was set at 40 kg and 25 kg for the incremental bilateral and unilateral test (respectively) for all participants and was progressively incremented at a rate of 5-10kg until they were unable to complete another lifting. When participants failed one lift, the load was reduced in a range of 0.5 to 2.5 kg to determine their 1-RM with a high degree of precision. The number of total sets in the incremental tests ranged 4–7 sets, with an average of 5.0 ± 1.2 sets. Mean velocity during the bilateral leg extension exercise was measured for each repetition by using the validated My Lift v.8.1 iOS app [12], which was installed on an iPhone 8 with the iOS 12.2 operative system (Apple Inc., USA). The app was used to record the ascent of the weight plates of the leg extension machine at 240 frames per second at a quality of FullHD (1080p). The iPhone was mounted on a tripod and positioned at 1.5m from the weight plates of the leg extension machine (see Fig 1). Then, mean velocity was calculated using Eq 1 from the fundamental laws of motion:

$$v = \frac{d}{t},\tag{1}$$

where v is the mean velocity of the concentric phase of the movement, t is its duration and d is the vertical displacement of the weight plates (ROM). The app measured the duration of the concentric phase of the movement by manually selecting the frame in which the weight plates started their vertical ascent (i.e., beginning frame) and the frame in which the weight plates stopped their vertical ascent (i.e., end frame). ROM was measured with a metric tape as the distance from the rest position of the weight plates to their maximum vertical position at each subject's full knee extension. In that final position, a mark was made in the machine using a tape. A certified strength and conditioning coach carefully supervised each repetition to guarantee that participants moved the weight plates until their individual mark (meaning that ROM was the same at each load), and if there was any doubt, the movement of the weight plates was further analyzed by visually inspecting the slow-motion video recorded with the smartphone. The use of a metric tape to measure ROM was validated and successfully implemented in different studies, both with barbell and machine-based exercises [3,12,15].

Then, the following parameters from the load-velocity relationships were computed in order to compare bilateral and unilateral tests: R², the standard error of the estimate (SEE), the slope and the intercept with the Y-axis of the regression line, and the theoretical velocity at 40, 70 and 100% of the 1-RM.

After 48-h from the bilateral test, subjects followed the same procedure to measure the 1-RM of each leg in the unilateral leg extension exercise. Participants reported which leg did they felt more comfortable with when kicking a ball to register leg dominance. Then, the test was started with the dominant or non-dominant leg randomly. Each load was performed with each leg before moving to the next incremental set. Thirty seconds of passive rest were allowed between sets with each leg, and 3 minutes of passive rest were allowed until performing the next incremental load. Finally, sets below 90%1-RM were computed to create individual load-velocity relationships in order to estimate the 1-RM. Maximal loads were not included in the computation of the load-velocity relationships in order to test its estimation of the 1-RM with submaximal loads, as has been previously studied [3,16].

Statistical analyses

Data are presented as means and standard deviations (SD), and normal distribution for all variables (Shapiro–Wilk test) and the homogeneity of variances (Levene's test) were confirmed (p > 0.05). The coefficient of determination (\mathbb{R}^2) and the standard error of the estimate (SEE) were used to assess the goodness of fit of the generalized and individualized load-velocity relationships for the bilateral and unilateral tests using a linear regression model. Linear rather than polynomic regressions have been proposed as a simpler and more reliable method to analyze the load-velocity relationship [7]. Cohen's *d* effect size (ES) with 95% confidence intervals (CI) were used to assess the magnitude of the differences between bilateral and unilateral load-velocity relationships. The criteria for interpreting the magnitude of the ES were: trivial (<0.2), small (0.2–0.6), moderate (0.6–1.0) and large (>1.0) [17]. One-way ANOVA was used to analyze the differences between bilateral load-velocity relationships. Finally, Pearson's product-moment correlation coefficient, SEE, Cohen's *d* with 95%CI and paired T-Test were computed to study the associations between actual and estimated 1-repetition maximum. The level of significance was set at 0.05. All statistical analyses were performed using JASP 0.9.2 for macOS (University of Amsterdam, Netherlands).

Results

Bilateral and unilateral load-velocity relationships

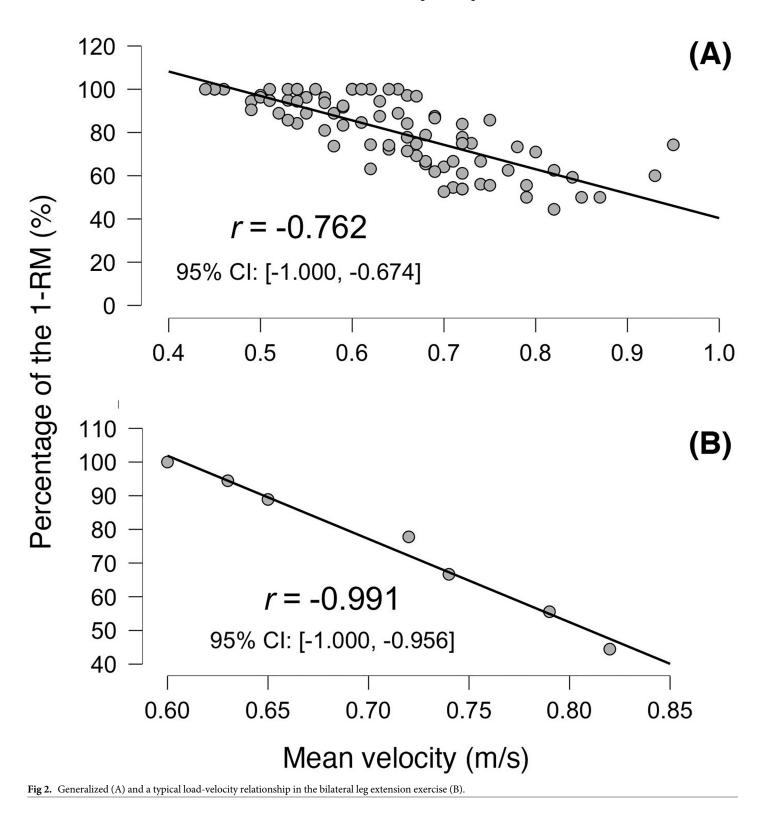
When analyzing each individual load-velocity relationship, high associations between mean velocity and load (in terms of %1-RM) were observed both for the bilateral ($R^2 = 0.96 \pm 0.02$; SEE = 3.60 ± 1.19%1-RM, p< 0.001) and unilateral test ($R^2 = 0.93 \pm 0.07$; SEE = 4.27 ± 2.44% 1-RM, p< 0.001). Finally, when comparing bilateral and unilateral relationships via a one-way ANOVA, significant differences were found in the intercept of the regression line with the Y-axis (p<0.05) and the velocity associated to 40%, 70% and 100% 1-RM (p<0.05) between the bilateral test and the dominant and non-dominant legs' tests. No significant differences were observed between dominant and non-dominant legs. See Fig 2 and Table 1 for more details.

Comparison of actual vs. estimated 1-RM

When comparing actual and estimated 1-RM, non-significant differences, with trivial to small ES were observed between the actual 1-RM and the 1-RM estimated using individual load-velocity relationships (Bilateral: ES = 0.02, 95% CI = -0.69, 0.73, p = 0.953; Dominant leg: ES = -0.25, 95% CI = -1.00, 0.48, p = 0.499; Non-dominant leg: ES = -0.33, 95% CI = -1.17, 0.51, p = 0.443). Actual and estimated 1-RM were very highly correlated as revealed by Pearson's product-moment correlation coefficient (Bilateral: r = 0.96, SEE = 3.4kg, p <0.05; Dominant leg: r = 0.88, SEE = 3.6kg, p <0.05). See Fig 3 for more details.

Discussion

This study aimed to analyze the goodness of fit of bilateral and unilateral load-velocity relationships in the leg extension exercise, and to analyze the differences between actual and estimated 1-RM. Specifically, results in our study showed that both bilateral and unilateral individual load-velocity relationships had similar levels of fit to what was previously investigated in different multi-joint exercises, with values of R² higher than 0.93. Generalized loadvelocity relationships showed weaker levels of agreement both in the bilateral and unilateral test (R² = 0.52–0.58) and higher standard errors of the estimate (SEE = 10.3–10.7%1-RM) than those observed when computing individual equations. This is in line with previous research



that showed that individual equations are more appropriate when analyzing the load-velocity relationships in different resistance exercises; thus, in order to calculate more accurate

	Bilateral	Dominant leg	ES (95% CI)	Non-dominant leg	ES (95% CI)
R ²	0.96 ± 0.02	0.94 ± 0.06	-0.49 (-1.23, 0.25)	0.84 ± 0.28	0.68 (-0.12, 1.48)
SEE (%)	3.6 ± 1.19	4.3 ± 2.28	0.43 (-0.34, 1.2)	3.79 ± 2.89	-0.09 (-0.87, 0.68)
Intercept	1.13 ± 0.27	$0.91 \pm 0.10^{*}$	-1.02 (-1.79, -0.23)	$0.82 \pm 0.28^{*}$	1.11, (0.26, 1.94)
Slope	-0.005 ± 0.002	-0.004 ± 0.001	0.52 (-0.22, 1.26)	-0.004 ± 0.002	-0.67 (-1.47, 0.12)
V40 (m/s)	0.90 ± 0.16	$0.73 \pm 0.06^{*}$	-1.31 (-2.10, -0.49)	$0.72 \pm 0.05^{*}$	1.32 (0.43, 2.20)
V70 (m/s)	0.72 ± 0.09	$0.59 \pm 0.05^{*}$	-1.68 (-2.52, -0.82)	$0.58 \pm 0.04^{*}$	1.76 (0.80, 2.69)
V1-RM (m/s)	0.54 ± 0.07	0.44 ± 0.07 *	-1.36 (-2.16, -0.54)	0.43 ± 0.05 *	1.69 (0.77, 2.59)

Table 1. Parameters of the load-velocity relationships in the bilateral and unilateral tests.

 $^{*}p{<}$ 0.05 in comparison with the bilateral test.

SEE = standard error of the estimate; V40 = mean velocity at 40%1-RM; V70 = mean velocity at 70%1-RM; V1-RM = mean velocity at 100%1-RM; ES (95% CI) = effect size of the differences with respect to the bilateral test (with 95% confidence interval)

estimations of the 1-RM by measuring movement velocity, practitioners are encouraged to perform individual load-velocity relationships rather than using generalized equations [3,7,10,18].

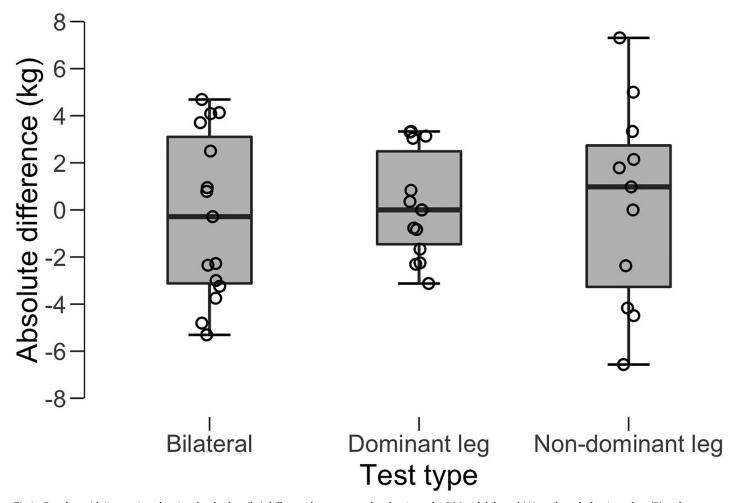


Fig 3. Boxplots with jitter points showing the absolute (kg) difference between actual and estimated 1-RM with bilateral (A), unilateral -dominant leg- (B) and unilateral -non-dominant leg- tests.

Another relevant finding of the present study is that the goodness of fit was not significantly different between the bilateral and unilateral relationships (p > 0.05); however, significant, moderate to high differences were found in several parameters of the load-velocity relationship. Specifically, result in our study showed that unilateral relationships had lower intercepts in the Y-axis and, derived from that, lower mean velocities associated to low (40%1-RM), moderate (70%) and maximum (1-RM) loads. Previous research has observed that upper-body exercises produce lower mean velocities than lower-body exercises [5,6], and it was suggested that this might be due to the smaller muscle groups involved in the movement. Results in our study are in line with those investigations by showing that the unilateral leg extension exercise produces lower mean velocities at each %1-RM than the bilateral leg extension. However, other studies have observed that the levels of force, EMG activity and muscle coordination during a bilateral lower limb explosive contractions are significantly lower in comparison with unilateral efforts [19]. More research is needed to better understand the mechanisms of this bilateral deficit and its role in movement velocity at different loads during the leg extension exercise.

Finally, it was observed that the 1-RM estimated from individual load-velocity relationships was similar to the actual 1-RM, non-significant differences, with trivial to small ES between them (ES < 0.2, p> 0.05). However, given the width and location of the 95% confidence interval of the effect size, these results should be taken with precaution. For example, some studies have shown that V1-RM can remarkably vary between subjects, therefore producing high errors of the estimate in barbell exercises [6,20,21]. Thus, more studies are needed in order to confirm the accuracy of the load-velocity relationship in the leg extension exercise. Taken together, these results show that load-velocity relationships in a single-joint, machine-based exercise computed by measuring the mean vertical velocity of the weight-plates have similar levels of fit to traditional exercises and can provide accurate estimates of the 1-RM. This proof of concept could help researchers and strength and conditioning coaches who wish to study the load-velocity relationships in other popular machine-based exercises by using a simple smartphone application.

Conclusions

There is a high association between load (in terms of %1-RM) and the mean vertical velocity of the weight plates both in the bilateral and unilateral leg extension exercise ($R^2 > 0.93$). Also, it was shown that 1-RM can be accurately estimated by using the load-velocity relationships, as revealed by the small, non-significant differences observed in comparison with the actual 1-RM (ES < 0.2, p > 0.05). This proof of concept proved that load-velocity relationships can be measured in a machine-based, single-joint exercise by video-analyzing the vertical velocity of the weight plates using a smartphone application. To date, load-velocity relationships have been analyzed mostly in barbell exercises [1,5,6]. This study shows an alternative way to analyze the load-velocity relationships in machine-based exercises, like the biceps or hamstring curl, for example.

Author Contributions

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5 Sprint versus isolated eccentric training: Comparative effects on hamstring architecture and performance in soccer players

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Abstract

Aims

The purpose of this study was to compare the effects of hamstring eccentric (NHE) strength training versus sprint training programmed as complements to regular soccer practice, on sprint performance and its mechanical underpinnings, as well as biceps femoris long head (BFIh) architecture.

Methods

In this prospective interventional control study, sprint performance, sprint mechanics and BFIh architecture variables were compared before versus after six weeks of training during the first six preseason weeks, and between three different random match-pair groups of soccer players: "Soccer group" (n = 10), "Nordic group" (n = 12) and "Sprint group" (n = 10).

Results

For sprint performance and mechanics, small to large pre-post improvements were reported in "Sprint group" (except maximal running velocity), whereas only trivial to small negative changes were reported in "Soccer group" and "Nordic group". For BFIh architecture variables, "Sprint" group showed moderate increase in fascicle length compared to smaller augment for the "Nordic" group with trivial changes for "Soccer group". Only "Nordic" group presented small increases at pennation angle.

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Conclusions

The results suggest that sprint training was superior to NHE in order to increase BFIh fascicle length although only the sprint training was able to both provide a preventive stimulus (increase fascicle length) and at the same time improve both sprint performance and mechanics. Further studies with advanced imaging techniques are needed to confirm the validity of the findings.

Introduction

Performing soccer-specific actions at high speed is a paramount physical feature of high-level soccer players. Short accelerations and linear sprints are two of the most important actions in soccer since they frequently precede goals and other decisive actions [1]. On the other hand, the majority of hamstring injuries (57%) occur during high-speed sprinting actions [2]. Specifically, hamstring muscle injuries (HMI) are the most prevalent injuries in soccer accounting for 12–16% of all injuries [3] and have not shown signs of clear decrease over the last three decades. Therefore, it seems logical to expect sprinting to be a key parameter in soccer both from a performance and injury point of view. Both the swing and the stance phase of sprinting, where the hamstring muscles are put under tension while lengthening (eccentric musculotendinous contraction) to decelerate knee extension have been suggested as possible scenarios of injury occurrence [4,5] and have laid the foundations of current prevention methods (eccentric strength training) of HMI in soccer [6].

Research suggests that this type of eccentric training results in a multifactorial adaptative response possibly including increases in motor unit discharge rate and changes in muscle architecture such as hypertrophy and fascicle lengthening [7-9]. Specifically, repeated exposure to lengthening hamstring contractions through the nordic hamstring exercise (NHE) seems to protect muscles from injury in soccer [6]. The main mechanism proposed by the authors is the increase in fascicle length, supposedly induced by an increase in the number of sarcomeres in series, which in turn results in less overall strain and also lower susceptibility to damage [10]. Moreover, Timmins et al. [8] have recently observed such an adaptation after eccentric knee flexor training on an isokinetic dynamometer while also noting that concentric training caused fascicle shortening, despite occurring at long muscle lengths. Furthermore, the same group of authors recently reported that soccer players with shorter biceps femoris long head (BFlh) fascicles (<10.56 cm on average) were at fourfold greater risk of hamstring strain injury than players with longer fascicles [11]. It is however important to keep in mind that clear methodological limitations are associated with ultrasound methodology to infer muscle fascicle lengths [12,13] as will be discussed below. Given the effectiveness of the predominantly eccentric NHE in increasing eccentric hamstring strength when added to soccer training [14], it is important to examine the impact of this single exercise on BFlh fascicle lengths concomitantly to real soccer practice and not only in isolated conditions.

In addition to its potential role in preventing posterior thigh muscle strains, hamstring muscle strength has been suggested as an important factor to improve sprinting performance in soccer as a horizontal force producer [15,16]. During the acceleration phase of sprinting, forward orientation of ground reaction force (GRF) has been shown to be the most powerful determinant of field sprint performance compared to the overall magnitude of vertical or resultant GRF [17]. Recently, Morin et al. [17] have shown that hamstring EMG activity during the swing phase and eccentric knee flexor peak torque were related to the amount of horizontal

GRF produced during treadmill sprint accelerations converting the hamstrings as a key muscular determinant of sprint acceleration performance. These results suggest that the conjunction of hip extensors (hamstrings in particular) torque capability and degree of activation during the swing phase, is a key muscular determinant of sprint acceleration performance [17]. This important role of the posterior thigh muscles during sprinting could partly explain the altered capability to produce horizontal force at low speed during the first meters of the acceleration phase shown by soccer players after return to sport from a hamstring injury [18].

Different types of hamstring-focused strength training have been proposed in the literature to improve sprint performance in soccer players [14,16] but the direct, individual relationship between improvements in single joint hamstring strength and sprint performance and mechanics remains unclear. Interestingly, to date, no study exists about the effect of sprint practice (recently suggested as a potentially preventive method in adequate doses) [19,20], as a complementary training on the muscle architecture of soccer players. The velocity at which an athlete runs once at full speed or close is directly related to the velocity of the lower limb segments during the swing phase, and in turn the negative work done by the hamstrings [3] since they are significant contributors to human propulsion at very high speeds [17,21]. Furthermore, in addition to the significant length-tension sustained by the hamstring muscle-tendon unit during maximal velocity running [22], this specific exercise is the only one, by far, that elicits maximal levels of hamstring activity as assessed by surface electromyography [23,24]. It is expected that a comprehensive sprint training program may induce an overall improvement of sprint performance and underlying mechanical outputs, and BFlh structural adaptations associated to this eccentric-type overload for the muscle-tendon unit, including a greater fascicle length. Given the time constraint of modern soccer training, specific NHE or sprint training might not be systematically implemented [25], raising the question of their respective effectiveness as complementary interventions to the soccer training content.

The aim of this study was to compare the effects of hamstring eccentric (NHE) strength training versus sprint training programmed as complements to regular soccer practice, on sprint performance and its mechanical underpinnings, and BFlh architecture.

Materials and methods

Procedure

In this prospective interventional controlled study, sprint performance, mechanics and BFlh architecture variables were measured before and after six weeks of training during the first six preseason weeks in three different groups of soccer players. The "Soccer group" (controls) continuing their usual soccer practice, the "Nordic group" players performed a NHE program in addition to usual soccer practice, and the "Sprint group" performed a comprehensive sprint acceleration program in addition to usual soccer practice. All subjects were informed of potential risks associated with the experimental procedures before giving their written informed consent to participate and ethics approval was granted by the Faculty of Sports of the University of Porto, Portugal human research ethics committee, which conforms to the ethical standards established by the declaration of Helsinki.

Participants

Soccer players were recruited from two different soccer teams playing in the same Elite Division of Football Association of Porto, North of Portugal. Within each team, players were randomly assigned to the different groups for the study. The possible effect of the training load on the study outcomes were mitigated by having all players proportionally distributed, and both teams following very close training and game programs and physical demands. Soccer teams were initially contacted and informed about the project via email. Inclusion criteria were: 1) to be older than 18 years; 2) to have a competitive experience in soccer for at least 3 consecutive years prior to measurements; and 3) to start the preseason at the scheduled time. Exclusion criteria were: 1) to be involved in any additional strength training program; 2) to present a history of hip, knee, or lumbo-pelvic joints injury in the past three years confirmed by MRI and that required intervention by a health professional, and 3) to suffer a neurological, cardiorespiratory or systemic disorder. 32 soccer players (16±6 per team) were voluntarily recruited and randomly assigned to either "Soccer group" (n = 10), "Nordic group" (n = 12), or "Sprint group" (n = 10). To reduce potential confounding, a match-pair design was used in which athletes were matched depending on their position (i.e., defender, midfield, and forward), playing status (i.e., starting or substitute player), and previous hamstring injury.

Nine players dropped out from the study: two from the "Soccer group" due to retirement form soccer and change to another club; five from the "Nordic group" due to a compliance of <80% to the training program (n = 3), one semitendinosus injury and one ankle injury; and two from the "Sprint group" due to both knee injury and adductor longus tear. All players trained four times per week during 90 minutes and played at least 180 minutes of friendly matches during the preseason period.

Sprint performance and mechanics measurements

After a standardized warm-up, subjects performed two 50 m maximal sprints, separated by 6 min of passive rest, from a standing start on an artificial turf field with their habitual soccer boots. Tests were performed by the same investigator (FC), at the same time of the day (always before of their normal soccer training), under similar environmental conditions of temperature. Each sprint was measured by means of a Radar device with a 46.9 Hz sampling frequency (Stalker ATS II Version 5.0.2.1, Applied Concepts, Dallas, TX, USA), which was placed on a tripod 10 meters behind the subjects at a height of 1 meter corresponding approximately to the height of subjects' center of mass [26,27]. From these speed-time measurements, a macroscopic biomechanical analysis-based on the laws of motion [28] was used to calculate the maximal horizontal external power (P_{max} (W·kg⁻¹)), velocity (v_0 (m·s⁻¹)) and force (F_0 (N·kg⁻¹)) mechanical outputs during the acceleration. In addition, the ratio of force was calculated as the horizontal component of the ground reaction force divided by the resultant ground reaction force, and the maximal value of this ratio $(RF_{max}(\%))$ was used as an indicator of the players ability to orient the ground reaction force in the forward direction at the beginning of their acceleration. The higher the RF_{max} , the more forward the force orientation during the early phase of acceleration. Finally, sprint performance was described via the measurement of 5 m (s) and 20 m (s) times, as derived from the fitted speed-time curves (see [28] for more details) [28].

Assessment of the BFlh architecture

BFlh muscle architectural characteristics has been performed using ultrasound following previously published procedure [29,30]. Muscle thickness (Thickness BFlh), pennation angle (PA) and the estimation of fascile length (FL) were determined from ultrasound images obtained along the longitudinal axis of the muscle belly using a 2D B-mode ultrasound (12 Mhz frequency, 8 cm depth; 14 x 47 mm field of view) (GE Healthcare Logiq S7, Wauwatosa, USA). The measurement site was the halfway point between the ischial tuberosity and the posterior knee joint fold, along the line of the BFlh. Once the scanning site was determined in each participant, several anatomical landmarks were taken (ischial tuberosity, fibula head and midpoint of the posterior knee joint fold) and photographs were taken in order to ensure reproducibility for future assessment sessions. All architectural measurements were performed after at least 5 minutes of inactivity, with the participant in prone position, with the hip in neutral position, and the knee positioned passively in full extension with their feet laying off the bed for comfort and they were instructed to remain relaxed during image acquisition. To obtain the images, the transducer was then aligned to the fascicle plane, which was assumed to correspond to the image with the most continuous and visible muscle fascicles (~25% or more of the total estimated length as a minimum) while the superficial and intermediate aponeuroses remained parallel (less than 4° between aponeuroses angle) in order to meet the stablished inclusion criteria [30]. For all scans, the probe was handled carefully by the sonographer (MF) and transmission gel was used to improve the acoustic contact and to keep the transducer pressure on the skin to a minimum [31].

After the scan, an analysis was carried out off line by means of a custom-made image processing routine developed in Matlab 2016a software, (The Mathworks, Inc., Natick, 2016). Fascicle length was measured by manually outlining visible parts of muscle fascicles and the sections that were not visible were extrapolated linearly to the linearly projected line of the aponeurosis [29] (see Fig 1). The angle between the line marking the intermediate aponeurosis and the outlined fascicle was the measured, giving the PA. MT was measured as the distance between the superficial and intermediate aponeuroses.

All images were collected and analyzed by the same researcher (MF), who was blinded to the identity and group of the participants during the analysis.

Nordic exercise program

The NHE program was performed during six weeks only in the "Nordic group". The NHE and the program was the same as the one proposed by Petersen et al. [6], but only completed over 6 weeks instead of the 10 weeks proposed in the original study. The exercise was conducted always after regular training sessions and players were supervised by their physical coach, who was informed about the exercise orally and had received written descriptions and illustrations of the exercise. Training program compliance and adverse effects were registered for each team on a weekly basis by contact with the coaches during the 6-week training period. A minimum of 48 h separated each training session. All training sessions were supervised by either researchers, physiotherapists or coaches.

Sprint training program

The sprinting program was performed only by players assigned to the "Sprint group" during six weeks with two sessions of exercises per week separated by at least 72 hours (Table 1). Each session lasted about 30–35 min and the team head coaches decided when exactly the program was performed within the training session but they were advised to follow sprint training after a proper warm-up program. The first session of the week included sprint running exercises aiming at stimulating the entire force-velocity spectrum: normal sprint accelerations (5x30 m to 3x30 m), heavy sled sprints (sled load of 70% of body mass) from 1x10 m to 3x10 m, and 4x20 m (with 20 m run-up distance), and flying start sprints. The second session of the week included some ankle plantar flexors exercises with added load (from 50 to 70% body mass), unilateral bouncing exercises, plyometrics, and various athletics drills and acceleration exercises over short distances. All details are provided in Table 1.

Soccer training program

The soccer training program was performed by players assigned to all three groups during six weeks with four sessions of soccer training per week. Each session lasted about 90 minutes

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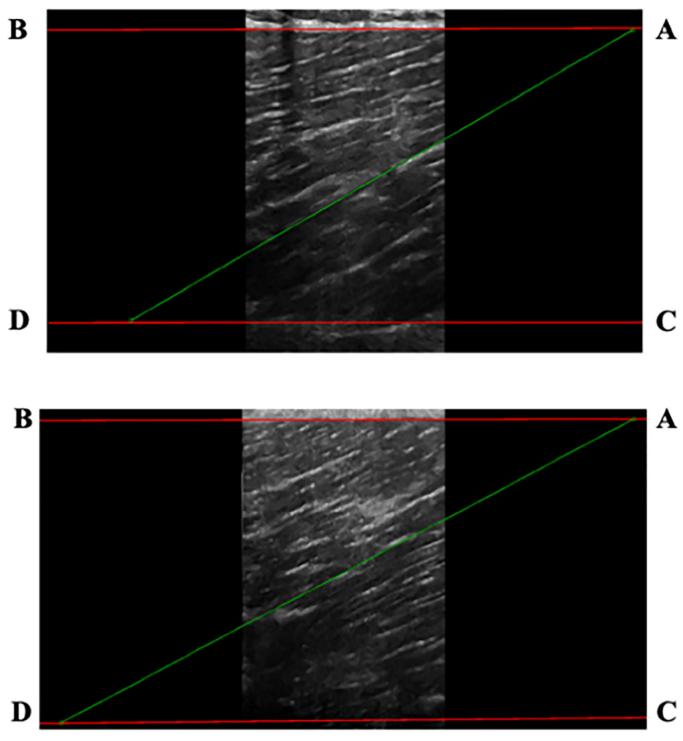


Fig 1. Two-dimensional ultrasound image of the pre-post intervention of Biceps Femoris long head (BFlh). In order to measure FL and PA, a line was placed along the length of a fascicle, which joined superficial aponeurosis (A-B line) and intermediate aponeurosis (C-D line). The FL was calculated as the length of this line. The PA was calculated as the angle between lines CD and DA.

including a warm-up, tactical work carried out with different types of possessions and small side games and ending with stretches of main muscle groups such as quadriceps, hamstring,

				Weeks							
	1	2	3	4	5	6	7				
FVP Exercises	First training session of the week										
	Very Heavy Sled (70% BW) 1x10-m	Very Heavy Sled (70% BW) 1x10-m	Very Heavy Sled (70% BW) 2x10-m	Very Heavy Sled (70% BW) 2x10-m	Very Heavy Sled (70% BW) 3x10-m	Very Heavy Sled (70% BW) 3x10-m	Very Heavy Sled (70% BW) 4x10-m				
	Sprint 5 x 30-m	Sprint 5 x 30-m	Sprint 4 x 30-m	Sprint 4 x 30-m	Sprint 3 x 30-m	Sprint 3 x 30-m	Sprint 2 x 30-m				
	Flying Run 4 x 20-m (20-m preparation)	Flying Run 4 x 20-m (20-m preparation)	Flying Run 4 x 20-m (20-m preparation)	Flying Run 4 x 20-m (20-m preparation)							
Gastro			Sec	cond training session o	of the week						
Exercises	Gastrocnemius Extensions (50% BW) 2x6	Gastrocnemius Extensions (50% BW) 2x6	Gastrocnemius Extensions (60% BW) 3x6	Gastrocnemius Extensions (60% BW) 3x6	Gastrocnemius Extensions (70% BW) 2x6	Gastrocnemius Extensions (70% BW) 2x6	Gastrocnemius Extensions (70% BW) 2x6				
	Gastrocnemius Rebounds (30% BW) 2x6	Gastrocnemius Rebounds (30% BW) 2x6	Gastrocnemius Rebounds (40% BW) 3x6	Gastrocnemius Rebounds (40% BW) 3x6	Gastrocnemius Extensions Unilateral (30% BW) 1x6	Gastrocnemius Extensions Unilateral (30% BW) 2x6	Gastrocnemius Extensions Unilateral (30% BW) 2x6				
					Gastrocnemius Rebounds (50% BW) 3x6	Gastrocnemius Rebounds (50% BW) 2x6	Gastrocnemius Rebounds (50% BW) 2x6				
					Gastrocnemius Rebounds Unilateral (10–20% BW) 1x6	Gastrocnemius Rebounds Unilateral (10–20% BW) 2x6	Gastrocnemius Rebounds Unilateral (10–20% BW) 2x6				
Acceleration			Sec	cond training session o	of the week						
Exercises	Wall Acceleration Drill (2 steps) 3 x 5	Wall Acceleration Drill (2 steps) 3 x 6	Wall Acceleration Drill (4 steps) 2 x 6	Wall Acceleration Drill (4 steps) 2 x 7	Wall Acceleration Drill (4 steps) 2 x 8	Wall Acceleration Drill (4 steps) 2 x 6	Wall Acceleration Drill (2 steps) 3 x 5				
	Free Sprint (10 m) 2 x 5	Free Sprint (10 m) 2 x7	Free Sprint (5 m) 2 x 4	10-m Weighted Sled Towing (15% BW) + 10-m Free Sprint 2 x 2	15-m Weighted Sled Towing (15% BW) + 10-m Free Sprint 2 x 2	15-m Weighted Sled Towing (15% BW) + 10-m Free Sprint 2 x 3	10-m Weighted Sled Towing (15% BW) + 10-m Free Sprint 2 x 2				
		Free Sprint (20 m) 1 x 4	Free Sprint (10 m) 1 x 2	Free Sprint (15 m) 1 x 2	Free Sprint (10 m) 1x 2	Free Sprint (5 m) 2 x 4	Free Sprint (10 m) 1 x 4				
		Alternate leg bounding (20m) 2x2	Free Sprint (15 m) 1 x 2	Alternate leg bounding(20m) 2x3		Alternate leg bounding (20m) 2x3					

Table 1. Training contents for the sprint training group.

hip flexors and calves. Two times per week, 15 to 20 minutes of aerobic capacity sessions were included. The training was supervised by the same experimenter (TP) and no additional strength or sprinting workout was allowed outside of the soccer practice established in each of the programs.

Statistical analysis

All data are presented as mean \pm standard deviation. In order to clearly assess the practical meaning of the results, data were analysed using the magnitude-based inference approach [32]. Changes in athlete scores were evaluated using effect sizes (ES) and 90% confidence limits. Within-group difference in pre and post-training of mechanical sprint properties and fascicle variables were assessed using standardised effect size (ES). The magnitude of the withingroup changes was interpreted by using values of trivial (< 0.20), small (0.20 -< 0.60), moderate (0.60 -< 1.20), large (1.20 -< 2.00) and extremely large of the between-athlete variation at pre (i.e. smallest worthwhile change SWC). The probability that these differences actually exist

was then assessed via magnitude-based qualitative inferences [33]. Qualitative inferences were based on quantitative chances of benefit outlined in [34]. Clinical chances are percentage chances that an observed effect is clinically positive/trivial/negative e.g. (40/40/20%) means an effect has 40% of chances to be positive, 40% to be trivial and 20% to be negative. Two separate statistical methods were used to assess the effectiveness of each method of training. To estimate inter-day reliability, intraclass correlation coefficient (ICC) and their 95% confidence intervals were calculated for variables related to biceps femoris architecture. Standar Error of Measurement (SEM) was calculated as the root mean square of total mean-square intrasubject variation. Pre- post-analysis was performed on each group's data, to provide a clear effect of whether there were substantial and clear changes as a result of the training intervention. A second parallel group trials assessment compared the interventions. Probabilities that differences were higher than, lower than, or similar to the smallest worthwhile difference were evaluated qualitatively as possibly, 25% to 74.9%; likely, 75% to 94.9%, very likely, 95% to 99.5%; and most (extremely) likely, >99.5%.

Since the findings of present study could be used for athletes considered in isolation, individual analyses were performed to quantify for each variable and each group the number of responders and non-responders. Monitoring progression of an athlete with performance requires taking into account the magnitude of the SWC in performance and the uncertainty or noise in the test result [34], SWC being computed as one-fifth of the between-athlete standard deviation (a standardized or Cohen effect size of 0.20 [35]. Individual training responses were then considered as decrease (individual change < -1 SWC), trivial (from -1 SWC to +1 SWC) or increase (+1 SWC) for each variable if interest.

Results

Mean \pm SD values for all sprint performance and mechanical variables pre- and post-training intervention are shown for all groups in Table 2, along with within-group changes qualitative inferences (Table 3). Substantial differences were found in mechanical sprint variables in the "sprint group" with small to large changes with a *very likely* inference for all the mechanical outputs except for v₀ (*possibly* inference). Contrastingly, these changes were less clear in "Nordic" and "soccer" groups, with small to trivial changes and *possibly* and *likely* inference post-training intervention.

The "sprint group" showed a small increase in v_0 (*possibly*) and RF_{max} (*very likely*), moderate in F₀ (*very likely*), 5 m and 20 m times (*very likely*, respectively), and large in Pmax (*very likely*), whereas the "nordic group" showed changes ranging between small and very large increase interaction (Table 3). Trivial and unclear changes were observed in most of the sprint mechanical variables analyzed between "sprint group" and "soccer group" except for v_0 and Pmax with small and moderate changes observed. Similar changes were observed between "Nordic" and "soccer" groups with small changes in v_0 , RF_{max} and 20 m time with *possibly* to *likely* inference, and moderate changes in P_{max} and 5 m time with *likely* inference, meanwhile a large change was observed in F₀ with *likely* inference (Table 3, Fig 2).

Furthermore, variables related to biceps femoris architecture such as fascicle length, pennation angle and muscle thickness pre- and post-training intervention are shown for all groups in <u>Table 4</u>, along with within-group changes qualitative inferences (<u>Table 5</u>). The ICC for variables related to biceps femoris architecture were 0.989 (0.959–0.998) for fascicle length, 0.964 (0.865–0.993) for pennation angle and 0.981 (0.929–0.996) for muscle thickness. SEM ranged from 1.68% to 2.83%. ICC was calculated with 7 sport sciences students in similar conditions (as described in Material and Methods section; 5 min rest and lying down before the measurement) separated by 24 hours.

	NORDIC GROUP $(n = 7)$					
	Pre	Post	Post	t—Pre	Inference	
	$\bar{x} \pm SD$	$\bar{\mathbf{x}} \pm \mathbf{SD}$	$\Delta \pm SD$	ES; ±90% CL		Individual Response Increase/Trivial/Decrease
$v_0 (m \cdot s^{-1})$	9.05 ± 0.24	9.04 ± 0.38	-0.11 ± 1.89	-0.03 ± 0.47	Trivial * (neutral)	1-2-4
F_0 (N·kg ⁻¹)	6.85 ± 0.44	6.66 ± 0.28	-2.76 ± 3.99	-0.40 ± 0.45	Small ^{**} (negative)	1-2-4
P_{max} (W·kg ⁻¹)	15.4 ± 1.0	14.9 ± 0.6	-2.77 ± 4.43	-0.39 ± 0.46	Small ^{**} (negative)	1-3-3
RF_{\max} (%)	46.9 ± 4.0	46.8 ± 2.3	-0.27 ± 5.13	-0.06 ± 0.60	Trivial * (neutral)	3-1-3
5 m (s)	1.42 ± 0.04	1.43 ± 0.03	1.02 ± 1.76	0.32 ± 0.41	Small [*] (positive)	1-1-5
20 m (s)	3.47 ± 0.07	3.50 ± 0.05	0.87 ± 1.59	0.35 ± 0.48	Small * (positive)	2-1-4
		SPRINT G	ROUP $(n = 8)$			
$v_0 (m \cdot s^{-1})$	8.91 ± 0.53	9.04 ± 0.55	1.46 ± 1.49	0.22 ± 0.16	Small * (positive)	5-3-0
F_0 (N·kg ⁻¹)	6.49 ± 0.57	6.97 ± 0.63	7.42 ± 3.20	0.75 ± 0.22	Moderate*** (positive)	8-0-0
$P_{\max} (W \cdot kg^{-1})$	14.4 ± 1.8	15.7 ± 1.8	8.93 ± 3.16	0.64 ± 0.15	Moderate*** (positive)	8-0-0
RF_{\max} (%)	44.3 ± 2.4	47.7 ± 2.5	7.80 ± 4.56	1.27 ± 0.49	Large*** (positive)	8-0-0
5 m (s)	1.46 ± 0.06	1.41 ± 0.06	-3.40 ± 1.43	-0.77 ± 0.22	Moderate*** (negative)	8-0-0
20 m (s)	3.55 ± 0.14	3.46 ± 0.14	-2.59 ± 1.05	-0.58 ± 0.16	Small *** (negative)	8-0-0
		SOCCER C	GROUP(n=8)			
$v_0 (\mathbf{m} \cdot \mathbf{s}^{-1})$	8.95 ± 0.36	8.87 ± 0.38	-0.85 ± 2.12	-0.19 ± 0.30	Trivial * (neutral)	3-1-4
F_0 (N·kg ⁻¹)	6.90 ± 0.79	7.01 ± 0.57	2.02 ± 5.38	0.12 ± 0.22	Trivial * (neutral)	3-4-1
$P_{\max} (W \cdot kg^{-1})$	15.3 ± 1.8	15.4 ± 1.4	1.08 ± 4.65	0.05 ± 0.21	Trivial ** (neutral)	1-6-1
RF_{\max} (%)	46.2 ± 4.6	48.0 ± 2.5	4.43 ± 7.07	0.35 ± 0.37	Small ** (positive)	6-0-2
5 m (s)	1.42 ± 0.07	1.41 ± 0.05	-0.83 ± 2.20	-0.16 ± 0.27	Trivial * (negative)	5-0-3
20 m (s)	3.48 ± 0.14	3.48 ± 0.10	-0.18 ± 1.41	-0.05 ± 0.22	Trivial** (positive)	3-1-4

Table 2. Sprint performance and mechanical output variables pre and post training for the control and intervention groups.

Values are mean \pm standard deviation, percent change \pm standard deviation and standardised effect size; \pm 90% confidence limits. Abbreviations: *n*, sample size; \bar{x} , mean; SD, standard deviation, % Δ , percent change; ES, effect size; 90% CL, 90% confidence limits; kg, kilogramme; ν_0 , theoretical maximal velocity; m, metre; s, second; F_0 , theoretical maximal horizontal force; N, newton; P_{max} , maximal power output; W, watt; RF_{max} , maximal ratio of force after 0.3 seconds. Qualitative inferences are trivial (< 0.20), small (0.20 -< 0.60), moderate (0.60 -< 1.20) and large (> 1.20)

* possibly, 25 -< 75

** likely, 75 -< 95%

*** very likely, 95 – < 99.5. Positive, neutral and negative descriptors qualitatively describe the change between post and pre values and its importance relative to the specific variable.

Substantial changes were observed in fascicle length, pennation angle and muscle thickness for "nordic group" with a *possibly* and *likely* inference after training intervention. Similarly, to "nordic group", the "sprint group" showed substantial changes in fascicle length and muscle thickness with moderate changes with a *likely* and *very likely* inference after training intervention, whereas a trivial effect was observed for pennation angle in this group. Finally, in the "soccer group", trivial changes were reported with a *possibly and likely* inference after the training intervention for all variables.

The between-group comparison showed that the "sprint group" had a *likely* moderate (fascicle length), a *possibly* trivial (pennation angle) and *likely* small (muscle thickness) changes in biceps femoris architectural variables in comparison with "nordic group" (Table 5, Fig 3). Similarly, *most likely* large (fascicle length), a *likely* moderate (pennation angle) and *very likely* moderate (muscle thickness) changes were found between "sprint" and "soccer" groups. "Nordic group" showed a *possibly* small change (fascicle length), a *likely* moderate (pennation angle) and a *likely* small change (muscle thickness) compared to soccer group (Table 5).

	Post – p	re group change	Post – j	ore group change	Post – pre group change		
	Sprint Group-Soccer group		Sprint Gr	oup-Nordic group	Nordic Group-Soccer group		
	ES; ±90% CL	Inference	ES; ±90% CL	Inference	ES; ±90% CL	Inference	
$\overline{\nu_0 (\mathbf{m} \cdot \mathbf{s}^{-1})}$	0.76 ± 0.96	Moderate ^{**} (positive)	0.26 ± 1.11	Small [*] (positive)	-0.38 ± 0.77	Small* (negative)	
F_0 (N·kg ⁻¹)	0.07 ± 0.78	Trivial * (positive)	1.18 ± 1.38	Moderate ^{**} (positive)	1.33 ± 1.19	Large** (positive)	
$P_{\rm max} ({\rm W} \cdot {\rm kg}^{-1})$	0.41 ± 0.98	Moderate** (positive)	1.53 ± 1.40	Large** (positive)	0.97 ± 1.50	Moderate** (positive)	
RF _{max} (%)	0.01 ± 1.01	Trivial * (positive)	0.38 ± 0.90	Small [*] (positive)	0.50 ± 0.85	Small [*] (positive)	
5 m (s)	0.11 ± 0.21	Trivial ** (neutral)	-1.10 ± 1.06	<i>Moderate</i> ^{**} (negative)	-0.99 ± 1.11	<i>Moderate</i> ^{**} (negative)	
20 m (s)	-0.41 ± 1.01	Small * (negative)	-1.08 ± 1.26	<i>Moderate</i> ** (negative)	-0.55 ± 1.40	Small* (negative)	

Table 3. Sprint performance and mechanical variables comparisons of between-group post-pre changes.

Values are mean \pm standard deviation, percent change \pm standard deviation and standardised effect size; \pm 90% confidence limits. Abbreviations: *n*, sample size; \bar{x} , mean; SD, standard deviation, % Δ , percent change; ES, effect size; 90% CL, 90% confidence limits; kg, kilogramme; ν_0 , theoretical maximal velocity; m, metre; s, second; F_0 , theoretical maximal force; N, newton; P_{max} , maximal power output; W, watt; RF_{max} , maximal ratio of force after 0.3 seconds. Qualitative inferences are trivial (< 0.20), small (0.20 -< 0.60), moderate (0.60 -< 1.20) and large (> 1.20)

 * possibly, 25 –< 75

** likely, 75 – < 95%. Positive, neutral and negative descriptors qualitatively describe the change between post and pre values and its importance relative to the specific variable.

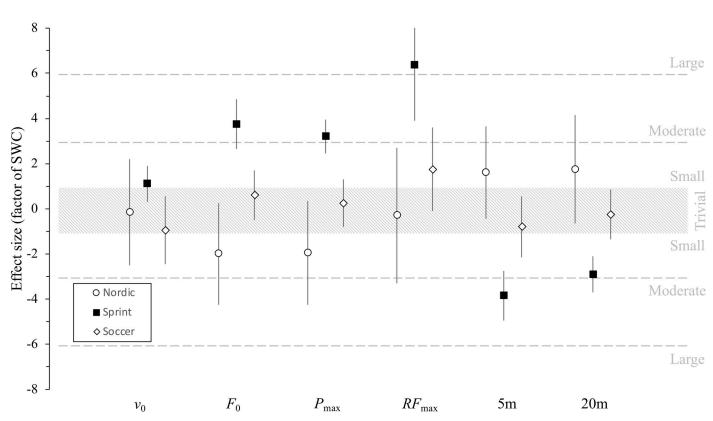


Fig 2. Magnitude of pre-post changes in the main sprint acceleration performance and mechanical outputs. The standardised differences are expressed as a factor of the smallest worthwhile change (SWC). Bars indicate the 90% confidence limits. v0: theoretical maximal velocity; F0: theoretical maximal horizontal force; Pmax: maximal power output; RFmax: maximal ratio of force; 5 m: 5 m sprint time; 20 m: 20 m sprint time.

	NORDIC GROUP (n = 7)					
	Pre	Post	Post—Pre		Inference	
	$\bar{\mathbf{x}} \pm \mathbf{SD}$	$\bar{\mathbf{x}} \pm \mathbf{SD}$	$\%\Delta \pm SD$	ES; ±90% CL		Individual Response Beneficial/Trivial/Harmful
FL-2legs Mean (cm)	9.93 ± 1.10	10.66 ± 1.01	7.38 ± 4.03	0.58 ± 0.33	Small ^{**} (positive)	5-1-1
PA-2legs Mean (°)	13.29 ± 2.61	14.52 ± 1.84	9.24 ± 8.60	0.41 ± 0.62	Small * (positive)	4-2-1
Thickness BFlh 2legs (cm)	2.28 ± 0.22	2.40 ± 0.19	5.04 ± 2.11	0.46 ± 0.40	Small **(positive)	4-1-2
	SPRINT GROUP $(n = 8)$					
FL-2legs Mean (cm)	10.23 ± 1.91	11.89 ± 1.16	16.21 ± 10.26	0.77 ± 0.67	<i>Moderate</i> ^{**} (positive)	7-0-1
PA-2legs Mean (°)	14.19 ± 1.82	14.26 ± 1.57	0.49 ± 2.47	0.03 ± 0.34	Trivial * (neutral)	4-2-2
Thickness BFlh 2legs (cm)	2.39 ± 0.16	2.52 ± 0.09	5.80 ± 2.11	0.76 ± 0.47	Moderate*** (positive	5-3-0
		SOCCER G	ROUP $(n = 8)$			
FL-2legs Mean (cm)	10.20 ± 1.08	10.17 ± 0.82	0.31 ± 1.69	-0.03 ± 0.26	Trivial ** (negative)	1-2-5
PA-2legs Mean (°)	12.47 ± 1.60	12.61 ± 1.60	1.12 ± 3.00	0.08 ± 0.34	Trivial * (positive)	2-4-2
Thickness BFlh 2legs (cm)	2.22 ± 0.23	2.25 ± 0.21	1.43 ± 1.98	0.12 ± 0.31	Trivial *(positive)	2-4-2

Table 4. BFlh muscle architectural variables pre and post training for the control and intervention groups.

Values are mean \pm standard deviation, percent change \pm standard deviation and standardised effect size; \pm 90% confidence limits. Abbreviations: *n*, sample size; \bar{x} , mean; SD, standard deviation, % Δ , percent change; ES, effect size; 90% CL, 90% confidence limits; FL-2legs Mean, fascial length mean for right and left legs; PA-2legs Mean, pennation angle mean for right and left legs; Thickness BFlh 2 legs, Thickness BFlh mean for right and left leg. Qualitative inferences are trivial (< 0.20), small (0.20 -< 0.60) and moderate (0.60 -< 1.20)

* possibly, 25 -< 75

** likely, 75 -< 95%

*** very likely, 95-<99.5. Positive, neutral and negative descriptors qualitatively describe the change between post and pre-values and its importance relative to the specific variable.

Discussion

The aim of this study was to compare the effects of two 6-week training programs added to the normal soccer training: eccentric hamstring strength using the nordic hamstring exercise versus a comprehensive sprint training program, on biceps femoris long head architecture, sprint

Table 5. BFlh muscle architectural variables between-grou	ps comparisons of post-pre changes.
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	Post – p	ore group change	Post – p	re group change	Post – pre group change		
	Sprint Group-Soccer group		Sprint Gro	oup–Nordic group	Nordic Group-Soccer group		
	ES; ±90% CL	Inference	ES; ±90% CL	Inference	ES; ±90% CL	Inference	
FL-2legs Mean (cm)	2.13 ± 0.96	Large**** (positive)	1.16 ± 1.18	Moderate** (positive)	-0.26 ± 0.91	Small [*] (negative)	
PA-2legs Mean (°)	1.11 ± 0.94	Moderate** (positive)	-0.11 ± 0.99	Trivial * (negative)	-0.86 ± 0.97	<i>Moderate</i> ** (negative)	
Thickness BFlh 2legs (cm)	1.14 ± 0.74	Moderate*** (positive)	0.55 ± 0.46	Small ^{**} (positive)	-0.57 ± 0.98	Smal* l* (negative)	

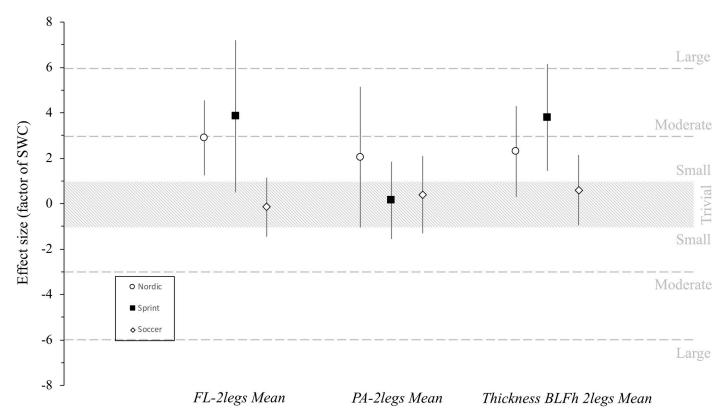
Values are mean \pm standard deviation, percent change \pm standard deviation and standardised effect size; \pm 90% confidence limits. Abbreviations: *n*, sample size; \bar{x} , mean; SD, standard deviation, % Δ , percent change; ES, effect size; 90% CL, 90% confidence limits; FL-2legs Mean, fascial length mean for right and left legs; PA-2legs Mean, pennation angle mean for right and left legs; Thickness BFlh 2 legs, Thickness Biceps Long Femoris Head mean for right and left leg. Qualitative inferences are trivial (< 0.20), small (0.20 -< 0.60), moderate (0.60 -< 1.20) and large (>1.20)

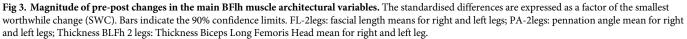
* possibly, 25 -< 75

** likely, 75 -< 95%

*** very likely, 95-<99.5

**** most likely, >99.5. Positive, neutral and negative descriptors qualitatively describe the change between post and pre values and its importance relative to the specific variable.





acceleration performance mechanical outputs. The main findings were (a) the addition of two weekly sessions of sprint training to regular soccer practice induced moderate improvements in biceps femoris long head fascicle length compared to the small increases showed after iso-lated eccentric strength training or not changes when practicing soccer training alone; (b) biceps femoris muscle pennation angle showed a small increase only when nordic hamstring exercise was added to soccer training, and not in the case of sprint training; (c) sprinting training added to regular soccer training produced small to large improvements in both sprint acceleration performance and the underlying mechanical outputs (except maximal running velocity), which contrasts with trivial or even small negative changes in the case of hamstring eccentric strength training or when practicing soccer training.

This study is the first to explore the architectural and morphological adaptations of the hamstrings in response to isolated knee flexor eccentric strength training versus sprint training programmed in addition to regular soccer training during pre-season, and not in isolated conditions without concomitant sport practice [8,11,36,37]. We think this point is an important feature of the current study, since it is more in line with the real sport practice, where soccer players train for soccer first, and any type of intervention is added to the basic sport practice. By definition, any complementary intervention comes in addition to the main sport practice, so it should not be studied separately. It is important to keep this more realistic scenario in mind when discussing the results of this study and previous works, since it may influence the practical conclusions of these works.

Sprint and eccentric exercises induce changes in BFlh fascicle length

Collectively, although our results suggest that both types of training added to soccer practice induced increases in the length of the fascicle BFlh, the sprint showed moderately superior adaptations (16%) compared to the NHE (7%). These adaptations may result from the addition of in-series sarcomeres [10]. It has been proposed that this increase in serial sarcomeres is associated with both a rightward shift in a muscle's force-length relationship, while also reducing its susceptibility to damage associated with strain [10]. However, fascicle lengthening due to increases in tendon stiffness is one possible alternative explanation [38]. Due to the limitations of the static method used in the present and previous studies[12,13], further research is clearly needed to fully understand the mechanism(s) responsible for these architectural changes (muscle-tendon interaction) and validate the suggested hypotheses in dynamic (not only isolated, static) actions.

The greater increase of BFlh FL observed after a comprehensive sprinting programme compared to NHE could be related to the continuous and increased intensity lengthening of the muscle-tendon unit induced by high-speed movements (for exercises targeting the velocity end of the sprint force-velocity spectrum) and sprint-specific strength overload (for exercises targeting the force-end of the spectrum). Similarly, studies on the architecture of other muscle groups, such as the vastus lateralis reported a significant greater increase in fascicle length without pennation angle changes (as in the present study) after a period of sprint/jump training alone compared to those who added different resistance training programs. [39]. Recently, sprint training has been shown effective for improving eccentric hamstring strength in adolescent athletes, in addition to the positive effects on sprint performance [40]. These arguments, among others, may partly explain recent preliminary research suggesting a protective effect of an adequate exposure to maximum velocity sprint efforts in different soccer codes that would place sprinting itself as part of a comprehensive strategy (including other evidenced strategies) to prevent soft-tissue injuries [41,42].

The observed increase in BFlh fiber length at rest after NHE was added to regular soccer training in the present study (7%) is similar to the results found in a group of elite young French soccer players following an eccentric-biased hamstring training program (~ 5%) [43] but considerably lower than the increases (between 16–32%) observed in other studies after 6-12 weeks of various strength training modalities (leg curl, isokinetic dynamometry and NHE, respectively) focused on knee flexor eccentric overload [11,36,37]. Even that architectural variables vary considerably between individuals, which may explain that the average changes in architectural variables differ between studies, the ultrasonography approach used [11,36,37,44] to extrapolate the changes in FL may also justify the large difference in FL changes observed between the current study and previous ones [11,36,37,44]. The static-image sonographic technique used to estimate the heterogeneous and non-uniform biceps femoris fascicle architecture presents clear limitations [12,13] that could affect the results obtained in studies, including the current one. Nonetheless, the method used in this study, manual linear extrapolation, has recently been recommended due to a lower fascicle estimation and greater accuracy with respect to trigonometric equation methods used in other studies if only conventional ultrasound imaging is available [30]. Thus, on this specific point, the present results, as those of previous studies, should be taken with caution until more research on the improvement of ultrasonographic approach for fascicle length measurement is available.

The discrepancy in results could also be related to the possibility that, in contrast with the more realistic training integrated to the usual soccer practice proposed in the present study [43,45], other studies included isolated eccentric training contractions targeting an increase in fascicle length, but not concomitant with the usual soccer practice, and the associated specific

movements patterns [11,36,37]. Based on the fact that soccer training alone induced increases in posterior thigh muscles concentric strength (but not eccentric) [14] and that different studies reported a decrease in fascicle length after concentric training [8,9], the present results suggest that soccer practice may mitigate the increase in FL associated with isolated eccentric contractions when these two interventions are programmed together, which is almost always the case in real training contexts.

Fascicle lengthening is one possible mechanism by which the NHE and other eccentric or long-length hamstring exercises have been proven effective on hamstring injury reduction. Timmins et al. [11] recently showed, prospectively, that professional soccer players with average BFlh fascicle length <10.56 cm were ~ 4 times more likely to suffer a hamstring strain than athletes with longer fascicle length and that the probability of injury was overall estimated to decrease by $\sim 74\%$ on average for every 0.5 cm increase in fascicle length. In the current study, we observed increases in BFlh fascicle length of ~ 0.7 cm after Nordic and ~ 1.6 cm after Sprint training programs added to the regular soccer practice. This would, according to the proportions described earlier, likely result in a higher reduction in hamstring injury risk after Sprint than Nordic interventions. However, although theoretically longer fascicles may enhance sprint performance by altering the operating range of muscles and increasing muscle force-generating capacity, only the Sprint intervention induced clear improvements in sprint performance and mechanics, which was not the case of the Nordic training program, as discussed below.

Regarding BFlh pennation angle, this study confirms previous findings by Lovell et al. [45] showing that the addition of knee flexor eccentric training through NHE post soccer training resulted in a similar hypertrophic response, identified by an increased pennation angle and muscle thickness (PA ~10%). This increase is considered to represent an increase of the physiological cross-sectional area with more myofibrils in parallel, enabling the improved transmission of force developed through the muscle-tendon unit, and in turn a higher architectural gear ratio [7,46]. The latter will allow the pennate biceps femoris to limit the strain experienced by active fascicles and provide some degree of protection during fast-velocity lengthening actions [46]. Mechanical tension and intramuscular metabolic stress, determine the hypertrophic signal of the muscle that may be amplified (as in this study) when resistance training was performed following high-intensity interval and endurance training, which has also been shown to trigger anabolic signaling pathways and hypertrophy [7].

Greater improvements of sprint performance and mechanics after sprint training

Concerning the sprint mechanics and performance outcomes, the ability to produce high acceleration and speed is considered an important quality for performance in soccer [1]. Although hamstrings play a role in the forward orientation of the ground reaction force, especially at high running speed (when their torque capability and electrical activity are both considered), the results of this study showed no benefits (and even small negative changes) of a NHE force program added to regular soccer practice on sprint mechanical outputs and performance. This is consistent with previous results [14], and contrasts with two very similar recent studies reporting small to moderate improvements (with high inter-individual variability) in sprint performance after NHE training in a group of soccer players [15,16]. Although sprint acceleration mechanical properties were not analyzed in these studies [15,16], the time of realization of the program (pre-season versus in-season), and match and training high running speed exposure, internal and external training load may explain the differences between studies. Ishøi et al. [15] reported concomitant average group changes, but not the direct

association, on an individual basis, between improvements in knee flexors strength and sprint performance. Furthermore, previous studies [14] reported substantial increases in hamstring strength with no or minimal concomitant changes in sprint acceleration mechanical outputs and performance. These two facts clearly question the idea that isolated posterior thigh strengthening alone (using for example the NHE modality) directly results in the improvement of such a complex neuromuscular task as maximal sprint acceleration, despite the fact that hamstring muscles play a role, especially when taking their sprint-specific activity as derived from electromyographical analysis [17]. This is not surprising given the large differences in the timing, velocity, length, hip-knee kinematics and overall intra- and inter-muscles coordination between any isolated hamstring strengthening exercise (a fortiori a single-joint, bilateral one like the NHE) and the sprint acceleration tasks.

In contrast, the specific comprehensive sprint program used in the present study resulted in moderate and very likely improvements on both sprint mechanics (mainly acceleration variables) and performance after 6 weeks during soccer preseason where the total workload is greater than in-season [47], comparatively favoring the increase of aerobic fitness to the detriment of anaerobic capacity [48]. Specifically, this program (likely due to the use of resisted and unresisted sprints) resulted in higher maximal force output in the horizontal direction (F_0 i.e. first meters of the acceleration phase), also evidenced by a greater RFmax. This specific variable of the sprint force-velocity profile, which was shown to markedly decrease after hamstring injury [18], increased here substantially in the Sprint group, whereas only a small increase was observed in the ability to produce horizontal force at high speeds (v_0) . The results of the Sprint group are similar to what Morin et al. [49] reported in a pilot study using very heavy sled resistance training in soccer players: specific F_0 and RF Max improvements with only trivial effect on v₀, and only trivial changes in the control group who performed unresisted sprints. Interestingly, this brings support to the use of a comprehensive sprint training that (a) covers/stimulate a large spectrum of force and velocity conditions (ranging from heavy resistance to flying start sprints) and (b) uses acceleration athletics drills and horizontal plyometric exercises. We think that this represents a more comprehensive (thus potentially more effective) overload than using unresisted sprints alone. Furthermore, soccer practice itself already includes unresisted, "classical" sprints, by definition. Our results suggest that this type of multifaceted sprint-specific program is effective to counteract the decrease in F_0 observed after soccer preseason [50] or hamstring injury [18]. Although it is still a hypothesis, a reverse thinking would make this increase in maximal sprint force output potentially beneficial in the return-to-performance, or even prevention process. This should be the topic of future studies, but it makes sense in light of the data previously published on the topic and the current results.

Some limitations associated with this manuscript should be acknowledged. No collection of match and training exposure, internal and external training load variables was performed during the study. Although all these variables were likely very similar among players, they are possibly confounding factors of fascicle length adaptation. With that being said, the present study is, to our knowledge, the first to integrate and compare, within the same randomized controlled protocol, a commonly used injury prevention method (using an isolated, single-joint hamstring strength exercise, NHE), and a comprehensive training program specifically targeting sprinting performance (through stimulation of the entire force-velocity spectrum) in a realistic soccer practice context.

Secondly, the use of two-dimensional ultrasound to estimate fascicle length, although previously validated against cadaveric measurements [51], has some associated methodological limitations mainly derived from a reduced field of view as a consequence of a too small transducer width resulting in a greater fascicle estimations, restricted region of interest analyzed, questionable mathematical extrapolations and omission of fascicle and aponeurosis 3-dimensional curvature [12,30,52,53]. Moreover, the fact that the assessment method is based on 2-dimensional static measurement conditions limits its extrapolation to dynamic tasks. Future research should consider the use of extended field of view ultrasonography and 3-dimensional measurements to minimize potential error and take into account fascicle rotation during dynamic conditions, beyond resting, static conditions [51–53]. Finally, we could not assess the hamstring force output in the current study (via for example isokinetic or Nordic exercise testing), but a recent study showed that a sprint program induced some positive changes in both sprint performance and hamstring eccentric strength output as measured during the Nordic exercise in young athletes [40].

Conclusions

Assuming fascicle length as a factor to be considered in the management of hamstring strain injuries, adding a sprint-focused program to regular soccer training induced greater increases in biceps femoris fascicle length than incorporation of Nordic hamstring exercise as a complementary intervention during the first 6 week of preseason period, compared with soccer practice alone. However, only the sprint comprehensive training provided this potentially preventive stimulus (increase fascicle length), and at the same time induced better sprint performance and mechanical outcomes, which could be considered a practical "win-win" strategy for the management of hamstring injuries. Due to the specificity of sprint training and low cost of testing methods (all variables can now be derived from split times or slow-motion videos with a validated App [54] of the sprints and online free computation spreadsheets (https://www.researchgate.net/publication/321767606_Spreadsheet_for_Sprint_acceleration_force-velocity-power_profiling), both players and staff can be more compliant to this type of inter-vention, compared to previously proposed methods. Finally, based on the current pilot results, further studies should test whether a comprehensive sprint training offers significant injury prevention advantages, as suggested recently [41,42].

Supporting information

S1 Raw data. (XLSX)

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6 Anabolic steroids among resistance training practitioners

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Abstract

Objective

To compare the use of anabolic steroids (AS), the motivation to use them, their side effects, the source of information and the form in which AS were obtained, the medical follow-up, and the periodic examinations in resistance training practitioners who are either current or former users of AS.

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Methods

A prevalence survey was performed in the gyms of the city of Curitiba, including 719 current and former AS users who self-administered a questionnaire. The chi-square and z of proportions (p < 0.05) statistical tests were conducted.

Results

Esthetics was the main motivation associated with AS intake, leading to satisfactory results. The information about the form in which to use AS was provided by doctors and AS were either purchased at the pharmacy with a prescription or illegally. Current users reported a higher number of cycles and doses, a longer duration of use, as well as larger economical investments into AS. This shows a higher consumption of such drugs, regardless of the medical follow-up and post-cycle therapy.

Conclusion

Given that a change in the usage pattern was observed when increasing the AS consumption, this should be considered in the elaboration of public policies to inhibit such a trend.

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

Introduction

Anabolic steroids (AS), including testosterone [1] are hormones that are usually used in a therapeutic setting [2–4]. However, they have also been illicitly employed as performance enhancers by professional competitors, school and amateur athletes [5], as well as by college students seeking improved Esthetics [5,6].

The AS medications can be administered either orally or intramuscularly [7] and their periods of use are denominated as cycles. Each cycle can range from 6 to 12 weeks, during which more than one AS administration is usually reported. The pyramid is one of the most common ways of performing a cycle. Specifically, while a gradual increase in the dosage occurs to ensure the adaptation of the body to the high doses, a gradual reduction follows to allow the recovery of the body [8].

The main testosterone drugs used as AS are as follows: $Durateston \mathbb{R}$, $Decadurabolin \mathbb{R}$, Winstrol \mathbb{R} and Landerlan \mathbb{R} [9–14].

Although the use of AS results in positive effects on performance, such as improvements in both strength and muscle mass [15], their use is also associated with changes in the anxiety and aggression patterns [6]. In addition, depression, personality and mood changes, sleep problems, irritability, and withdrawal symptoms are also common [8].Furthermore, while women may experience menstrual irregularities, clitoris hypertrophy, uterine and breasts atrophy, men may present a decrease in reproductive hormones, testicular atrophy, impotence, and gynecomastia [16].

Similarly, AS may cause acne, stretch marks, hair growth, voice alterations, pain, and abscesses after the application of injectable AS, liver changes (e.g., cholestasis, adenoma, and carcinoma), and cardiovascular events (e.g., hypertension, thrombosis, arrhythmias, systolic and diastolic dysfunction, left ventricular hypertrophy, and myocardial infarction) [8]. Moreover, the risk of sudden and unexpected death may also increase with AS usage [17, 18]. In addition, although the hospitalizations related to the use of AS are relatively low in Brazil, 1319 hospitalizations (age range: from 15 to 29 years old) were counted between 2000 and 2010, representing a burden for the health system. This number may be even higher given the failures seen in the hospitalization process [19].

Therefore, in addition to the sporting environment, the use of AS represents a problem also for the public health, considering the indiscriminate and non-therapeutic use of such drugs. The present study aims at comparing the use of AS, the motivation to use them, their side effects, the source of information and the form in which AS were obtained, the medical followup, and the periodic examinations in weight training practitioners who are either current or former users of AS. Given the possible side effects of the abuse of such drugs, the characterization of the AS form of use presented here is necessary. Furthermore, our results may be important for the foundation of public policies focused on informing and monitoring this publicly.

Materials and methods

An observational cross-sectional prevalence survey was conducted. The project was approved by the Research Ethics Committee of the Pontifical Catholic University of Paraná (PUCPR)— Curitiba—Paraná - Brazil, opinion no. 1,524,203 / 2016. The study met the ethical standards of Harriss et al., 2018 [20].

Specifically, the survey was conducted in the city of Curitiba (Brazil), which has approximately 1.9 million inhabitants and a human development index (HDI) of 0.823 [21]. Fig 1 shows the design of the sampling plan, which includes an initial survey of the number of gyms registered with the Regional Council of Physical Education, i.e., 680 gyms. Successively, only the resistance training centers were selected, leading to a total of 286 gyms. A sample

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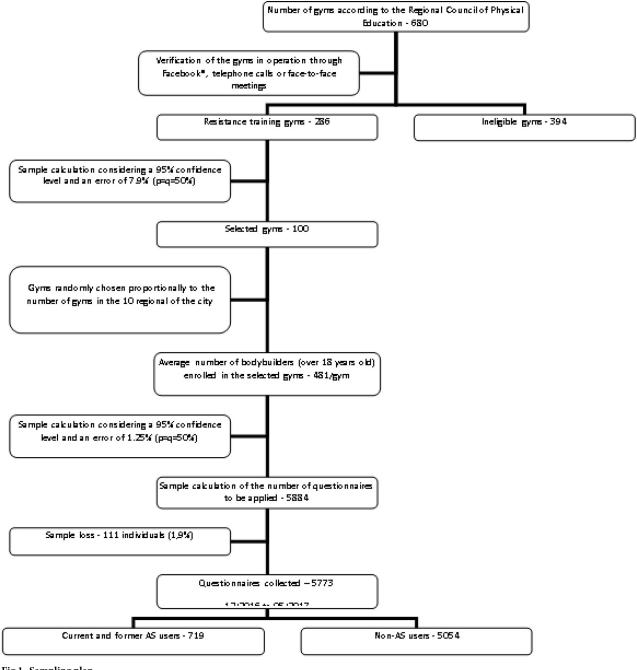


Fig 1. Sampling plan.

calculation was performed to determine the number of gyms required in this study (considering p = q = 50%), which resulted to be 100 (given an error of 7.9% and a confidence level of 95%). Successively, the average number of students (over 18 years old) enrolled in these gyms was identified, i.e., 481 practitioners. Thereafter, a new sample calculation was performed, considering an error of 1.25% and a 95% confidence level. The survey was applied out to individuals upon entering each gym. The participants signed a disclaimer that explained informed consent, confidentiality, and anonymity for subjects. A sample of 5884 individuals was obtained. Data were collected between December 2016 and May 2017, in a manner proportional to the number of practitioners present in each gym. Finally, after the exclusion of those individuals who did not use AS, a sample of 719 current and former users of AS resulted.

The questionnaire (S1 and S2 File) was self-administered by participants using the Kobo-Collect application (KoboCollect, Cambridge, Massachusetts, United States) on the Samsung Tablet, Tab 2 model (Samsung, Campinas, Brazil). This questionnaire was developed for this research and validated through the clarity, construct and content indices. The aspects of construction and content were validated by health professionals, while the clarity aspect was validated with individuals of the same class, age and lifestyle of the individuals who would be researched. A pilot study was conducted that the questionnaire could be used with the intended population. The questionnaire briefly, it comprised a first part to be answered by all the resistance training practitioners participating in this research and a second part to be answered only by current and former users of AS. This second part was analyzed in the present study, as it contained questions that addressed the age at AS use onset, the number of cycles performed, the cycle length, the weekly dosage, the type of AS used, the money invested in AS, AS used, the knowledge about the post-cycle therapy (PCT), the motivation to use AS, the satisfaction with the results, the source of information about AS, the form in which AS were obtained, the medical follow-up, the periodic exams and the changes in such exams.

Successively, all the data were transferred from the Kobocollect application (KoboCollect, Cambridge, Massachusetts) to the Excel (Microsoft, Redmond, Washington) and IBM SPSS 20.0 (IBM SPSS, Armonk, New York) software. The exploratory descriptive statistics of the frequency distribution and the percentages were performed using the results presented in the tables. With regards to the inferential analyzes, the chi-square test and the z-test of proportions were conducted, in addition to the student's t-test to compare the means (significant difference at p < 0.05).

Results

In the sample studied, 73% of the practitioners were former users of AS, while 27% were current users (men: 77.7%; women: 22.3%). However, when comparing the former and current users, differences between men and women were not observed (p = 0.09). Furthermore, the mean age of the participants was similar between men (30.4 ± 7.0 years) and women (30.8 ± 7.4 years) (p = 0.57), also when considering both former (30.8 ± 7.0 years) and current (29.8 ± 7.2 years) users (p = 0.08).

The majority of the current and former users began to use AS between their 18 and 29 years of age (73.1%), with 6.7% of them starting before their 18 years of age. Specifically, the mean age at onset among the former (24.8 \pm 6.0 years) and current (25.0 \pm 6.7 years) users was similar (p = 0.79).

The highest percentage of the former users consumed AS for a period of time longer than one year (66.3%): 19.2, 17.5, 29.6, 14.5, and 19.2% used it for more than five and three years, one year, and six and three months, respectively.

Table 1 compares the current and former users of AS. While a higher percentage of former users only performed one cycle of AS, six cycles or more were reported by current users. In addition, a shorter duration of one to two months per cycle was mainly observed in former users, whereas cycles with a duration longer than five months were mostly found among current users. Moreover, while a dosage of 100 mg per week was commonly reported by former users, dosages higher than 301 mg per week were described by current users. In addition, injectable AS were mostly employed by current users. Furthermore, a higher percentage of former users invested up to US\$ 134 for buying AS, whereas values over US\$ 134 were described by current users. However, the use of AS related to esthetic reasons and curiosity was higher

Table 1. Comparison of the number and duration of AS cycles, the dosage and type of AS used, the money invested, AS used, the motivation for the use of AS, and the subsequent satisfaction between former and current users of AS; Curitiba, 2016/2017.

Variables	Total n (%)	Former Users n (%)-n M-n F	Current Users n (%)–n M–n F	Р
Number of Cycles				
Only 1	331 (46%)	284 _a (54,1%)- 208-76	47 _b (24,2%)- 35-12	0,0001
2 to 5	281 (39,1%)	199 _a (37,9%)– 154–45	82 _a (42,3%)– 62–20	
6 or more	107 (14,9%)	42 _a (8,0%)- 38-4	65 _b (33,5%)- 62-3	
Duration of the cycle				
1 to 2 months	376 (52,3%)	315 _a (60,0%)- 242-73	61 _b (31,4%)- 47-14	0,0001
3 to 4 months	189 (26,3%)	128 _a (24,4%)– 100–28	61 _a (31,4%)– 48–13	
5 to 6 months	58 (8,1%)	33 _a (6,3%)– 27–6	25 _b (12,9%) 22–3	
7 to 12 months	36 (5,0%)	14 _a (2,7%)– 10–4	22 _b (11,3%)– 18–4	
More than 12 months	60 (8,3%)	35 _a (6,7%) 21–14	25 _b (12,9%) 24–1	
Dosage per week				
Unknown	176 (24,5%)	149 _a (28,4%)– 110–39	27 _b (13,9%)- 20-7	0,0001
Up to 100 mg	296 (41,2%)	241 _a (45,9%)- 178-73	55 _b (28,4%)- 32-23	
101 mg to 300 mg	108 (15,0%)	76 _a (14,5%)– 67–9	32 _a (16,6%)– 28–4	
301 mg to 500 mg	64 (8,9%)	34 _a (6,5%)- 31-3	30 _b (15,5%)- 30-0	
501 mg to 700 mg	22 (3,1%)	8 _a (1,5%)- 8-0	14 _b (7,2%)- 14-0	
Above 700 mg	53 (7,4%)	17 _a (3,2%)– 16–1	36 _b (18,6%)- 35-1	
Туре				
Injectable	566 (78,7%)	400 _a (76,2%)- 339-61	166 _b (85,6%)- 140-26	0,006
Oral	427 (59,4%)	301 _a (57,3%)- 204-97	126 _a (64,9%)- 100-26	0,065
Other	16 (2,2%)	11 _a (2,1%)– 7–4	5 _a (2,6%)- 3-2	0,697
Value invested				
Up to US\$ 134	362 (56,3%)	299 _a (64,6%)- 207-92	63 _b (35,0%)- 46-17	0,0001
US\$ 135 to US\$ 269	139 (21,6%)	86 _a (18,6%)- 78-8	53 _b (29,4%)- 43-10	_
More than US\$ 270	142 (22,1%)	78 _a (16,8%)– 65–3	64 _b (35,6%)- 60-4	_
Motivation				
Esthetic	538 (74,8%)	406, (77,3%)- 302-104	132 _b (68,0%)- 109-23	0,011
Sports Performance	262 (36,4%)	185 _a (35,2%)- 155-30	77, (39,7%)-67-10	0,271
Bodybuilding	131 (18,2%)	56 _a (10,7%)- 43-13	75 _b (38,7%)- 65-10	0,0001
Curiosity	85 (11,8%)	75, (14,3%)-64-11	10 _b (5,2%)- 8-2	0,001
Therapeutic	18 (2,5%)	12 _a (2,3%)- 10-2	$6_a(3,1\%) - 6 - 0$	0,539
Other	10 (1,4%)	7 _a (1,3%)- 6-1	$3_a(1,5\%) - 2 - 1$	0,829
Satisfaction after use				
Yes	613 (85,3%)	428 _a (81,5%)- 329-99	185 _b (95,4%)- 151-34	0,0001
No	106 (14,7%)	97 _a (18,5%)-71-26	9 _b (4,6%)- 8-1	
AS used				
Stanozolol	436 (60,6%)	308 _a (58,7%)- 258-50	128 _a (66,0%)- 112-16	0,075
Phenylpropionate, isocapronate, propionate and decanoate testosterone	332 (46,2%)	217, (41,3%)-214-3	115 _b (59,3%)- 112-3	0,0001
Oxandrolone	329 (45,8%)	214, (40,8%)- 127-87	115 _b (59,3%)- 86-29	0,0001
Nandrolone decanoate	235 (32,7%)	142 _a (27,0%)– 136–6	93 _b (47,9%)- 87-6	0,0001
Methandrostenolone	165 (22,9%)	89 _a (17,0%)- 84-5	76 _b (39,2%)- 76-0	0,0001
Testosterone cypionate	163 (22,7%)	85 _a (16,2%) - 84-1	78 _b (40,2%)-77-1	0,0001
Trembolone	162 (22,5%)	$75_{a}(14,3\%) - 70 - 5$	87 _b (44,8%)- 84-3	0,0001
Boldenone Undecylate	129 (17,9%)	$51_a (9,7\%) - 40 - 11$	78 _b (40,2%)- 68-10	0,0001
Drostanolone propionate	109 (15,2%)	$46_a (8,8\%) - 37 - 9$	63 _b (32,5%)- 58-5	0,0001
Oxymethalone	94 (13,1%)	$59_{a}(11,2\%) - 57-2$	35 _b (18,0%)- 34-1	0,0001

(Continued)

Table 1. (Continued)

Variables	Total n (%)	Former Users n (%)–n M–n F	Current Users n (%)–n M–n F	Р
Other	43 (6,0%)	30 _a (5,7%)– 20–10	13 _a (6,7%)– 9–4	0,620

Proportion z test. The different letters in the lines indicate significant differences (p < 0.05). US\$: American dollar (exchange of 10/31/2018). n M: number of male. n F: number of female.

among former users. Specifically, of those who were curious, 60.1% only performed one cycle (p = 0.029). In contrast, the use of AS for bodybuilding was greater in current users. Of the satisfied individuals, there was a higher percentage of current users.

Winstrol® (stanozolol) was the most commonly used AS by both current and former users. However, a greater diversity of AS was consumed by current users, including Durateston® (phenylpropionate, isocaproate, propionate and decanoate testosterone), Landerlan® (oxandrolone), Deca-Durabolin® (nandrolone decanoate), Dianabol® (methandrostenolone), Deposteron® testosterone), Parabolan® (trenbolone), Boldenone® (boldenone undecylated), Masteron® (drostanolone propionate), and Hemogenin® (oximethalone).

Table 2 shows the presence of collateral symptoms in both current and former users, as well as their disappearance after the end of the cycle. A higher percentage of the following symptoms was observed among current users: increased libido, acne appearance, irritability/aggressiveness, hypertension, depression, and dependence.

Table 3 illustrates that a higher percentage of current users obtained the information related to AS from doctors and nutritionists. In addition, a higher percentage of current users received AS either through a prescription in the pharmacy or in other ways, including the black market or imports. Furthermore, a higher percentage of current users reported medical follow-ups and periodic exams (e.g., total testosterone dosage) given their usage of AS. Moreover, changes in the results of such tests were identified, mainly among the current users. Finally, a higher percentage of current users was aware of the PCT and performed it similarly to previous users.

Discussion

One of the strengths of the present study relies on its large sample size, i.e., 719 weight training practitioners who are either current or former users of AS. Our results reveal differences between these groups, which were mainly related to the form of use: the number and duration of the AS cycles, the amount of money invested in them, the type of AS, the dosage used, and their motivation to consume them. Furthermore, differences were observed in the source of information related to the drugs, the way in which AS were obtained and the conduction of medical follow-ups.

In the current study, a higher number of former rather than current users of AS was observed, in accordance with previous literature [22, 23]. While Silva & Moreau (2003) [22] and Leifman et al., (2011) [23] separated current (17 and 5, respectively) from former (23 and 62, respectively) users into two categories, all the other surveys grouped them into a single category, making their comparison not possible. Furthermore, a larger number of current and former male users was here found, in accordance with previous literature [24, 13, 25, 26].

Practitioners' mean age was similar between men and women, as well as between current and former users. However, although our averages are above the age ranges described in other studies [27, 10, 9], the mean corroborates with the values seen in the literature if the age at onset of AS use is considered. It is also worth mentioning that 6.7% of the individuals started the use of AS at an age younger than 18 years old, as evidenced in other studies [28–31].

Variables	Total n (%)	Former Users n (%)	Current Users n (%)	Р
Collateral Symptoms				
Yes	643 (89,4%)	466 _a (88,8%)	177 _a (91,2%)	0,338
No	76 (10,6%)	59 _a (11,2%)	17 _a (8,8%)	
Symptoms				
Increased Libido	327 (45,5%)	213 _a (40,6%)	114 _b (58,8%)	0,0001
Acne	308 (42,8%)	213 _a (40,6%)	95 _b (49,0%)	0,043
Irritability / Aggressiveness	233 (32,4%)	157 _a (29,9%)	76 _b (39,2%)	0,018
Headache	159 (22,1%)	119 _a (22,7%)	40 _a (20,6%)	0,557
Decreased Libido	111 (15,4%)	77 _a (14,7%)	34 _a (17,5%)	0,346
Gynecomastia	97 (13,5%)	63 _a (12,0%)	34 _a (17,5%)	0,054
Hypertension	91 (12,7%)	55 _a (10,5%)	36 _b (18,6%)	0,004
Change in Menstrual Cycle	73 (10,2%)	58 _a (11,0%)	15 _a (7,7%)	0,191
Deepening of the Voice	71 (9,9%)	50 _a (9,5%)	21 _a (10,8%)	0,604
Depression	50 (7,0%)	26 _a (5,0%)	24 _b (12,4%)	0,001
Dependency	31 (4,3%)	16 _a (3,0%)	15 _b (7,7%)	0,006
Vomiting / Nausea	25 (3,5%)	17 _a (3,2%)	8 _a (4,1%)	0,545
Other	59 (8,2%)	48 _a (9,1%)	11 _a (5,7%)	0,132
Symptoms Disappeared After Cycle				
Yes	555 (77,2%)	415 _a (79,0%)	140 _a (72,2%)	0,025
No	98 (13,6%)	71 _a (13,5%)	27 _a (13,9%)	
Some	66 (9,2%)	39 _a (7,4%)	27 _b (13,9%)	

Table 2. Presence of collateral sy	ymptoms in both current and former use	rs, as well as their disappearance after the end of the cycle.

Proportion z test. The different letters in the lines indicate significant differences (p <0.05).

Our results identified that a higher percentage of former, as opposed to current, users only conducted one AS cycle, with a shorter duration (1 to 2 months) and smaller dosages (100 mg). This strengthens the hypothesis of a curiosity-driven AS use, given that the percentage of former users who reported such a reason was also higher. In contrast, the opposite was observed in current users, who performed six or more cycles, with longer durations (5 months or more) and higher dosages (301 mg or more) than former users. While the satisfaction associated with the results can be one of the motivating hypotheses behind the regular use of AS, the practice of bodybuilding may also be an explanation to this phenomenon, since the motivation to consume AS was also greater in current users.

Injectable AS were mostly used, mainly among current users. To the best of our knowledge, this is the first study identifying the frequency of AS administration forms in this population. With regards to injectable AS, a lack of proper asepsis care during the application increases the risk of infections. As a consequence, this may lead to hospitalizations given the practitioners' progression to abscesses, which may turn into more severe conditions, such as muscular necrosis or sepsis [32]. With regards to oral AS, high hepatoxicity is expected [33, 16].

In accordance with the literature, testosterone and stanozolol were the most commonly used AS [22, 34, 35]. Although a previous study cited testosterone as commonly used AS [33], oxandrolone, which had a high prevalence in this study, was not widely assessed in other studies.

Stanozolol was the most used AS among both groups, in accordance with Silva and Moreau (2003) [22], possibly given its popular brands that can be found both in the oral and injectable forms [36]. Similarly, nandrolone decanoate was also commonly consumed in other studies [9, 11, 22, 35, 37], possibly considering its higher accessibility (i.e., lower cost in trade) and greater

Table 3. Comparison of the source of information related to AS, the way in which the AS was obtained, the medical follow-up and exams, alterations in such exams,
and the knowledge and conduction of the PCT between former and current users of AS; Curitiba, 2016/2017.

Variables	Total n (%)	Former Users n (%)	Current Users n (%)	Р
Information Source				
Doctors	331 (46,0%)	214 _a (40,8%)	117 _b (60,3%)	0,0001
Friends	254 (35,3%)	193 _a (36,8%)	61 _a (31,4%)	0,185
Coaches	226 (31,4%)	156 _a (29,7%)	70 _a (36,1%)	0,103
Internet	155 (21,6%)	119 _a (22,7%)	36 _a (18,6%)	0,234
Nutritionist	109 (15,2%)	68 _a (13,0%)	41 _b (21,1%)	0,007
Other	34 (4,7%)	25 _a (4,8%)	9 _a (4,6%)	0,945
Way of obtaining the AS				
Friends	394 (54,8%)	291 _a (55,4%)	103 _a (53,1%)	0,576
Pharmacy with prescription	271 (37,3%)	183 _a (34,9%)	88 _b (45,4%)	0,010
Pharmacy without prescription	77 (10,7%)	55 _a (10,5%)	22 _a (11,3%)	0,739
Other	119 (16,6%)	77 _a (14,7%)	42 _b (21,6%)	0,025
Medical follow-up				
Yes	281 (39,1%)	157 _a (29,9%)	124 _b (63,9%)	0,0001
No	438 (60,9%)	368 _a (70,1%)	70 _b (36,1%)	
Conducting examinations				
Yes	521 (72,5%)	358 _a (68,2%)	163 _b (84,0%)	0,0001
No	198 (27,5%)	167 _a (31,8%)	31 _b (16,0%)	
Exams				
Total Testosterone	402 (55,9%)	257 _a (49,0%)	145 _b (74,7%)	0,0001
Cholesterol	399 (55,5%)	272 _a (51,8%)	127 _b (65,5%)	0,001
High density lipoprotein—HDL	378 (52,6%)	254 _a (48,4%)	124 _b (63,9%)	0,0001
Low density lipoprotein—LDL	357 (36,0%)	238 _a (45,3%)	119 _b (61,3%)	0,0001
Cortisol	259 (36,0%)	161 _a (30,7%)	98 _b (50,5%)	0,0001
Alanine Aminotransferase—ALT	246 (34,2%)	146 _a (27,8%)	100 _b (51,5%)	0,0001
Aspartate Aminotransferase—AST	243 (33,8%)	146 _a (27,8%)	97 _b (50,0%)	0,0001
Follicle Stimulating Hormone— FSH	243 (33,8%)	147 _a (28,0%)	96 _b (49,5%)	0,0001
Progesterone	218 (30,3%)	129 _a (24,6%)	89 _b (45,9%)	0,0001
Other	68 (9,5%)	41 _a (7,8%)	27 _b (13,9%)	0,013
Do not Know	51 (7,1%)	34 _a (6,5%)	17 _a (8,8%)	0,289
Exams alterations				
Yes	201 (28,0%)	112 _a (21,3%)	89 _b (45,9%)	0,0001
No	518 (72,0%)	413 _a (78,7%)	105 _b (54,1%)	
Knowledge PCT				
Yes	567 (78,9%)	391 _a (74,5%)	176 _b (90,7%)	0,0001
No	152 (21,1%)	134 _a (25,5%)	18 _b (9,3%)	
Realization PCT				
Yes	288 (40,1%)	176 _a (33,5%)	112 _b (57,7%)	0,0001
No	431 (59,9%)	349 _a (66,5%)	82 _b (42,3%)	

Proportion z test. The different letters in the lines indicate significant differences (p <0.05).

disclosure [36]. Both stanozolol and nandrolone decanoate are frequently used for muscle growth, given their higher anabolic characteristics compared to androgenic AS [36]. Although more than one AS is usually used during a cycle [8], veterinary drugs, such as boldenone undecylation and trenbolone, have also been consumed [12]. A report of fulminant

heart attack due to the use of boldenone in humans was reported [38]. In contrast, trenbolone may affect the liver, causing cholestatic hepatitis [39, 40] and may be associated with the proliferation of tumor cells in prostate cancers [41]. In addition, it can result in dermatitis, including severe inflammatory acne with pustules and hemorrhagic ulcerations [42].

Collateral symptoms were reported by both current and former users, as widely reported in previous literature [5, 8, 16]. However, while most symptoms disappeared after the end of the cycle, given their acuity, the chronic symptoms observed may cause slow and irreversible changes [8].

In accordance with previous studies, increased libido, acne, and irritability/aggressiveness were the main collateral symptoms reported by both current and former users [22, 9]. Increased libido may be classified as an acute side effect, given that it again decreases after the cycle [15], and it is associated with high serum levels of testosterone resulting from the use of supraphysiological dosages [5]. In contrast, considering that acne usually occurs during puberty and not in adulthood, the use of AS increases the activity of the sebaceous glands, which leads to higher oil concentrations present in the skin [8]. Furthermore, irritability/aggressiveness are often associated with the AS use [8]. Although increased aggression may lead to heightened violence, such a hypothesis was not previously proven, given that the individuals involved in cases of violence also used other drugs, alcohol or had personality tendencies [24].

Transient hypertension, an acute symptom, was more frequent in current users [16]. However, this acute symptom may become chronic, since the use of AS for long periods is associated with cardiovascular diseases, including hypertension, heart attack, and stroke. The most commonly used oral AS alter the levels of lipoproteins that carry the cholesterol in the blood, increase the level of low-density lipoprotein (LDL), and decrease the high-density lipoprotein (HDL) instead [8].Therefore, their assessment is important for controlling health-related risks [22].Moreover, current users were noticed to be more cautious compared to former users, possibly because they underwent more medical follow-ups.

Dependency was also more frequent in current users. The use of AS for long periods of time may eventually affect the brain as other addictive illicit drugs, acting primarily on the dopamine, serotonin, and opioid systems [8]. However, dependency may also be associated with the presence of body image disorders, such as "muscular dysmorphism", where excessive preoccupation is laid onto the musculature [24]. Moreover, the stories of success disseminated by the means of communication related to image alteration and muscular bodies motivates such body transformations [12].

A higher percentage of current users obtained information about AS from doctors. In fact, the emergence of anti-aging treatments increases the number of doctors who encourage AS therapies [43]. However, attention is drawn to the fact that doctors are prescribing such therapies to youngsters, who fail in conducting a medical follow-up, representing a big issue [13]. Therefore, the increased number of doctors prescribing the use of AS may be stimulating its use. In fact, the acquisition of such drugs in the pharmacy with a prescription was high, as described in previous literature [22, 33]. Moreover, friends, coaches, and nutritionists also influence the use of AS [44, 9]. In fact, coaches encourage their clients to consume AS for better results in the shortest time possible to improve their reputation in the academy [27].

This study did not find a definite protocol for the use of AS. Various combination, dosages, durations, and cycles were in fact used by practitioners. An empirical culture on how to best use AS according to the final objective exists, which can either be obtained from manuals or transmitted orally between users (based on their own experiences) [22]. Furthermore, this information, as well as the AS products, are widely found on the internet.

In accordance to previous studies, in addition to pharmacies, AS were found to be also illegally marketed (black market) and easy to access [33, 45, 9]. In fact, AS are marketed freely on the internet and in the gyms themselves [36]. About 1/3 of the illicitly imported products derive from Paraguay, according to the federal police of Brazil. It should be noted that these products are not regulated and that they may be falsified [46], leading to possible serious health damages.

Although most of the individuals do not conduct a medical follow-up, a higher percentage of current users performed monitoring and tests to control for the risks associated with the use of AS, including changes in vital organs (e.g., heart and liver) and dosage of testosterone. However, performing these tests does not eliminate health risks [22].

Although a great knowledge about PCT exists, a small number of individuals actually perform such a therapy. Briefly, PCT involves the use of certain medications aimed at reversing the suppression of endogenous production of testosterone at least temporarily. Its abrupt interruption, without returning to the endogenous production, can lead to a state of hypogonadism characterized by a substantial loss of muscle mass, reduced energy levels, depression, and loss of libido. However, a higher percentage of current users are performing PCT, which may be a result of the increased number of medical follow-ups conducted in this group⁷. In the present study, the following drugs used during PCT were identified: tamoxifen, human chorionic gonadotropin hormone (CGH), Clomiphene, Anastrozole, Saw Palmetto, Legalon, and Proviron. These drugs were prescribed for such a therapy in previous literature [7].

The limitations of the current study will now be highlighted. Firstly, a response bias may have affected our results, considering that the answers provided in the self-administered questionnaire depended on the participant's honesty and that confirming their veracity was not possible. In addition, as some of the information refer to a period in the past (even years before the current investigation), especially with regards to former users, uncertainties regarding the information provided may be present.

Conclusions

Most current users performed between two to five and up to six or more cycles of AS, with a duration of five months or more and a dosage higher than 301 mg per week. They consumed the injectable type of AS, invested an amount of money higher than US\$ 134 and used stanozolol. Esthetics was found to be the main reason associated with the use of such drugs and individuals were satisfied with the obtained results. Although they presented side-effects during the period of use (mainly increased libido, irritability/aggressiveness, and acne appearance), such symptoms disappeared after the use. The information related to the use of AS was mainly obtained through doctors and the drugs were purchased either through friends or at a pharmacy with a prescription. Individuals conducted medical follow-ups, had a knowledge of PCT and performed it. In contrast, most former users only conducted one cycle of AS, for a duration of one to two months, with dosages lower than 100 mg per week. They invested less than US\$ 134 in AS, did not conduct medical follow-ups and did not perform PCT.

Overall, we noticed some changes in the pattern of use between former and current users of AS. Specifically, the latter reported a higher number of cycles of AS, a longer duration, increased dosage and money invested in AS, and a consequent higher and diverse consumption of AS. However, the presence of collateral symptoms does not inhibit such a consumption, possibly due to the safety provided by the medical follow-ups, which were conducted by a high number of current users. Finally, an increase in both the number of doctors providing such services and the acquisition of AS in pharmacies with a prescription was observed. This implies the need for public policies ensuring both improved information, given the symptoms and risks presented, and better control, considering that the abuse of such drugs is associated with a number of health risks.

Supporting information

S1 File. Portuguese questionnaire. (DOC)

S2 File. Questionnaire. (DOC)

Author Contributions

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7 Effects of remote limb ischemic conditioning on muscle strength in healthy young adults: A randomized controlled trial

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Abstract

Remote limb ischemic conditioning (RLIC) is a clinically feasible method in which brief, sublethal bouts of ischemia protects remote organs or tissues from subsequent ischemic injury. A single session of RLIC can improve exercise performance and increase muscle activation. The purpose of this study, therefore, was to assess the effects of a brief, two-week protocol of repeated RLIC combined with strength training on strength gain and neural adaptation in healthy young adults. Participants age 18-40 years were randomized to receive either RLIC plus strength training (n = 15) or sham conditioning plus strength training (n = 15). Participants received RLIC or sham conditioning over 8 visits using a blood pressure cuff on the dominant arm with 5 cycles of 5 minutes each alternating inflation and deflation. Visits 3-8 paired conditioning with wrist extensors strength training on the non-dominant (non-conditioned) arm using standard guidelines. Changes in one repetition maximum (1 RM) and electromyography (EMG) amplitude were compared between groups. Both groups were trained at a similar workload. While both groups gained strength over time (P = 0.001), the RLIC group had greater strength gains $(9.38 \pm 1.01 \text{ lbs})$ than the sham group $(6.3 \pm 1.08 \text{ lbs})$, P = 0.035). There was not a significant group x time interaction in EMG amplitude (P =0.231). The RLIC group had larger percent changes in 1 RM (43.8% vs. 26.1%, P = 0.003) and EMG amplitudes (31.0% vs. 8.6%, P = 0.023) compared to sham conditioning. RLIC holds promise for enhancing muscle strength in healthy young and older adults, as well as clinical populations that could benefit from strength training.

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Introduction

Ischemic conditioning describes a phenomena in which an organ exposed to a controlled, short-term, local, sublethal ischemia will be protected from subsequent ischemia [1–4]. Remote ischemic conditioning is another more practical approach where transient ischemia and reperfusion applied to a remote organ or tissue, protects other organs or tissues from further episodes of lethal ischemia/reperfusion injury. For example, ischemia on the limb protects the brain from subsequent ischemic injury [5–7]. Ischemic conditioning can be delivered before (preconditioning), during (perconditioning) or after (postconditioning) the ischemic insult [8]. Remote limb ischemic conditioning (RLIC) is a clinically feasible way of performing remote ischemic conditioning where alternating, brief ischemia and reperfusion is delivered with cyclic inflation and deflation of a blood pressure cuff on the arm or leg [9].

In 1986, ischemic conditioning was first found to protect cardiac myocytes from ischemia [10–12]. Since then a number of studies have found that ischemic conditioning also protects skeletal muscles from infarction in porcine and rodent models [13, 14]. Subsequent studies in humans have shown that RLIC improves exercise performance in healthy young adults [15–18]. Specifically, a single session of repeated 5-min bouts of RLIC, not combined with any training, increases muscle force [19], power output [20], and muscular endurance [21, 22], delays muscle fatigue [23], and improves recovery time between tasks that require maximum force generation [24]. In stroke survivors, also within a single session, RLIC has shown to increase paretic muscle force and activation [25]. In a similar paradigm delivered over two weeks, RLIC improved walking capacity and decreased neuromuscular fatigability, despite failing to increase muscle force [26].

Although the specific mechanisms for improving skeletal muscle performance are incompletely understood, the beneficial effects of RLIC on skeletal muscles occurring secondary to humoral, neural and metabolic mechanisms are generally accepted [27]. Specifically, RLIC improves metabolic efficiency by reducing adenosine triphosphate (ATP) and glycogen depletion [13, 14, 28–30], increasing blood flow by inducing vasodilation [20, 31, 32], and decreasing lactate production [15, 18]. RLIC also lowers sensitivity to fatigue signals [17], and increases neural drive, and the recruitment of motor units [33, 34].

These studies open the possibility that RLIC, when paired with skeletal muscle training, could enhance strength gains in humans. Improving muscle strength is important for many populations such as those undergoing rehabilitation [35–37], prolonged inactivity [38, 39], chronic diseases [40, 41], and aging [42]. In short, if RLIC can be harnessed to enhance strength training, many people around the world would benefit. Considering the beneficial effects of RLIC alone on skeletal muscle, pairing RLIC with strength training merits further investigation.

The purpose of this study, therefore, was to determine whether RLIC could enhance a brief period of muscle strength training in healthy young adults. We note that paring RLIC with strength training is a different technique than the blood flow restriction (BFR) exercise paradigms popularized by high performance athletes [43–47]. The latter are characterized by limiting venous blood flow to the working muscle, but do not generate ischemic bouts in the muscle [48–50]. BFR exercise paradigms target the muscle that receives the flow restriction, whereas RLIC operates systemically [22, 48]. A short-duration, high-intensity strength training protocol was selected in this early phase study and thus is not intended as a comprehensive evaluation. We chose to test healthy young adults to determine if benefits occur with healthy physiology before moving to specific, co-morbid patient populations. We hypothesized that combining RLIC with strength training would enhance strength gains compared to sham conditioning. Results from this study can be used to inform future, more definitive studies of this combinatorial therapy.

Materials and methods

Trial design

This study was an early phase, prospective, single-blind, randomized controlled trial with a repeated-measures design. The study was approved by Washington University Human Research Protection Office on 09/12/2017 and conducted from 11/08/2017 to 12/18/2018. The trial was registered at https://www.clinicaltrials.gov/ (NCT03512028). The definition of a clinical trial and the rules for registration changed after the study was initiated. At the time of design, funding, and initiation, the study was considered as an experiment. Due to the changes in the definition of clinical trials at NIH, we have registered the study as clinical trial after enrolling few participants. All related trials for this intervention are registered at clinicaltrials.gov.

The study included ten total visits to assess the combined effects of remote limb ischemic conditioning (RLIC) combined with muscle strength training in healthy young adults. All participants provided informed consent and received compensation for their time and effort.

Participants

Healthy young adults were recruited through advertisements in the greater St. Louis community. Inclusion criteria were age 18 to 40 years and intact cognitive-motor functions to actively participate in the study. Exclusion criteria were: (1) a history of any neurological condition (i.e. stroke, i.e. stroke, Alzheimer's disease, Parkinson's disease), attention deficit disorder, attention deficit hyperactivity disorder, depression, bipolar disorder, balance impairment, or vestibular disorder; (2) history of depression or bipolar disorder; (3) recent wrist, hand or forearm injury that would affect their ability to lift weights; (4) any extremity, soft tissue, orthopedic, or vascular injury (i.e. peripheral vascular disease) which may contraindicate RLIC; (5) a history of sleep apnea which could confound the effects of RLIC [51, 52]; (6) any cognitive, sensory, or communication problem that might prevent completion the study; (7) current intensive weight lifting or interval training exercise, which could confound the effects of RLIC [53, 54]; (8) current substance abuse or dependence; (9) current use of medications, such as selective serotonin reuptake inhibitors, that could decrease nervous system excitability [55]; (10) participation in previous RLIC studies; and (11) an inability or unwillingness to travel for all study visits. As per the study protocol, the inclusion criteria of visual acuity of 20/50 with corrected vision and exclusion criteria of moderate to severe motion sickness were specific to cognitive testing (simulated driving task). Since this study reports only strength related measures, we stated inclusion and exclusion criteria specific to strength testing and training.

Fig 1 shows the CONSORT diagram, describing the flow of participants through the study. A total of 115 participants were assessed for eligibility and 34 participants were eventually randomized. Nineteen participants were assigned to receive RLIC and 15 to receive sham conditioning. Per the CONSORT diagram, 30 participants were included in the final analysis (RLIC: n = 15; sham: n = 15).

Data were collected in the Neurorehabilitation Research Lab of Washington University School of Medicine in St. Louis. Sample size was estimated based on previous studies of the effects of RLIC on a motor leaning task [56, 57]. Based on motor learning (change in balance score), 20 participants in each group was estimated to provide 80% power to detect a mean difference of 3 seconds change (posttest-pretest) between two treatment groups (RLIC vs. Sham) based on a two-sample t-test (significance level of 0.05). The standard deviations for change scores are assumed to be 2.5 seconds and 1.9 seconds for the RLIC and sham groups, respectively. Since we did not have pilot data for the effects of RLIC on strength, we performed post-

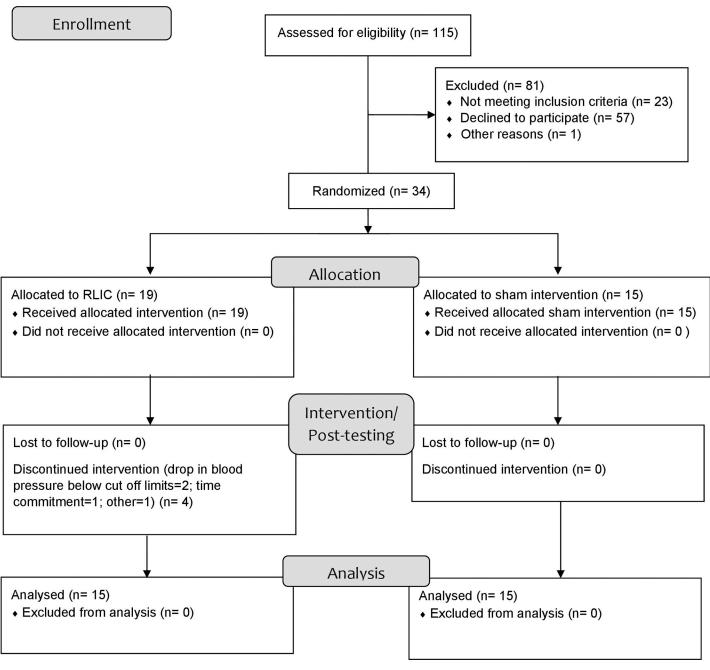


Fig 1. CONSORT flow diagram.

hoc analysis to detect study power for strength measure. 15 participants in each group provided 82.6% power based on the strength measure.

Experimental procedure

The experimental procedure is shown in Fig 2. There were 10 total visits. Visits 1–3 occurred on consecutive weekdays. Visits 4–8 occurred on alternating weekdays to allow sufficient time between strengthening visits [58, 59]. Visit 9 occurred 1 weekday after visit 8, and visit 10, was

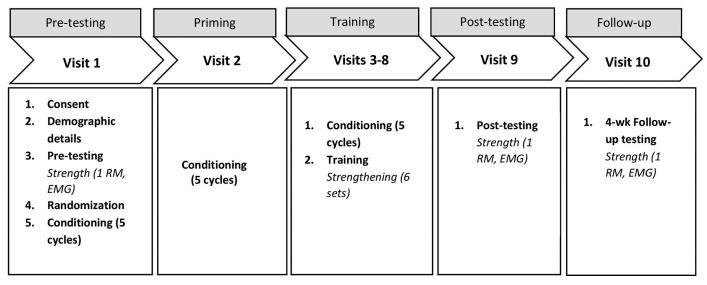


Fig 2. Order of experimental procedures.

a 4-week follow-up. During the first visit, participants provided demographic details including height and weight for the calculation of body mass index (BMI), and health and exercise history. After pre-testing, participants were randomly assigned to the RLIC or the sham group using a randomization list generated by the study statistician. Participants were blinded to group assignment until the completion of all 10 study visits.

Intervention. *Remote limb ischemic and sham conditioning*. Before beginning conditioning, we recorded resting heart rate, blood pressure, and oxygen saturation. Conditioning was performed by cyclic inflation and deflation of a blood pressure cuff on the dominant upper extremity. We specifically chose to perform conditioning on the dominant upper extremity (not the limb undergoing strength training), because the RLIC has systemic effects [60, 61]. Conditioning for the RLIC and sham conditioning groups was delivered as 5 cycles of 5 min blood pressure cuff inflation followed by alternating 5 minutes of deflation resulting in total conditioning time of 45 minutes [6, 25, 56, 62]. Consistent with our previous studies, the conditioning pressure in the RLIC group was \geq 20 mmHg above that visit's resting systolic blood pressure since this pressure was sufficient to induce ischemia and shown as effective as standard 200 mmHg, with fewer side effects [56, 57]. The conditioning pressure in the sham group was 10 mmHg below that visit's diastolic BP since it gives the sensation of cuff inflation, but does not induce arterial occlusion [63–65]. All the participants received conditioning for the first 8 visits (Fig 1).

The experimenter continuously monitored the presence or absence of ischemia in the RLIC and sham conditioning groups, respectively, by monitoring a pulse oximeter placed on the index finger of the conditioning arm and by visually inspecting the color of the conditioning arm. A reading of "0" for pulse and oxygen saturation on the pulse oximeter and the presence of pale dusky appearance of the conditioning arm confirmed the presence of ischemia in the RLIC group. Oxygen saturation and pulse equivalent to baseline or prior to initiation of conditioning and unchanged color of the conditioning limb confirmed the absence of ischemia in the sham conditioning group. In the RLIC group, if the pulse or oxygen saturation reading appeared on the pulse oximeter anytime during the inflation cycle, the assessor increased the inflation pressure until confirmation of ischemia on the conditioning arm and the total time was adjusted to be consistent with 5 minutes of inflation cycle. Similarly, in the sham

conditioning group, if oxygen saturation and pulse dropped below baseline measures, the conditioning pressure was decreased until preconditioning pulse and oxygen saturation was achieved and the arm showed no visible evidence of ischemia.

Conditioning safety was monitored through measurement of blood pressure, heart rate, oxygen saturation and pain on the non-conditioning arm before, during, and after each session. For both groups, conditioning was terminated if systolic BP was <85 or >160 mmHg; diastolic BP was <40 or >100 mmHg; heart rate was <60 or >100; oxygen saturation was <90%; or pain was >6 on Likert pain scale.

Strength training. We chose short duration (2 weeks), high intensity (80% of 1 RM) strength training in healthy young adults. The aim was to probe the effects of RLIC on muscle strength and early neural adaptation to short-term strength training, rather than to comprehensively evaluate strength, which would require a long-duration strength training programs [66–70]. Participants performed strength training within 5–25 minutes of conditioning. Therefore, strength training was performed within the early phase of the RLIC response [71].

A single wall-mounted pulley with stackable weights was used for strength training. The dynamic contraction strength of the wrist extensor muscles on the non-dominant extremity was trained. For strength training, participants were seated in a chair with trunk upright, feet firmly positioned on the floor, shoulder abducted to 45°, forearm pronated and strapped to a cushioned arm tray mounted on a table to avoid compensatory movements, wrist was in 80°-85° flexion and participant firmly grasped the handle of a pulley, consistent with the previous study set up from our lab [72]. A total of 6 strength training sessions (visits 3-8) occurred on alternate weekdays across 2 weeks. Prior to each strength training session, participants performed warm-up for 2-3 minutes that included gentle wrist oscillations, stretching of wrist extensors, isometric contractions of wrist extensors, and wrist movements. The high intensity, progressive strength training protocol followed standard American College of Sports Medicine guidelines for intensity, frequency, duration, and progression [73]. Specifically, the participants completed 6 sets of wrist extension movement with 6-8 repetitions in each set. Resistance was set at 80% of the participant's 1 Repetition Maximum (1 RM). Participants were verbally cued to slowly move the wrist, resulting in the concentric and eccentric phases of wrist movement for 5–6 seconds each. If the participant was unable to perform 6 repetitions per set with 80% of 1 RM intensity, the load was slightly reduced to ensure adequate stimulus for maximizing strength gains. Training was progressed over 6 visits by increasing resistance in subsequent visits when 8 repetitions were achieved in 4 out of 6 sets. Between each set, 1-3minutes rest was provided since such inter-set rest intervals reduce metabolic stress and enhance strength gains [74]. Training load was calculated as the total repetitions in 6 sets during that visit multiplied by average weight across 6 sets.

Strength outcomes. *a. One Repetition Maximum (1 RM).* Wrist extensor muscle strength of the non-dominant extremity was measured using 1 RM with the single column wall-mounted pulley with stackable weights. 1 RM was quantified as the maximum weight that a participant could lift only once from a position of full wrist flexion to full extension. Prior to the testing, participants were familiarized with the testing protocol and performed warm-up exercises as described previously in strength training.

The set-up for 1 RM testing and the participants' positioning was the same as described above. Prior to each attempt, participants were instructed to contract with full effort, and move through the full active range from flexion to extension. Standardized verbal encouragement was given to enhance efforts and was consistent across participants. The measurement of 1 RM was considered valid and complete if the participant lifted the weight through full range of motion and then lowered the weight back to the starting position. One to three minutes of rest was allocated between each attempt to minimize fatigue and maximize performance [75,

76]. Consistent with the literature, strength changes were also expressed and analyzed as percent change [58].

b. Electromyography (EMG) recording and analysis. To assess neural adaptation to strength training, we recorded wrist extensor muscle activation using EMG. Raw EMG was obtained using a 2-channel EMG system (Noraxon Inc, USA) with the sampling frequency set at 1000 Hz. The target for the EMG signal activity was the extensor carpi radialis longus (ECRL) muscle on the non-dominant extremity during maximum voluntary isometric contraction (MVIC). Because of the close proximity of the wrist muscles, the recorded signals likely include some activity from adjacent wrist extensor muscles. Before placing the surface electrodes, skin was cleaned with an alcohol swab for the removal of dead cells and oil, thereby reducing skin impedance. Bipolar Ag-AgCl, disc shape surface electrodes with 5 mm diameter and 20 mm interelectrode distance were placed at the muscle belly in the direction of muscle fibers, according to surface EMG for non-invasive assessment of muscles (SENIAM) guidelines (http://www.seniam.org). The ground electrode was placed on the lateral epicondyle of the elbow. To ensure reliability of positioning of electrodes across pre-, post-, and follow-up testing, placement of electrodes was measured and recorded between two reference points: 1) distance between medial and lateral epicondyles and 2) intersection point between vertical distance from the first electrode to medial distance from lateral epicondyle.

EMG signals were recorded during a MVIC, using a similar position as the 1 RM testing, with the wrist in neutral position. Participants were instructed to produce maximum isometric wrist extension force within 30° wrist extension range by pushing against a handheld dynamometer placed on the dorsum of the wrist for 3 maximal effort contractions lasting 5 seconds each. One to three minutes rest between each contraction was given to prevent muscle fatigue [75, 76]. EMG recording was performed for each participant at the pre-, post, and follow-up testing.

EMG data were processed off-line in MATLAB R2016a (MathWorks, Natick, MA) with custom-written software. Signals were full-wave rectified and smoothed with a second-order Butterworth-filter using cutoff frequencies of 20 and 250 Hz for lower and upper band-pass, respectively [77]. EMG data were quantified by averaging the amplitude of the EMG activation during the middle 3000 ms for each trial. The three trials were then averaged to yield a single value representing ECRL activation for a given assessment point. EMG activation from pre-and post-testing was also expressed and analyzed as % change.

The assessor that performed conditioning also did strength training and testing. Post-test strength assessments were performed within 24–36 hours after the conditioning i.e. within the delayed phase of the RLIC response [71].

Statistical analysis

Data were managed and stored in a secure REDCap database (Vanderbilt University, Nashville, TN) [78] and statistical analyses were performed in SPSS Statistics 24 (Version 24.0, IBM Corporation, Armonk, New York) with two-sided tests at a significance level of 0.05. Descriptive statistics for continuous variables (mean, standard deviation, median and range) and for categorical variables (frequency tables) were obtained for the study sample. Normality of the data was assessed using normal distribution plots. Since the data were normally distributed, independent t-test tests were used for the continuous variables of age, weight, height, BMI, pain, conditioning pressure, oxygen saturation, systolic and diastolic BP. Fisher Exact Tests were used for categorical variables such as gender, dominance, and race. A mixed model analysis of variance (ANOVA) with groups (RLIC and sham) as between-subject, and time (pre-, post-, and follow-up) as within-subject, factors was used to determine if there was a significant

Participants			
Characteristics	RLIC (n = 15)	Sham (n = 15)	Main effect of group (p)
Age (years)	25.5 ± 0.99	27.3 ± 1.11	0.225
Female/Male	10/5	10/5	1.00
Dominant side (R/L)	14/1	15/0	1.00
Weight (lbs)	140.4 ± 8.26	151.26 ± 6.86	0.321
Height (inches)	65.8 ± 1.01	65.93 ± 1.21	0.933
BMI (kg/ (m*m))	22.55 ± 0.79	24.56 ± 1.00	0.125
Resting Systolic BP (mmHg)	111 ± 3.09	117 ± 2.23	0.139
Resting Diastolic BP (mmHg)	71 ± 1.77	75 ± 1.98	0.239
Race			0.931
Caucasian	8 (53.3%)	8 (53.3%)	
African American	1 (6.7%)	2 (13.3%)	
Asian	5 (33.3%)	4 (26.7%)	
Other	1 (6.7%)	1 (6.7%)	

difference in 1 RM and EMG amplitude. A separate mixed model ANOVA with groups (RLIC and sham) as between-subject, and time (visits 3-8) as within-subject, factors was used to determine if there was a significant difference in training load. We were specifically interested in the group by time interaction effects. Significant interaction effects were followed by a Bon-ferroni post-hoc analysis. Independent t-tests were used to determine if significant differences existed between groups in percent change for 1 RM and EMG amplitude. P values equal to or less than 0.05 alpha were considered significant. Results in the text and graphs are presented as mean \pm standard error. Post-hoc power analyses were performed using G*Power [79].

Results

Values are numbers or mean ± SE. RLIC = remote limb ischemic conditioning; BMI = body mass index.

Table 1 shows baseline characteristics of the participants included in the study. There were no differences (all P values > 0.05) in the baseline characteristics between the RLIC and sham groups. There were no adverse events in either group, but two participants were withdrawn from the RLIC group. The baseline BP for these two participants was 85/65 and 91/6 mmHg. At the end of the conditioning, BP dropped to 83/62 and 77/49 for the participant 1 and 2 respectively. These BP were below our cuff-off threshold of systolic BP (<85 mmHg) and diastolic BP (<40 mmHg). Therefore, participants were withdrawn from the study. However, both the participants did not have any signs of distress. We followed-up with both the participants twice within 24 hours, who did not report any unusual symptoms.

For the remaining subjects, conditioning was delivered as planned. Average cuff inflation pressure for the RLIC group $(143 \pm 3 \text{ mmHg})$ was, as expected, significantly higher than the sham conditioning group $(65 \pm 2 \text{ mmHg}; P = 0.001)$. Ischemia occurred during conditioning cycles in the RLIC group. Oxygen saturation during conditioning on the conditioning arm was $0 \pm 0\%$ in the RLIC group and $97.93 \pm 0.11\%$ in the sham group (P = 0.001). Pain reports from the RLIC group (2.43 ± 0.20) were higher than those in the sham group $(1.69 \pm 0.23, P = 0.001)$. Individual ratings of pain were variable; no participant reported pain higher than 4/10. Average systolic and diastolic BP data for both groups across 8 visits is shown in S1 Appendix.

Strength

Overall, RLIC paired with strength training resulted in greater strength gains than sham conditioning paired with strength training. Average training load in the RLIC and sham groups during the 2 weeks is shown in Fig 3A. Both groups were trained at a similar workload over the 6 training sessions (visits 3–8) (P = 0.99).

Fig 3B shows 1 RM data at pre-, post-, and follow-up testing for each group. Both groups gained strength over time (main effect of time, P = 0.001). The RLIC group had a greater gain in strength compared to the sham group (group x time interaction, P = 0.035). No significant differences between groups were found overall (main effect of group, P = 0.844), or at specific time points (pre-, post-, and follow-up; P = >0.005). The greater strength gain was verified by a greater percent change in strength in the RLIC vs. sham group (P = 0.002, Fig 3C).

The RLIC group also exhibited a trend towards greater neural adaptations to strength training as seen in Fig 4A. Both groups showed increased neural drive over time (main effect of time, P = 0.006). No significant differences in EMG amplitude were found between groups (main effect of group, P = 0.590), nor group by time interaction (P = 0.231). The RLIC group had greater % change in EMG amplitude than the sham group (P = 0.023, Fig 4B). Altogether, these results indicate that, as an adjunct to training, RLIC enhances strength gain.

Our post-hoc power analysis showed that with average 1 RM (Fig 3B), a standard deviation of 11 and a correlation of 0.97 among repeated measures of 1 RM from the observed data, a total sample of 30 (15 in each group) provided 82.6% power to detect a significant group x time interaction using two-sided tests based on mixed model analysis of repeated measures at alpha level of 0.05. With the observed average EMG amplitude (Fig 4A), a large standard deviation of 43 and a correlation of 0.69 among repeated measures, a total sample of 30 (15 in each group) provided only 29% power to detect a significant group x time interaction using two-sided F-tests based on mixed model analysis of repeated measures at significance level 0.05. Thus, there was sufficient statistical power to detect interactions for the strength measure, but not the neural adaptation measure.

Discussion

This study investigated the effects of RLIC combined with strength training on strength gains in healthy young adults. The major finding of the present study is that combining RLIC with strength training increased wrist extensor muscle strength by 44%. The strength gains also appeared to be parallel with increases in muscle activation, potentially due to greater neural adaptations to strength training when combined with RLIC. Altogether these preliminary results suggest that RLIC holds promise to enhance skeletal muscle strength training.

To the best of our knowledge, this study represents the first time that RLIC paired with high intensity strength training has been tested and shown to facilitate muscle strength gain. This study applied RLIC to the dominant arm prior to strength training, and strength training and testing were performed on the non-dominant, non-conditioned arm. Thus, systemic effects of RLIC improved muscle strength, not local effects [22, 48]. Results of our study open the possibility that RLIC has the potential to enhance strength gains in a short period of time in individuals undergoing rehabilitation for various conditions that result in muscle weakness as well as to improve muscle performance in athletes. If the promise of this combinatorial therapy were to hold true in future, larger, later phase studies, then this clinically feasible, inexpensive paradigm could be easily implemented in clinics and exercise facilities.

The 44% strength gain seen in the RLIC group is notable with a 2-week strengthening duration. Strength training with longer durations (6–12 weeks) and similar intensities (> 70% of 1 RM) reported an average of 35% strength gains [80]. Two studies that used strength training

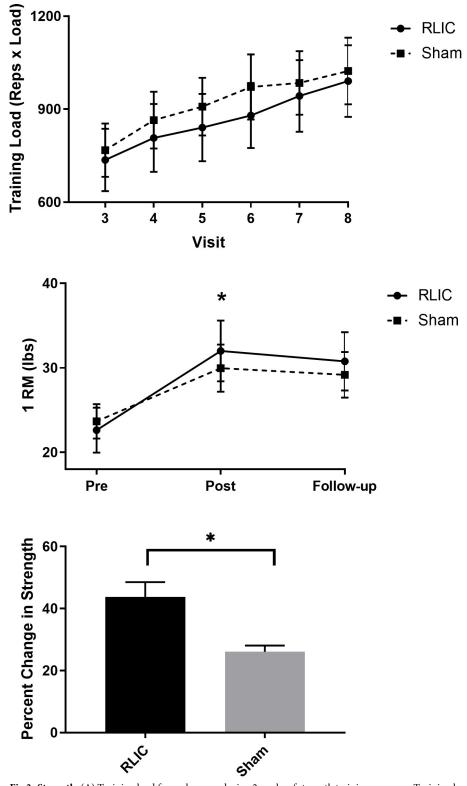


Fig 3. Strength. (A) Training load for each group during 2 weeks of strength training program. Training load was calculated as total number of repetitions by average load during each visit. Training visits 3–8 occurred on alternate weekdays. (B) 1 Repetition Maximum (1 RM) of the wrist extensor muscles on the non-dominant arm for each group. From pre- to post-test, mean change score in 1 RM in the RLIC group was 3.07 ± 0.75 lbs greater than the sham group. * indicates P<0.05 (group x time). (C) Percentage change in strength from pre- to post-test between groups. On

average, the RLIC group demonstrated 17.67 \pm 10.75% relative increase in wrist extensors strength compared to the sham group. * indicates P<0.05.

protocols with short durations, and similar intensity, frequency, and progression showed 10% and 17% gains in ankle dorsiflexors over 2 weeks [58, 81], and 33% gains in hand muscles over 4 weeks [82]. Rapid, large improvements in strength could be highly relevant for many clinical populations. Our results from RLIC plus strength training are in line with previous reports showing the beneficial effects of a single session of RLIC on muscle performance in the absence of training [17, 18, 23, 25, 33]. It should be noted that our results showed significant group x time interaction in absolute strength gain; however, there were no significant group differences. Although RLIC group had a 3.03 lbs greater gain in strength compared to sham group from pre- to post-testing, we speculate that 2 weeks of strength training might not be sufficient for detecting group differences at post-intervention.

Given the exploratory nature of this study, we can only speculate about the possible vascular and cellular mechanisms underlying strength gains. Previous studies show that RLIC causes vasodilation due to release of several humoral factors [83, 84], increasing blood flow to skeletal muscles [85]. Such increased vasodilation might improve delivery of oxygen and nutrients to the increasing metabolic demands of muscles during strength training [20], thereby improving the performance of muscles [30, 86]. Secondly, RLIC attenuates lactate production [18], which potentially lowers sensitivity to fatigue signals [23]. Third, RLIC enhances muscle efficiency in ATP usage via ATP sparing and by improving efficiency of excitation-contraction coupling [12, 15, 87]. All these mechanisms might have contributed to the enhanced strength gains we witnessed when RLIC was paired with strength training.

Strength gains are often partially a function of neural adaptation to training [88]. Although the strength gains here were accompanied by a > 30% change in neural drive, these changes in neural adaptation achieved statistical significance between groups only when considered as a percent of baseline values. It is possible that the lack of group difference seen in EMG amplitudes stem from insufficient duration of training [59, 89]. More likely however, is that the large standard deviations in the RLIC group reduced our ability to find differences. Indeed, the percent change in EMG in RLIC group is comparable to changes elicited by 3–4 weeks of similar training, reflecting increased neural drive [59, 88–91]. Although the EMG results are mixed, the data are consistent with the findings from single-session studies that RLIC augments neural drive [25, 33, 92]. Others have shown that group III and IV afferent neurons in skeletal muscle are blocked by humoral factors released by RLIC [93, 94], which increases central motor drive and facilitates efferent neural drive to increase motoneuron activation [95, 96]. This is one potential underlying mechanism that might have facilitated greater neural adaptation to strength training when combined with RLIC.

The cardioprotection literature suggests short/early (0–12 hours) and long/delayed (24–72 hours) phase protection response to ischemic conditioning, which is mediated by different mechanisms [71]. In this study, we performed strength training within 5–25 minutes after the RLIC, i.e. within the early phase of RLIC response. The post-testing of strength was performed within 24–36 hours after the RLIC session, i.e. within the delayed phase of RLIC response. Although the mechanisms of the early and delayed phase of RLIC response to improve muscle performance are unknown, we speculate that RLIC mediated changes in the strength measures might differ between the early and delayed phases and should be investigated in the future studies. Moreover, it would be interesting to explore the effects of strength training performed during delayed phase on the strength outcomes. Future trial designs should include strength training after 24 hours of the RLIC session.

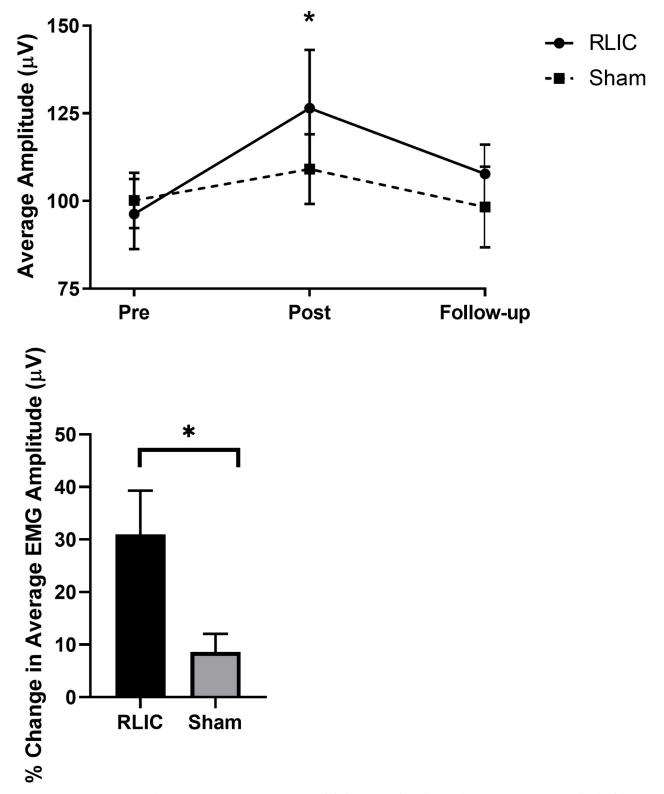


Fig 4. Electromyography (EMG). (A) EMG activity in extensor carpi radialis longus muscle on the non-dominant arm. Average amplitude of the EMG signal from pre- to post-test was increased in the RLIC compared to the sham group. (B) Percent change in EMG amplitude between from pre- to post-test between groups. On average, the RLIC group demonstrated $22.36 \pm 4.89\%$ relative increase in EMG amplitude compared to the sham group. Values are means \pm SE. * indicates P<0.05.

We recognize four main study limitations and propose future study directions based on these preliminary results. First, this is an early phase trial that combined RLIC with a shortduration strength training protocol. Although the data are promising, future studies combining RLIC with longer duration of strength training are warranted in healthy young and older populations. Biweekly assessments of strength during such studies are recommended in order to assess the time course over which benefits accrue. A second limitation is that the conditioning pressure used in the sham group was 10 mmHg below diastolic BP. Although this pressure did not cause ischemia in the conditioned arm, the average pressure in the sham group was comparable to pressure used in a blood flow restriction study [49]. The average 23% strength gains in the sham group, which was higher than expected [58, 81], could be a result of venous blood flow restriction on the conditioned arm affecting the training of the unconditioned arm. Thus the sham conditioning used here may not be a true sham group. A third limitation is that RLIC alone has been shown to increase strength and muscle performance, without any training [15, 17–22, 26], and we did not include such a group. Future study designs could include four groups (RLIC alone, RLIC + training, BFR + training, and a true sham + training) to compare the effects of all four protocols. Lastly, although we used standardized verbal encouragement for strength testing and training for all the participants, lack of accessor blinding could introduce potential bias in the strength measures. Future studies should incorporate doubleblind design where both the participants and assessors are blinded to the intervention group assignment.

Conclusions

When paired with strength training, RLIC facilitates greater strength gains in healthy young adults. RLIC is a safe, inexpensive, and clinically feasible method. Future randomized controlled trials and mechanistic studies are warranted in various populations that could benefit from this therapy. Testing this intervention in different populations such as older adults, and individuals with neurological injuries and/or chronic disease will help in understanding if the responses to RLIC vary among different populations, and whether this adjunct intervention can help these patients.

Supporting information

S1 Checklist. CONSORT 2010 checklist of information to include when reporting a randomised trial*.

(DOC)

S1 Appendix. (DOCX)

S1 Data. (XLS)

S1 Protocol. (RTF)

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8 Kinematic and electromyographic analysis of variations in Nordic hamstring exercise

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Abstract

The purpose of this study was to present and biomechanically evaluate several variations of the Nordic hamstring exercise (NHE), achieved by altering the slope of the lower leg support and by asumming different hip flexion angles. Electromyographic and 2D kinematic measurements were conducted to analyse muscle activity (biceps femoris, semitendinosus, gluteus maximus, erector spine and lateral head of the gastrocnemius), knee and hip joint torques during 6 variations of NHE. The study involved 18 adults (24.9 ± 3.7 years) with previous experience in resistance training, but with little or no experience with NHE. Increasing the slope of the lower leg support from 0° (standard NHE) to 20° and 40° enabled the participants to perform the exercise through a larger range of motion, while achieving similar peak knee and hip torques. Instructions for increased hip flexion from 0° (standard NHE) to 25°, 50° and 75° resulted in greater peak knee and hip torgue, although the participants were not able to maintain the hip angle at 50° nor 75°. Muscle activity decreased or remained similar in all modified variations compared to the standard NHE for all measured muscles. Our results suggest that using the presented variations of NHE might contribute to optimization of hamstring injury prevention and rehabilitation programs, by providing appropriate difficulty for the individual's strength level and also allow eccentric strengthening at longer hamstring lengths.

Introduction

Nordic hamstring exercise (NHE) is commonly used in hamstring conditioning protocols, especially for injury prevention. Studies have shown that implementation of NHE into the training process can significantly reduce the incidence of hamstring strain injuries in high-speed running sports [1,2]. Moreover, numerous positive neuromuscular adaptations after performing the NHE have been demonstrated. For instance, significant improvement in eccentric hamstring strength was reported following an implementation of 4–10 weeks of NHE training [3–5]. Iga et al. [4] have also reported an improvement in eccentric hamstring strength at three angular velocities (60°/s, 120°/s and 240°/s) after a 4-week NHE intervention,

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even though the NHE was performed at a relatively slow pace. Improvements at higher angular velocities are likely one of the reasons why NHE is effective for hamstring strain injury prevention, considering that most hamstring strain injuries occur at high movement velocities (i.e., sprinting). Furthermore, significant lengthening effect on hamstring muscle fascicles resulting from an eccentric hamstring strengthening protocol have been shown [6–11]. Consequently, several research groups reported peak knee torque shifts towards position closer to complete knee extension (i.e., towards longer hamstring length) [5,8,12,13], which is another mechanism that is likely to contribute to decreased incidence of hamstring strain injuries, as these most often occur at longer hamstring lengths.

Although the effectiveness of NHE is well documented, several authors have pointed out that NHE has potential disadvantages. Brughelli and Cronin [14] have expressed doubt about whether NHE causes sufficient hamstring activity for optimal eccentric strengthening in its final phase (at smaller knee flexion angles). In addition, Ditroilo et al. [15] reported that the peak electromyographic (EMG) activity of hamstrings can be observed at $65.4 \pm 8.4^{\circ}$ knee flexion during the NHE and that a marked decrease is seen at 45° of knee flexion. Tillaar et al. [16] reported similar knee flexion angle at peak hamstring activation. They also showed an increase in hip flexion angle at the position of peak hamstring activity, which indicates difficulty to perform the NHE with an optimal form. Thus, the potential problem of NHE is the general difficulty of the exercise. Only sufficiently strong athletes are able to take full advantage of the exercise, with an active descend lasting until nearly fully extended knee position. Since the majority of the hamstring muscles also cross the hip in addition to the knee, performing NHE with neutral hip position does not enable strengthening the hamstrings at the longer lengths. This could be important drawback of NHE, since most of the hamstring strain injuries during sprinting [17] occur in the final part of the swing phase when the hamstring muscle-tendon complex reaches a significantly larger length compared to NHE, due to the significantly greater hip flexion (55–65°). [18] Therefore, the standard version of NHE might not be an optimal exercise for prevention of the hamstring strain injuries. Recently, attempts have been made to modify NHE in order to remove its drawbacks [19], however, the effects of different NHE variations on biomechanical parameters and muscle activity have not been thorougly tested yet.

The purpose of this study was to present and biomechanically evaluate potentially improved NHE variations that were obtained with (i) changing the slope of the lower leg support and (ii) changing the hip joint angle instructions. Such adjustments could possibly eliminate the existing disadvantages of the standard NHE. Including different variations of NHE in an individual's training regimen could contribute to the larger or quicker hamstring muscle adaptations and further enhance its effectiveness in hamstring strain injury prevention. In our evaluation of different variations of NHE, we were primarily focused on differences in peak torques and angles at the knee and the hip joints, as well as peak EMG activity. We hypothesized that both approaches of NHE modifications (i.e. increasing the slope of the lower leg support and instructing to maintain larger hip flexion angle during NHE) would allow the participants to reach similar peak knee and hip joint torques at longer estimated hamstring lengths (reflected in larger knee and/or hip joint angles), compared to the standard NHE. Furthermore, we hypothesized that peak EMG activity of all measured muscles would also remain similar for all of the variations of NHE.

Methods

Participants

Eighteen healthy volunteers (5 females, 13 males) participated in the study. The sample characteristics were (mean \pm SD): age 24.9 \pm 3.7 years, body mass 74.1 \pm 14.1 kg, body height

Competing interests: NŠ and GM were employed by commercial companies, S2P, Ltd. and Motus Melior, Ltd. The companies did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. 176.0 ± 8.9 cm, BMI 23.7 ± 2.6 kg/m², body fat $15.9 \pm 4.3\%$, muscle mass $79.9 \pm 4.2\%$. Minimal sample size of 15 participants was determined a priori for 80% statistical power, an alpha error of 0.05 and an effect size of 0.5. The inclusion criteria were: performing regular physical activity, experience with strength training, little or no experience with NHE and the ability to descend actively to at least 50% of the range of motion in the standard NHE. The exclusion criteria were: neural, muscular, skeletal or connective tissue injuries during the last 12 months in the area of the back, hips and legs. All participants were informed about the purpose and content of the study and gave written informed consent prior to participation. The individual pictured in Fig 1 and Fig 2 has provided written informed consent to publish their image alongside the manuscript. The study was approved by the National Medical Ethics Committee (0120-690/2017/8) and conducted according to the Decla-ration of Helsinki.

Study protocol

To assess biomechanical differences between NHE variations, a single-visit cross-sectional study design was used. Before the warm-up, body mass, body fat percentage and muscle mass percentage were measured using a bio-impedance scale (Tanita MC-980MA, Tanita, Tokyo, Japan). Then, the participants performed a warm-up, consisting of light aerobic activity (6 minutes of alternating stepping on a 25 cm high box), 8 repetitions of dynamic stretching exercises (hip circles, forward, backward and side hip bending, leg swings) and 10 repetitions of strength bodyweight exercises (squats, heel rises, hip bridges, Jackknife sit-ups and hip extensions). After the warm-up, EMG electrodes and kinematic markers were attached (detailed locations are described in further paragraphs). Five repetitions of each variation were performed, with 2 additional familiarization repetitions before each NHE variation. The rest between NHE variations was 3 minutes while the rest between familiarization and actual trials of the same variations was 2 minutes. The rest between each repetition within each NHE variation was sufficent for the subject to comfortably return into the starting position (5-10 seconds). The order of the variations was randomized between participants. After performing all NHE variations, the kinematic markers were removed, and participants performed maximal voluntary isometric contractions (MVC) for the purpose of EMG normalization. For each muscle, 3 repetitions of 3-second maximal isometric exertion against external resistance were performed as follows: trunk extension in isometric dynamometer (S2P, Science to practice, Ltd., Ljubljana, Slovenia) in an upright stance with fixed pelvis for the erector spine muscles, hip extension in a prone position on a physio bed against fixed straps, placed just above the knee, with 90° knee flexion for gluteus maximus, plantar flexion against external resistance in neutral ankle position in an upright stance (attempting to lift overloaded Olympic bar on a smith machine, using only ankle joint) for the lateral head of gastrocnemius and knee flexion in a prone position on a physio bed against fixed straps, placed above the calcaneus bone, with knee flexed at 45° for the biceps femoris and semitendinosus. The knee angle was determined in view of the previous studies [20-22], showing highest knee flexion torque during isokinetic tests and a similar EMG activity of the biceps femoris and semitendinosus at 45° of knee flexion. Loud verbal encouragement by the examiner was provided during all MVC trials.

Nordic hamstring exercise variations

For the implementation of all variations of NHE, a custom-designed device with adjustable length and slope of the lower leg support was used (S2P, Science to practice, Ltd., Ljubljana, Slovenia). Table 1 and Fig 1 show key differences between NHE variations used in this study.

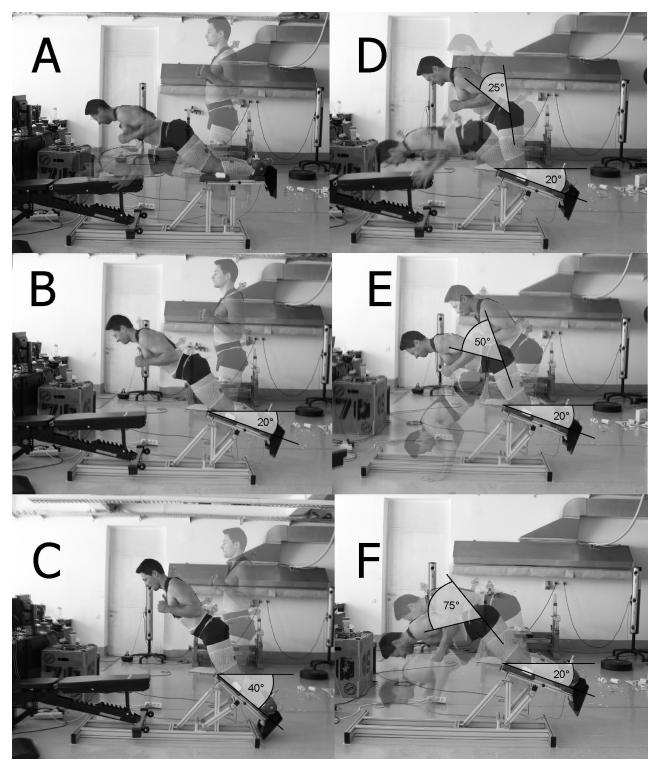


Fig 1. Depiction of NHE variations. Standard NHE (A) was modified by changing the slope of the lower leg support ($B-20^\circ$, $C-40^\circ$) and by instructing the participants to maintain different hip flexion angles throughout the movement ($D-25^\circ$, $E-50^\circ$, $E-75^\circ$).

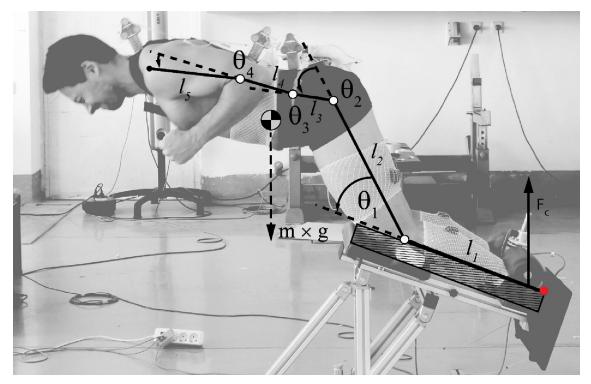


Fig 2. Planar inverse dynamics model. The participant performing the task motion was represented by a five-segment model. Segment angles for the shank (l_1) , thigh (l_2) , pelvis (l_3) , lumbar spine (l_4) and thoracic spine (l_5) were defined as shown. The origin of the model, which is also the point where the model is fixed to the base, is marked with a red dot. F_c (contact force) was measured by the built-in dynamometer.

Three different slopes $(0^{\circ}, 20^{\circ}, 40^{\circ})$ of lower leg support and four hip flexion positions $(0^{\circ}, 25^{\circ}, 50^{\circ} \text{ and } 75^{\circ})$ were used. For all variations with different hip angle instructions, the slope of the lower leg support was set at 20°. Note that the amount of hip flexion refers to researcher's instruction and was not necessarily maintained by the participants to the end of the range of motion. Before each repetition, the appropriate hip flexion was determined using a goniometer. Participants were instructed to position the hands along the body, with an approximately 130° elbow flexion in all variations (Fig 1).

Data acquisition and processing

Contact forces were measured at a sampling rate of 500 Hz at the ankle support (Fig 2) using a built-in dynamometer (Optoforce 3D, Budapest, Hungary). Three-dimensional marker

Variations in Fig 1	Slope of the lower leg support	Hip flexion instruction	
A	0°	0°	
В	20°	0°	
С	40°	0°	
D	20°	25°	
E	20°	50°	
F	20° 75°		

Table 1. Key differences between NHE variations.

trajectory data were collected at a sampling rate of 100 Hz, using the 3D Optotrak Certus motion capture system with 2 cameras (NDI Inc., Ontario, Canada). Active markers were placed (unilaterally) on the bony landmarks of at the ankle (lateral malleoli), knee (lateral condyle of tibia) and hip (greater trochanter). Additionally, five rigid marker clusters were secured to the pelvis (cluster at the sacrum), lumbar region (cluster near the thoracic level of T12), upper body (cluster near the cervical level of C7) and mid-upper arm and mid-lower arm. Small gaps of missing data were filled in using linear interpolation method. Signals were smoothed using low frequency fourth-order Butterworth filter, with 5 Hz cut-off frequency [23]. Joint torques were calculated by using a planar (2D) inverse dynamics model, built with a segmental method [24,25] and by using the segments' inertial parameters from de Leva [26]. The model consisted of 5 segments (shank, thigh, pelvis, lumbar and thoracic spine) and 4 joints (knee-q1, hip-q2, lumbar-q3 and thoracic-q4) and it was fixed to the base at the beginnig of the first segment (Fig 2). 2D joint moments were then computed in MATLAB 2015b (The MathWorks, Natick, USA) in which we used the Spatial v2 package as by Featherstone [27]. Main outcome measures were peak knee torque, peak hip torque (not necessarily achieved at the same time point during the exercise) and peak hip + knee torque (the higest sum of the torques at the same time point). Additionally, we calculated knee, hip, hip + knee and lumbo-pelvic angles at time point of peak hip + knee torque.

For EMG activity assessment, Trigno Delsys Wireless System was used (Delsys Inc., Massachusetts, USA), with pre-amplified self-adhesive wireless electrodes (dimensions: 27 x 37 x 15 mm; mass: 14.7 g; electrode material: silver; contact dimension: 5 x 1 mm) placed bilaterally on erector spine muscles, gluteus maximus, biceps femoris, semitendinosus and lateral head of the gastrocnemius. Prior to sensor placement, the skin over the muscles was shaved, abraded and cleaned with alcohol. EMG sensors were placed according to SENIAM recommendations [28], as shown in Table 2. Their location was confirmed with palpation and isometric muscle contractions.

The EMG data was acquired at 2000 Hz and processed in the following order: 1) band pass filtration using Butterworth second-order filter (20–500 Hz), 2) rectification, using root mean square function (0.05 second window length and point-by-point overlap), 3) smoothing, using moving average function (0.05 second window length and point-by-point overlap). The main outcome measure was peak EMG activity for all muscles, which was determined as highest mean value on 0.25 window length and expressed as percentage of maximal EMG activity during MVC trials (processed in the same order and calculated as maximal value on a 0.25 window length, which is in line with previous studies) [29].

Muscle	Location	Orientation		
Erector Spinae	At 2 finger width lateral from the proc. spin. of L1.	Vertical.		
Gluteus Maximus	At 50% on the line between the sacral vertebrae and the greater trochanter.	In the direction of the line from the posterior superior iliac spine to the middle of the posterior aspect of the thigh.		
Biceps Femoris	At 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia.	In the direction of the line between the ischial tuberosity and the lateral epicondyle of the tibia.		
Semitendinosus	At 50% on the line between the ischial tuberosity and the medial epycondyle of the tibia.	In the direction of the line between the ischial tuberosity and the medial epycondyle of the tibe		
Lateral head of the gastrocnemius	At 30% on the line between the head of the fibula and the heel.	In the direction of the line between the head of the fibula and the heel.		

Table 2. Locations and orientations of electromyographic sensors for different muscles, as recommended by SENIAM [28].

Statistical analysis

The data were statistically processed in the SPSS 22 computer program (IBM, New York, USA). Descriptive statistics were calculated and reported as mean \pm standard deviation. Shapiro-Wilk test was used for testing of normality and Levene's test for equality of variances. Differences among corresponding variables obtained from different NHE variations were tested with the analysis of the variance for repeated measurements. For pair-wise comparisons, paired 2-tailed post-hoc t-tests with Bonferroni's correction were used. Furthermore, the effect sizes were calculated (Cohen's d) and interpreted as small (d = 0.2), moderate (d = 0.5) and large (d = 0.8) [30]. The level of statistical significance was set at p < 0.05 for all analyses.

Results

All participants performed all variations of the NHE. An example of joint torque, joint angles and raw EMG activity signals for one repetition is shown in Fig 3.

Peak joint torques

Changing the slope of the lower leg support did not affect peak torque at the knee ($F_{(2)} = 0.651$; p = 0.528; d = 0.037), hip ($F_{(2)} = 0.607$; p = 0.551; d = 0.034) or hip + knee ($F_{(2)} = 1.073$; p = 0.353; d = 0.059). Instructing the participants to maintain different hip flexion angles had a statistically significant effect on peak knee, hip and hip + knee torques (F = 15.008-74.101; all p < 0.001, d = 0.496-0.813). Lowest peak knee torque was achieved in 0° hip flexion variation (297.69 ± 69.98 Nm) and highest in 75° hip flexion variation (355.54 ± 78.82 Nm). Similar trend was observed for hip and hip + knee peak torques. Changes in peak torque data are shown on Fig 4.

Joint angles

Joint angles were analysed at the time point of peak hip + knee torque. Note that 0° represents knee angle at the starting position of NHE and thus it increases during the descend towards the full knee extension. This method was used to enable for calculation of the estimated hamstring length, i.e. hip + knee angle, in which greater values represents longer hamstring length. Knee angle at the moment of peak hip+knee torque was significantly increased in NHE with 20° slope $(75.01 \pm 7.30^\circ)$ and 40° slope $(87.91 \pm 7.45^\circ)$ of lower leg support, compared to the standard NHE (56.10 \pm 9.08°) (F₍₂₎ = 100.3; p < 0.001; d = 0.855). Knee angle remained similar as changing hip flexion instructions ($F_{(3)} = 2.510$; p = 0.069; d = 0.129). Hip flexion angle at the moment of peak hip+knee torque was significantly decreased in NHE with 20° slope (5.64 ± (6.76°) and 40° slope $(3.94 \pm 8.06^{\circ})$ of lower leg support, compared to the standard NHE $(9.39 \pm 8.36^{\circ})$ (F₍₂₎ = 67.31; p < 0.001; d = 0.798). Hip flexion angle at the moment of peak hip +knee torque was significantly increased at larger hip flexion instructions ($F_{(2)} = 46.23$; p < 0.001; d = 0.731). However, no differences were shown by pairwise tests between 50° and 75° variation ($t_{(17)} = 1.61$; p = 0.125; d = 0.133). Participants did not maintain the instructed hip flexion angles in 50° and 75° variations at the moment of the peak torque ($26.94 \pm 9.46^\circ$ and $25.23 \pm 16.62^\circ$, respectively). Lumbar-pelvic angle at the moment of peak hip+knee torque increased with increasing angle of hip flexion instructions ($F_{(3)} = 28.08$; p < 0.001; d = 0.623), but not with changing the slope of lower leg support ($F_{(2)} = 2.76$; p = 0.077; d = 0.140). Changes in joint angles at the time point of peak hip + knee torque are shown on Fig 5.

Muscle activity

For all measured muscles in all NHE variations, peak EMG activity was detected at the moment (\pm 25 ms) of peak hip + knee torque. Increasing the slope of lower leg support

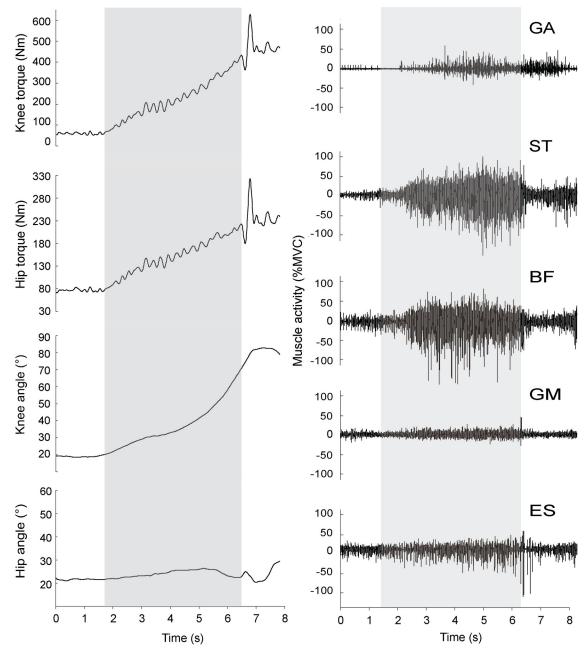
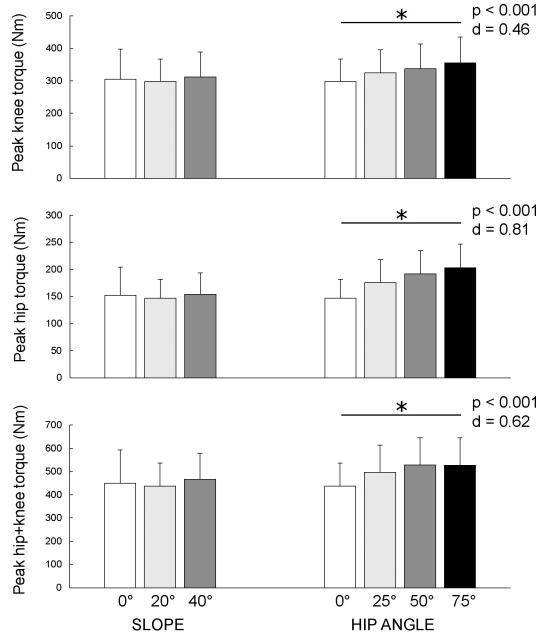
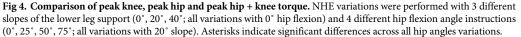


Fig 3. A representation of a typical kinematic values and raw EMG signals. Data is presented for one repetition during the variation with 20° slope of lower leg support and instructions to maintain 25° of hip flexion. The grey area represents the analyzed timeframe, after which the participants ceased to maximally contract the muscles and dropped down. Signals were manually inspected and time of actual peak torques were determined. GA-gastrocnemius, ST-semitendinosus, BF-biceps femoris, GM-gluteus maximus, ES-erector spinae.

significantly decreased EMG activity of all analyzed muscles ($F_{(2)} = 8.36-22.29$; p = 0.001-0.002; d = 0.343-0.567) (Fig 5). Pairwise comparisons revealed statistically significant differences between 0° and 20° slopes for all muscles except semitendinosus (p = 0.06) and between 20° and 40° slopes for all muscles except gluteus maximus (p = 0.495). Changing the instructed hip flexion position decreased EMG activity of all muscles ($F_{(3)} = 4.58-79.15$; p = 0.000-0.007;





d = 0,223-0,744), except gluteus maximus (p = 0.287). With the exception of gastrocnemius, there was a trend for EMG activity to drop with increasing hip flexion angle. Changes in peak EMG activity are shown on Fig 6.

Discussion

The main purpose of this study was to evaluate biomechanical differences between the six different NHE variations. We hypothesized that the increased slope of the lower leg support and

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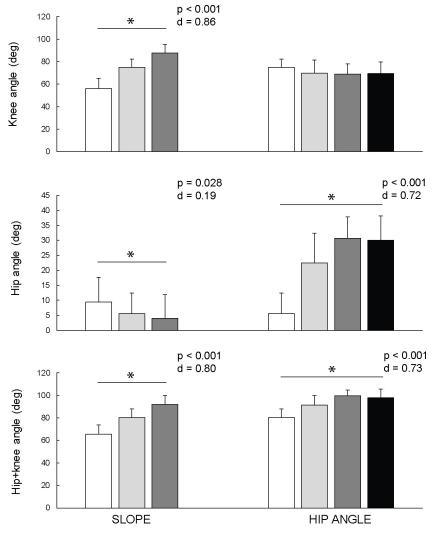


Fig 5. Comparison of joint angles at the time point of peak hip + knee torque. Nordic hamstring exercise variations were performed with 3 different slopes of the lower leg support $(0^{\circ}, 20^{\circ}, 40^{\circ}; all variations with 0^{\circ} hip flexion)$ and 4 different hip flexion angle instructions $(0^{\circ}, 25^{\circ}, 50^{\circ}, 75^{\circ}; all variations with 20^{\circ} slope)$. 0° knee angle represents knee angle at the starting position of NHE. 0° hip angle represents neutral hip position. The sum of hip and knee angle represents estimated hamstring length. Asterisks indicate significant differences across all hip angles variations.

instructions to maintain larger hip flexion would allow the participants to perform the exercise through greater amplitude and reach peak joint torques at longer hamstring lengths, compared to the standard NHE. The results confirm that modifying the slope of the lower leg support allowed the participants to perform the movement through greater amplitude in controlled manner, since the peak joint torques occurred closer to the full knee extension (Fig 5). Morever, compared to the standard NHE, peak joint torques were reached at longer hamstring lengths in all modified variations (Fig 5). The level of the peak torque remained similar in the variations with different inclination of the lower leg support or was increased in the variations with the instruction to sustain hip flexion position (Fig 4). Despite that, peak EMG activity of all measured muscles significantly decreased in all modified variations, compared to the standard NHE. Furthermore, the participants in our study were only able to maintain small to modrate hip flexion throughout the exercise (Fig 5), indicating that our results demonstrate

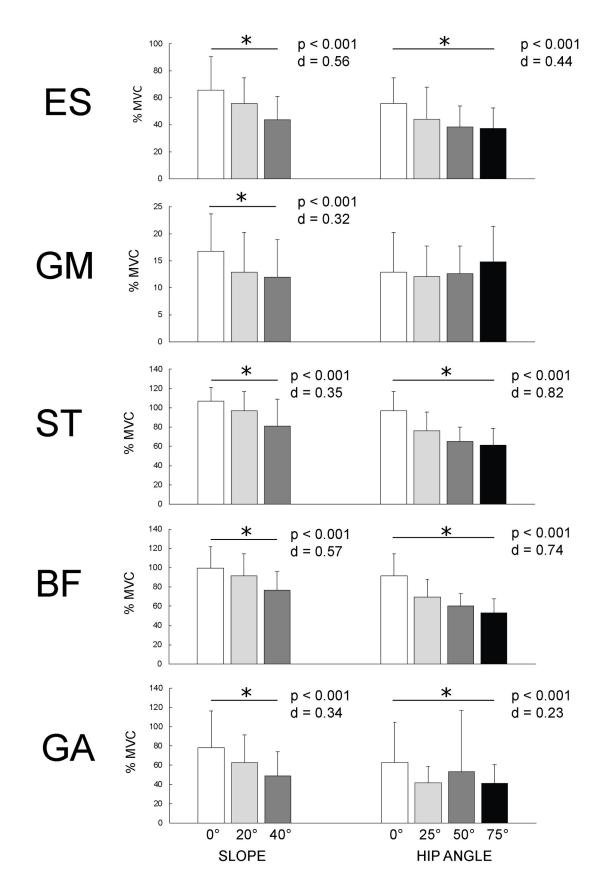


Fig 6. Comparison of EMG activity. Measured muscles were: GA (lateral head of the gastrocnemius), BF (biceps femoris), ST (semitendinosus), GM (gluteus maximus) and ES (erector spinae) in 3 different slopes of the lower leg support $(0^{\circ}, 20^{\circ}, 40^{\circ}; all variations with 0^{\circ} hip flexion) and 4 different hip flexion angle instructions <math>(0^{\circ}, 25^{\circ}, 50^{\circ}, 75^{\circ}; all variations with 20^{\circ} slope)$.

only the effect of different instructions to participants, not the effect of the actual hip flexion angle. Variations with instructions to maintain 50° and 75° of hip flexion turned out to be difficult to perform. In particular, the participants often substantially flexed the lumbar spine, despite being instructed to maintain neutral spine position.

Peak joint torques and joint angles

The rationale behind changing the slope of the lower leg support was to decrease the knee flexion angle at the moment of peak knee torque, in order to allow an individual to perform NHE through greater range of motion in a controlled manner. Furthermore, performing the exercise with flexed hips could additionally lengthen the hamstring muscles. That would enable eccentric strengthening at hamstring lengths similar to those at which most strain injuries occur during sprinting. Our results show that all modified variations of NHE allowed the participants to reach peak hip and knee torques at longer estimated hamstring lengths (Fig 5). Specifically, during the standard NHE, similar peak joint torques occurred at a smaller knee angle $(56.10 \pm 9.08^{\circ})$, compared to the modified variations, during which participants could achieve nearly complete knee extension (75.01 \pm 7.30° at 20° slope and 87.91 \pm 7.45° at 40° slope). Moreover, when participants were instructed to maintain larger hip flexion angles, their average estimated hamstring length (hip + knee angle) at the moment of peak torques increased, mostly as a result of increased hip angle (Fig 5). At the same time, this type of modification of NHE proved to be effective for increasing peak knee torque, which is in line with the results of previous research that reported larger knee flexion strength when the hip is flexed [20,31]. It is known that training at longer hamstring lengths is effective for hamstring strain injury rehabilitation [32] and can favorably affect several architectural and functional characteristics of the hamstring muscles, but the underlying mechanisms are not yet completely understood. Recently, Guex et al. [8] compared eccentric hamstring conditioning protocols at shorter and longer lengths, and reported no significant differences in changes in fascicle length nor pennation angle. Although we demonstrated favorable effects of NHE variations on kinematic variables, further studies are needed to confirm whether performance of these variations lead to different architectural and functional adaptations of the hamstring muscles, compared to the standard NHE.

It is necessary to stress the discrepancy between the instructions for keeping a particular hip flexion angle (0°, 25°, 50°, 75°) during the NHE and the hip flexion value that was actually maintained at the moment of peak hip + knee torque. Namely, the average hip flexion achieved at the moment of the peak hip + knee torque during variations with the instruction for 50° and 75° hip flexion were only $30.74 \pm 7.10°$ and $29.99 \pm 8.19°$, respectively. Kinematic data also shows that the average hip flexion during 50° and 75° NHE variations was already 10-20° lower than instructed in the starting position (despite using the goniometer and constant verbal warnings) and that it rapidly declined in the last 20% of the range of motion. In contrast, lumbar-pelvic angles significantly increased with increased level of the instructed hip flexion, which implies that the participants did not manage to maintain neutral lumbar curvature as instructed. By attempting to follow the instructions for larger hip flexion (50° and 75°), the participants actually performed a combination of hip flexion, pelvic rotation and spine flexion. Future studies are needed to define what level of NHE strength is needed to perform these variations of NHE without lumbar flexion. Based on these results and the fact that a big

proportion of high-level athletes and most of the recreational athletes are not able to perform the standard NHE through full range of motion [4,15], we propose that those individuals start with with neutral hip position on either 20° or 40° slope of the lower leg support (depending on their strength level). The next step in progression would be gradually increasing hip flexion, while lowering the slope of the lower leg support to 0°. When an individual is capable to perform the NHE at the 0° slope of the lower leg support and with 75° of hip flexion, he/she can then progress to the weighted variations.

Muscle activity

Despite the fact that the peak knee and hip torques remained similar (in variations with increased lower leg support slope) or were increased (in variations with increased hip flexion angle) compared to the standard NHE, the peak hamstring activity significantly decreased during all modified NHE variations. Moreover, the largest peaks in knee and hip torque were achieved concomitantly with the lowest peak hamstring EMG activity. A possible explanation for this phenomenon is that the non-contractile elements contributed a larger proportion of the force, due to the longer length of the hamstrings. The results are consistent with the findings of Higashihara et al. [22] and Lunnen et al. [33], who reported inverse relationship between hamstring EMG activity and hamstring length during eccentric and isometric knee flexion contractions. However, two recent studies [31,34] reported no differences in peak EMG activity during maximal voluntary knee flexion between different hamstring lengths, achieved by altering hip angle. In the present study, lower EMG activity during modified variations of NHE was also observed for other muscles, suggesting that these were unloaded as well. The only exception was the gluteus maximus activity, which was relatively low during all variations of the NHE and did not significantly change with hip angle modification. Comparable (<20% MVC) level of activity during standard NHE for gluteus maximus were reported recently by Narouei et al. [35]. However, the activity of erector spine muscles was lower in their experiment (35-40% MVC) compared to our results (65% MVC in the standard NHE).

During the standard NHE, a higher peak in EMG activity of semitendinosus compared to biceps femoris was observed, with a difference of 6.5% (106.7 \pm 15.5% MVC for semitendinosus and 99.7 \pm 22.2% MVC for biceps femoris). Similar or even larger differnces in muscle activity between semitendinosus compared to biceps femoris during the standard NHE were reported by other researchers [16,36,37]. This variability in findings could be related to differences in normalization procedure of peak hamstring EMG (e.g. maximal isometric voluntary contraction or maximal sprinting) and to different methodological approaches to quantification of hamstring activity, namely functional MRI [37] and EMG [16]. Since biceps femoris is injured more often than semitendinosus, other exercises for hamstring conditioning should be considered to be included within a training regimen. For instance, supine leg curls and stiffleg deadlifts were shown to target biceps femoris [29,38,39] more than the other hamstring muscles. It was also shown that lateral rotation of the tibia increases biceps femoris activity compared to semitendinosus during isometric knee flexion [40]. However, such adjustment is not possible for NHE, since the rotation of the tibia cannot be maintained when approaching knee extension.

Several limitations of the study should be acknowledged. In the present study, a familiarization session was not performed before the trials. While all participants had previous experience with resistance exercise and were instructed to perform two familiarization repetitions of each variation, we cannot rule out the presence of the learning effect. Although the variations of NHE were performed in a randomized order, such effect could nonetheless influence our outcomes. Furthermore, participants in our study were not able to maintain all of the instructed hip flexion positions during NHE. This way, the part of the experiment that was conducted to reveal the effects of different hip flexion positions merely showed the effects of instructions to the participants, not the effect of actual hip angles. Participants often flexed the lumbar spine instead of the hip, which could impose undesirable forces on the spine. Lastly, a large number of eccentric repetitions of the same muscle group in the single session could lead to a significant level of fatigue, which could affect peak torque or EMG activity during NHE variations and MVC procedures carried out at the end of the session. Considering all of the above, the results of this study need to be verified in an experiment that includes trained athletes with high level of NHE strength.

Conclusion

The presented modifications of NHE can be used for the purpose of individualization and optimization of strength and conditioning interventions, injury prevention and rehabilitation. It is likely for athletes to progress faster and more efficiently using suggested variations and appropriate progression. In particular, this study has demonstrated that an increase in the slope of the lower leg support allows more controlled descending throughout larger range of motion while reaching similar peak knee and hip torque as in the standard NHE. Individuals who are unable to perform the standard NHE through full range of motion will therefore probably benefit from adjusted slope support, before progressing towards the standard (i.e. horizontal) position of the lower leg. Performing NHE with an increased hip flexion angle may also be effective; however, athletes may change the position of the spine as well as the hip when larger hip flexion angles are instructed to be maintained during NHE.

Author Contributions

Conceptualization: Nejc Šarabon, Jan Marušič, Goran Marković.

Data curation: Jan Marušič, Žiga Kozinc.

Formal analysis: Jan Marušič, Žiga Kozinc.

Investigation: Nejc Šarabon, Jan Marušič, Žiga Kozinc.

Methodology: Nejc Šarabon, Jan Marušič, Goran Marković.

Visualization: Goran Marković.

Writing – original draft: Nejc Šarabon, Jan Marušič, Žiga Kozinc.

Writing - review & editing: Nejc Šarabon, Jan Marušič, Goran Marković, Žiga Kozinc.

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9 Does squatting need attention?—A dual-task study on cognitive resources in resistance exercise

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Abstract

Introduction

Accumulating evidence shows that acute resistance exercises and long-term resistance training positively influence cognitive functions, but the underlying mechanisms have been rarely investigated. One explanatory approach assumes that the execution of resistance exercises requires higher cognitive processes which, in turn, lead to an 'indirect' training of higher cognitive functions. However, current knowledge on the engagement of higher cognitive functions during the execution of resistance exercises is relatively sparse. Hence, the purpose of this study was to examine to what extent cognitive resources are needed to perform a resistance exercise in the form of barbell back squatting.

Methods

Twenty-four young adults performed a cognitive task (serial subtraction of 7's) during standing and during barbell back squatting on a Smith machine. The total number and the number of correct responses were analyzed and taken as indicators of the cognitive load imposed by the experimental condition (squatting) and the control condition (standing). Additionally, participants' perceived exertion, mean heart rate, and the number of squats they were able to perform were assessed.

Results

While accuracy scores were found not to be significantly different between conditions, the numbers of total and of correct responses were significantly lower during squatting than during standing. Additionally, during squatting a higher number of total answers was given in the fifth set compared to the first set. We attribute this phenomenon to a learning effect. Furthermore, there was no statistically significant correlation between cognitive measures and perceived exertion.

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Conclusion

Results suggest that perceived exertion cannot explain the higher dual-task costs observed during squatting. They rather reflect that more cognitive resources are needed to perform low-load barbell back squats than during standing. However, further research is necessary to confirm and generalize these findings.

Introduction

There is growing evidence in the literature that acute resistance exercises and long-term resistance training improve cognitive functions [1–4]. However, the underlying mechanisms for these cognitive improvements are not fully understood yet, although they seem to rely on changes at multiple levels [5–7]. One assumption is that resistance exercises may act as an 'indirect' form of cognitive training since for their execution subjects need to constantly engage cognitive resources as they have to pay attention to perform the movement with an appropriate technique (e.g., squat), to produce an appropriate level of force, and to observe the surroundings in order not to harm themselves or others [3]. Engaging specific cognitive resources to execute a specific motor task (e.g., resistance exercise such as squatting) is deemed a necessary prerequisite to guide the facilitation effects of physical exercises. The latter provides the basis for cognitive improvements in response to physical training interventions [8]. However, to our current knowledge, there is currently no study that investigated the cognitive resources needed to execute dynamic resistance exercises (e.g., squats). Therefore, the assumption that resistance exercises 'indirectly' train cognitive functions due to the engagement of cognitive resources requires further exploration.

An established behavioral approach to quantify the cognitive resources which are needed to execute a motor task is the dual-task paradigm. For instance, the dual-task paradigm is frequently utilized to investigate the amount of cognitive resources required during walking or postural tasks [9–13]. Using the dual-task paradigm, an individual's performance during a single-task condition (e.g., performing a cognitive task) is compared with his/her performance during a dual-task condition (e.g., performing a motor task [e.g., squatting] and a cognitive task simultaneously). The changes in performance from single-task to dual-task (also known as dual-task costs) are used to probe the amount of cognitive resources needed to execute the motor task (e.g., squatting). In this study, we aimed to investigate whether higher cognitive resources are required to perform barbell back squatting. To do so, a dual-task paradigm was applied and the relative increase in cognitive resources needed to perform the resistance exercise 'barbell back squats' was examined.

Materials and methods

Twenty-four (10f/14m) healthy adults participated in this randomized study (mean age (\pm SD): 24.38 \pm 3.15 years; mean height: 173.92 \pm 8.29 cm; mean body mass: 70.25 \pm 11.56 kg). All study procedures were in accordance with the Declaration of Helsinki (1964) and were approved by the local ethics committee of the Medical Faculty of the Otto von Guericke University Magdeburg (181/18). Each participant was asked to visit the laboratories for two sessions at least 48 hours apart. At the first session, the participants were informed about the experimental procedures and had to complete the German version of the Physical Activity Readiness Questionnaire (PARQ) which screens for individuals at increased health risk when

exercising physically [14–16]. All individuals interested in participating in this study verified by self-reports that they were not suffering from musculoskeletal, cardiovascular, and/or neurological disorders. Based on the results of the PARQ and self-reports, individuals at an increased health risk while exercising were excluded from this study. Furthermore, all participants gave their written informed consent to participate in this study and received a compensation of $24 \in$.

In addition, all participants completed the Minimal Mental State Examination (MMSE) [17], Trail Making Test (TMT A&B) [18], the Beck Depression Inventory (BDI-II) [19], and a physical activity questionnaire (BSA; derived from the German Bewegungs- und Sportaktivitätsfragebogen) [20] at their first visit. The MMSE consists of 11 items and screens for cognitive impairments (indicated by lower scores) [17]. The TMT A is considered to measure abilities of visual search while TMT B quantifies the performance of higher cognitive abilities such as cognitive flexibility [21,22]. The difference between the performance of TMT B and TMT A is presumed to be a measure of shifting ability [23]. BDI-II reflects a measure of depressive symptoms whereas higher scores reflect a higher severity of depression [19]. The BSA measures the level of physical activity and physical exercise engaged within the last four weeks [20]. Additionally, the participants' experience in resistance training was quantified using a visual analogue scale ranging from 0 (i.e., no experience) to 100 (i.e., strong experience). After the completion of the questionnaires, a standardized warm-up was conducted to prepare the participants for a one-repetition maximum (1-RM) test. The warm-up consisted of five minutes of stationary cycling (1 W per kilogram body weight at 60 to 80 revolution per minute) and one set of barbell back squats with ten repetitions at light loads [24]. Then, based on established testing protocols [25], several sets of barbell back squats were performed until a load was found which the participants could lift exactly one time with a proper technique (stance width: shoulder width / squat depth: at least horizontal thighs). Between the testing sets, participants rested for at least three minutes and immediately after each set the perceived exertion was quantified using the Repetitions in Reserve scale (RIR) [26]. The RIR was used as countermeasure to verify that the 1-RM is the maximal load that the participants can lift. The 1-RM of the participants was determined within four (± 1) sets and the corresponding mean RIR was ten. The barbell back squats were performed using a Smith machine (integrated in the squat rack by MAXXUS[®]; version 9.1).

During the second visit, the participants performed a single-task condition (solving a cognitive task during standing, [ST]) and a dual-task condition (squatting while solving a cognitive task, [DT]). The conditions were separated by a rest period of ten minutes and conducted in a randomized order (balanced permuted block randomization) by using the Web site 'Randomization.com' (http://www.randomization.com). To make ST and DT comparable, the time of each set was limited to 30 seconds and between the sets, a rest in a standing position of 60 seconds was given (see Fig 1). In this study, a set is defined as the 30 seconds in which the participants solve the cognitive tasks while standing or performing barbell back squats. In DT ten seconds just before the end of the rest period, a cue was given that enable the participants to take the starting position of the barbell back squats to make sure that they had have the full 30 seconds to solve the cognitive task as in ST. After finishing the set in DT, the barbell was placed back into the squat rack. In DT the participants were allowed to squat with their preferred repetition velocity. In each condition, five sets were conducted and in DT the load of the barbell was set to of 40% of 1-RM. We choose 40% of 1-RM because it was shown that even this low load leads to cognitive improvements [27]. As cognitive task, we used the serial subtraction of 7's from a three-digit number [28]. A new, randomly assigned three-digit number was given to the participants at the beginning of each set in ST and DT. Furthermore, as shown in Fig 1, the

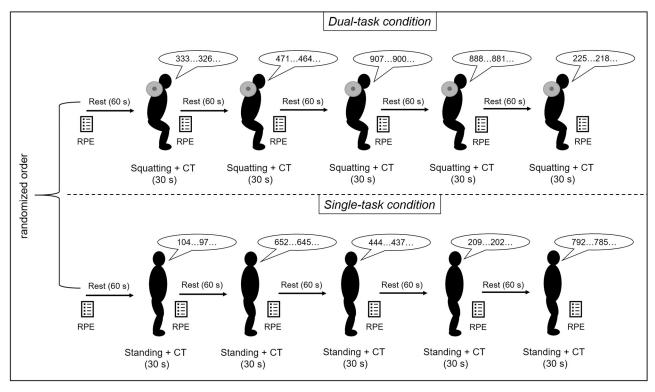


Fig 1. Overview of the experimental protocol in the second session and the time points of the assessment of RPE. The cognitive task was solved in a standing position (single-task) and during squatting (dual-task). Both tasks were performed for 30 seconds and afterwards the participants rested in a standing position for 60 seconds. RPE: Rating of perceived exertion.

rating of perceived exertion (RPE) using a RPE scale which ranged from 6 (no exertion) to 20 (maximal exertion) was administered during the second visit [29].

Furthermore, we measured mean heart rate (HR) continuously with a portable heart rate (HR) monitor (V800, Polar Electro Oy^(R), Kempele, Finland) and analyzed HR data using 'Kubios HRV' (Biosignal Analysis and Medical Imaging Group, Universität Kuopio, Finland; Version 3.3.1) [30]. In Kubios artefacts were removed by applying the threshold-based artefact correction algorithm. Therefore, we set the threshold to a medium level (i.e., values that differ more than 0.25 s from average were replaced with interpolated values using a cubic spline interpolation) [30–32]. Furthermore, the HR time series was detrended by using the smoothness-priors-based detrending approach (smoothing parameter, $\lambda = 500$) [30]. Thereafter, the mean heart rate was calculated from corrected and detrended HR time series using whole 30 seconds of task periods (i.e., Squatting + DT and Standing + DT; see Fig 1) and the middle 30 seconds of the first rest period (for 'Pre'; see Fig 1 and Table 1).

Additionally, during the sets in DT the number of squat repetitions was counted.

Statistical analysis

The statistical analysis was performed using IBM SPSS (Statistical Package for social science, Version 22, Chicago, IL, USA) and non-parametric tests were conducted because not all data were normally distributed. To compare performance in single-task conditions versus performance in dual-task conditions, the Wilcoxon test was performed. To identify a possible main effect of time (respectively set), a Friedman test with post-hoc analyses (i.e., Wilcoxon tests)

Table 1. Personal data for the characterization of the participants and results of the screening test in the investigated sample; BDI: Becks Depression Inventory; BSA: Physical activity questionnaire, derived from German 'Bewegungs- und Sportaktivitätsfragebogen'; MMSE: Minimal Mental State Examination; PA: Physical activity; PE: Physical exercise; 1-RM: One-repetition maximum; TMT: Trail Making Test.

Parameters	Mean ± SD
Years of education [years]	15.8 ± 3.0
MMSE score	29.60 ± 0.63
BDI-II	3.29 ± 3.43
BSA [min per week]	PA: 313.60 ± 237.33 / PE: 338.95 ± 223.25
TMT A time [sec] / errors	$20.87 \pm 5.16 / 0.04 \pm 0.20$
TMT B time [sec] / errors	41.46 ± 9.25 / 0.08 ± 0.40
TMT B-A time [sec]	20.59 ± 7.77
1-RM [kg]	97.70 ± 27.26
1-RM normalized to body mass	1.39 ± 0.41
Self-rated experience in resistance training	49.79 ± 25.43
Resistance training sessions per week	2.06 ± 1.69

were conducted. Outliers were not removed from statistical analyses because non-parametric test are relatively robust against the effects of those [33,34]. The effect sizes for the Wilcoxon tests were calculated using following formula $r = \frac{|z|}{\sqrt{N}}$ and were rated as follows: 0.5 large effect, 0.3 medium effect, and 0.1 small effect [35,36].

Furthermore, in order to examine possible relationships between cognitive measures and RPE, mean HR, number of squat repetitions, or self-rated experience in resistance training, correlation analyses were performed. Therefore, Spearman's Rho (r_s) was calculated and rated as follows: 0.00 to 0.19 no correlation; 0.20 to 0.39 low correlation; 0.40 to 0.59 moderate correlation; 0.60 to 0.79 moderately high correlation; > 0.8 high correlation [37]. The level of significance was initially set to $\alpha = 0.05$ for all statistical analyses. In order to account for the multiple comparison problem in post-hoc tests and correlation analyses (correction within one tested condition between the five sets), the Holm correction method was applied [38]. Therefore, the $n p_{raw}$ -values (where *n* is the number of p_{raw} -values corresponding to one hypothesis) were ordered in an ascending order starting with the smallest praw-value $(p_{raw(1)}, \ldots, p_{raw(n)})$. Afterwards, the ordered p_{raw} -values are compared to the threshold α_j calculated as follows: $p_{(i)} \le \alpha_j = \alpha/(n - (i - 1))$. The Holm correction will stop at the ith test for which the first non-rejection occurs (i.e., the *i* for which $p_{raw(i)} > \alpha_i$) [38,39]. Furthermore, the corrected p-values ($p_{corrected}$) were calculated by using the following formula [40,41]: $p_{corrected} = p_{(i)raw} \times /(n - (i - 1))$. Please note that for the calculation of $p_{corrected}$ the $p_{raw values}$ were ordered as described in Holm correction.

Results

The general characteristics of the participants are displayed in Table 1.

Cognitive measures

A descriptive overview about the number of total answers and number of correct answers in ST and DT for the task serial subtraction of 7's is provided in <u>Table 2</u>.

To evaluate the effect of DT on the number of total answers and number of correct answers, performance in DT was compared to the performance in ST.

With regard to the number of total answers in the first set (Z (N = 24) = -3.759, $p_{corrected} = 0.001$; r = 0.77), the second set (Z (N = 24) = -3.462, $p_{corrected} = 0.002$; r = 0.71), the third set

Parameter	Pre	1 st set	2 nd set	3 rd set	4 th set	5 th set	
Single-task condition							
Total number of answers	n.a.	12.0 (5.0) *	12.0 (6.0) *	14.0 (5.0) *	12.0 (5.0) *	13.0 (5.0) *	
Number of correct answers	n.a.	12.0 (7.0) *	12.0 (8.0) *	13.0 (6.0) *	12.0 (5.0) *	12.5 (5.0) *	
Accuracy score (in %)	n.a.	100.0 (9.1)	100.0 (0.0)	100.0 (9.2)	100.0 (0.0)	100.0 (7.7)	
RPE score	6.0 (1.0) ^b	7.0 (3.0) * ^{, b}	8.0 (4.0) * ^{, b}	7.5 (3.0) ^{*, b}	7.5 (4.0) ^{*, b}	8.0 (4.0) * ^{, b}	
Mean HR	87.0 (19.5)	92.5 (24.3) *, ^b	86.0 (22.8) *	87.0 (23.3) *	86.5 (20.5) *	87.5 (25.0) *	
Dual-task condition							
Total number of answers	n.a.	9.0 (4.0) *, ^a	10.0 (3.0) *	10.0 (5.0) *	11.0 (4.0) *	11.0 (4.0) *, ^a	
Number of correct answers	n.a.	9.0 (3.0) *	9.5 (4.0) *	9.5 (4.0) *	10.5 (5.0) *	10.0 (4.0) *	
Accuracy score (in %)	n.a.	100.0 (9.1)	100.0 (10.0)	96.2 (11.1)	100.0 (12.2)	100.0 (9.8)	
RPE score	6.0 (1.0) ^b	13.0 (2.0) *, ^b	13.0 (1.0) *, ^{b, c}	14.0 (2.0) *, ^b , ^c , ^d	15.0 (3.0) *, b, c, d, e	15.5 (3.0) *, b, c, d, e, f	
Mean HR	80.5 (17.5)	121.0 (17.3) *, ^b	127.5 (25.8) *, ^{b, c}	130.5 (34.0) *, b, c, d	131.0 (36.3) *, ^{b, c, d, e}	139.5 (41.0) *, b, c, d, e, f	
Number of squat repetitions	n.a.	11.0 (3.0)	11.0 (2.0)	12.0 (3.0)	12.0 (2.0)	12.0 (2.0)	

Table 2. Median (interquartile range) of cognitive measures and RPE in single-task condition and dual-task condition are shown. Additionally, the number of repetitions of barbell back squats in dual-task condition is presented.

^a: indicates a significant difference between 1st and 5th set;

^b: indicates significant difference between 'Pre' and sets;

^c: indicates significant difference between 1st set and the respective set;

^d: indicates significant difference between 2nd set and the respective set;

^e: indicates significant difference between 3rd set and the respective set;

^f: indicates significant difference between 4th set and respective set;

n.a.: not applicable; HR: heart rate; RPE: Rating of relative perceived exertion.

*: indicates a significant difference between single-task condition and dual-task-condition.

(Z (N = 24) = -3.505, $p_{corrected}$ = 0.002; r = 0.72), the fourth set (Z (N = 24) = -3.034, $p_{corrected}$ = 0.005; r = 0.62), and the fifth set (Z (N = 24) = -2.414, $p_{corrected}$ = 0.016; r = 0.50) a lower number of total answers was given in DT compared to ST.

The statistical comparison concerning the number of correct answers shows that in the first set (Z (N = 24) = -3.324, $p_{corrected} = 0.003$; r = 0.68), the second set (Z (N = 24) = -3.576, $p_{corrected} = 0.001$; r = 0.73), the third set (Z (N = 24) = -3.633, $p_{corrected} = 0.001$; r = 0.74), the fourth set (Z (N = 24) = -3.304, $p_{corrected} = 0.002$; r = 0.67), and the fifth set (Z (N = 24) = -2.476, $p_{corrected} = 0.013$; r = 0.51) a lower number of correct answers was given in DT compared to ST.

Furthermore, a significant effect of time was observed for the number of total answers in DT ($X^2 = 13.741$ (df = 4, n = 24), p = 0.008) but not in ST. After post-hoc tests and the following Holm adjustment, it was observed that in DT in the fifth set a higher number of total answers was given compared to the first set (Z (N = 24) = -3.143, p_{corrected} = 0.017; r = 0.64).

A significant effect of time was registered for correct answers in DT ($X^2 = 10.047$ (df = 4, n = 24), p = 0.040) but not in ST. However, the effects observed in post-hoc tests in DT did not remain their statistical significance after the application of Holm correction method.

With regard to the accuracy score, we did neither observe significant differences between ST and DT ($p_{corr} > 0.05$) nor was a statistically significant effect of time in ST ($X^2 = 6.194$ (df = 4, n = 24), p = 0.185) and in DT ($X^2 = 0.712$ (df = 4, n = 24), p = 0.950) noticed.

Psychophysiological measures

Ratings of perceived exertion. A descriptive overview about RPE ratings obtained in ST and DT is provided in Table 2. The difference between RPE values obtained prior to ST

or DT were not statistically significant (Z (N = 24) = 0.000, $p_{corrected} = 1.000$; r = 0.00), whereas after first set (Z (N = 24) = -4.214, $p_{corrected} < 0.001$; r = 0.86), second set (Z (N = 24) = -4.296, $p_{corrected} < 0.001$; r = 0.88), third set (Z (N = 24) = -4.295, $p_{corrected} < 0.001$; r = 0.88), fourth set (Z (N = 24) = -4.295, $p_{corrected} < 0.001$; r = 0.88), and fifth set (Z (N = 24) = -4.291, $p_{corrected} < 0.001$; r = 0.88) the RPE score were significantly larger in DT (see Table 2).

In the ST condition, a main effect of time was observed ($X^2 = 36.684$ (df = 5, n = 24), p > 0.001) and post-hoc test indicate that the RPE scores obtained prior the sets was significantly lower than after first set (Z (N = 24) = -2.953, p_{corrected} = 0.035; r = 0.60), second set (Z (N = 24) = -3.324, p_{corrected} = 0.012; r = 0.68), third set (Z (N = 24) = -3.219, p_{corrected} = 0.015; r = 0.66), fourth set (Z (N = 24) = -3.453, p_{corrected} = 0.008; r = 0.70), and fifth set (Z (N = 24) = -3.321, p_{corrected} = 0.012; r = 0.68). Between the sets no significant changes in RPE scores were observed (p_{corrected} > 0.05).

In the DT condition we observed a significant main effect of time ($X^2 = 104.526$ (df = 5, n = 24), p < 0.001). The post-hoc analyses show that the RPE score obtained prior the sets was lower than after first set (Z (N = 24) = -4.219, $p_{corrected} < 0.001$; r = 0.86), second set (Z (N = 24) = -4.313, $p_{corrected} < 0.001$; r = 0.88), third set (Z (N = 24) = -4.308, $p_{corrected} < 0.001$; r = 0.88), fourth set (Z (N = 24) = -4.299, $p_{corrected} < 0.001$; r = 0.88) and fifth set (Z (N = 24) = -4.295, $p_{corrected} < 0.001$; r = 0.88). Furthermore, following statistically significant changes between sets were observed: first set vs. second set (Z (N = 24) = -3.466, $p_{corrected} = 0.002$; r = 0.71), first set vs. third set (Z (N = 24) = -3.685, $p_{corrected} = 0.001$; r = 0.75), first set vs. fourth set (Z (N = 24) = -4.140, $p_{corrected} < 0.001$; r = 0.85), first set vs. fifth set (Z (N = 24) = -4.220, $p_{corrected} < 0.001$; r = 0.86), second set vs. third set (Z (N = 24) = -2.758, $p_{corrected} = 0.006$; r = 0.56), second set vs. fourth set (Z (N = 24) = -4.162, $p_{corrected} < 0.001$; r = 0.85), third set vs. fourth set (Z (N = 24) = -3.499, $p_{corrected} = 0.002$; r = 0.71), third set vs. fifth set (Z (N = 24) = -3.858, $p_{corrected} = 0.001$; r = 0.79), and forth set vs. fifth set (Z (N = 24) = -3.858, $p_{corrected} = 0.001$; r = 0.79), and forth set vs. fifth set (Z (N = 24) = -3.858, $p_{corrected} = 0.001$; r = 0.79), and forth set vs. fifth set (Z (N = 24) = -3.858, $p_{corrected} = 0.001$; r = 0.79), and forth set vs. fifth set (Z (N = 24) = -3.858, $p_{corrected} = 0.001$; r = 0.79), and forth set vs. fifth set (Z (N = 24) = -3.211, $p_{corrected} = 0.003$; r = 0.66).

Mean heart rate. The difference between mean HR obtained prior to ST or DT were not statistically significant (Z (N = 24) = -1.121, $p_{corrected} = 0.262$; r = 0.23), whereas during the first set (Z (N = 24) = -4.258, $p_{corrected} < 0.001$; r = 0.87), second set (Z (N = 24) = -4.286, $p_{corrected} < 0.001$; r = 0.87), third set (Z (N = 24) = -4.288, $p_{corrected} < 0.001$; r = 0.88), fourth set (Z (N = 24) = -4.287, $p_{corrected} < 0.001$; r = 0.88), and fifth set (Z (N = 24) = -4.287, $p_{corrected} < 0.001$; r = 0.88) the mean HR was significantly larger in DT (see Table 2).

Furthermore, we observed in ST a significant main effect of time ($X^2 = 15.524$ (df = 5, n = 24), p = 0.008). The post-hoc analyses show that the mean HR obtained prior to the sets was lower than during the first set (Z (N = 24) = -2.992, p_{corrected} = 0.042; r = 0.61).

In DT we observed a significant main effect of time regarding the change in mean HR (X² = 96.037 (df = 5, n = 24), p < 0.001). The post-hoc analyses showed that the mean HR score obtained prior the sets was lower than during the first set (Z (N = 24) = -4.292, p_{corrected} < 0.001; r = 0.88), second set (Z (N = 24) = -4.286, p_{corrected} < 0.001; r = 0.87), third set (Z (N = 24) = -4.287, p_{corrected} < 0.001; r = 0.88), fourth set (Z (N = 24) = -4.286, p_{corrected} < 0.001; r = 0.87) and fifth set (Z (N = 24) = -4.287, p_{corrected} < 0.001; r = 0.88). The following statistically significant changes were observed: first set vs. second set (Z (N = 24) = -3.409, p_{corrected} = 0.003; r = 0.70), first set vs. third set (Z (N = 24) = -3.653, p_{corrected} = 0.001; r = 0.75), first set vs. fourth set (Z (N = 24) = -3.803, p_{corrected} = 0.001; r = 0.78), first set vs. fifth set (Z (N = 24) = -4.201, p_{corrected} < 0.001; r = 0.86), second set vs. third set (Z (N = 24) = -3.036, p_{corrected} = 0.005; r = 0.62), second set vs. fourth set (Z (N = 24) = -3.361, p_{corrected} = 0.002; r = 0.69), second set vs. fifth set (Z (N = 24) = -4.124, p_{corrected} < 0.001; r = 0.84), third

set vs. fourth set (Z (N = 24) = -2.910, $p_{corrected} = 0.004$; r = 0.60), third set vs. fifth set (Z (N = 24) = -4.033, $p_{corrected} < 0.001$; r = 0.82), fourth set vs. fifth set (Z (N = 24) = -3.983, $p_{corrected} < 0.001$; r = 0.81).

Number of repetitions

Regarding the number of repetitions, there was no significant differences between the sets (main time effect: $X^2 = 8.846$ (df = 4, n = 24), p = 0.065) and, hence, no post-hoc tests were performed.

Correlation between specific outcome variables

We neither found statistically significant correlations between measures of cognition (i.e., total number of answers, number of correct answers, and accuracy score) and RPE or mean HR in each of the five sets nor were there statistically significant correlations between measures of cognition and self-rated experience in resistance training or resistance training sessions per week (see Table 3). A significant moderate positive correlation between number of squats and total number of correct answers was observed in the first set of DT condition (see Table 3).

Discussion

The aim of this study was to quantify the amount of cognitive resources needed to perform resistance exercises in the form of low-load barbell back squats. To that end, the dual-task paradigm was applied. The observed behavioral performance costs during low-load barbell back squatting would allow to draw conclusions about the necessary higher cognitive resources [9– 12]. Based on the observed decrease in total number of answer and number of correct answers in the DT condition (squatting while performing serial subtraction of 7's), our results suggest that higher cognitive resources are required to perform low-load barbell back squats on a Smith machine. Furthermore, our results imply that the observed dual-task effect is rather quantitative than qualitative in nature because the accuracy of the answers remains unaltered. Our findings are in line with previously published studies reporting a significant decrease in cognitive performance (e.g., number of solved items) in DT conditions involving dynamic motor actions (e.g., walking) [42–44]. Such a decrease in cognitive performance in DT conditions could be explained by the 'limited resource hypothesis' which postulates that the pool of available cognitive resources is restricted [45,46]. In DT situations, the motor task and the cognitive task compete for limited cognitive resources. When the resources do not suffice in a way that the demands of both tasks are fully satisfied, this could lead to a decrease in the performance of the motor and/or cognitive task [45]. In the light of the limited resources hypothesis, our results suggest that the execution of low-load barbell back squats (as motor task) withdraw a considerable amount of cognitive resources (significant lower cognitive performance in conjunction with high effect sizes) from processes required to solve the cognitive task (serial subtraction of 7's).

Furthermore, the positive moderate correlation between the number of squat repetitions and the number of correct answers in the first set suggests that some synchronization between the motor task and the cognitive task has occurred. Speculatively, the synchronization between motor tasks and cognitive tasks could be a strategy to better cope with the cognitive demands imposed by the simultaneous solving of specific motor task (i.e., squatting) and cognitive task (i.e., serial subtraction of 7's) [47]. However, this finding should be threated cautiously as it (i) was not persistent across the second to fifth set and (ii) could not been observed regarding other parameters of cognition (i.e., total number of answers and accuracy score). Moreover, the observed DT effect cannot merely be a result of a higher physical exertion in the DT condition but rather a consequence of the execution of the low-load barbell back squat itself. This

Table 3. Overview of correlation coefficients (Spearman's Rho [r,]) and corresponding p-values / RPE: Rating of relative perceived exertion; sERT: Self-rated expe-
rience in strength training; Reps: Number of squat repetitions; RTS: Resistance training sessions per week.

Correlation	1 st set	2 nd set	3 rd set	4 th set	5 th set
		Single-task condition			
RPE and total number of answers	r _s = -0.19	r _s = -0.13	r _s = -0.04	r _s = -0.08	r _s = -0.17
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.84)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$
RPE and number of correct answers	r _s = -0.14	r _s = -0.11	r _s = -0.04	r _s = -0.08	r _s = -0.12
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.86)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$
RPE and accuracy score	$r_{s} = -0.30$	r _s = -0.08	r _s = -0.01	r _s = 0.05	r _s = 0.14
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$
Mean HR and total number of answers	r _s = -0.00	$r_{s} = 0.06$	$r_{s} = 0.06$	r _s = 0.13	$r_{s} = 0.17$
	$(p_{corrected} = 0.99)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$
Mean HR and number of correct answers	r _s = -0.03	$r_{s} = 0.06$	$r_{s} = 0.01$	$r_{s} = 0.08$	r _s = 0.09
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.96)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$
Mean HR and accuracy score	$r_s = -0.03$	$r_s = -0.02$	$r_s = -0.31$	$r_s = -0.28$	$r_s = -0.44$
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.94)$	$(p_{corrected} = 0.57)$	$(p_{corrected} = 0.56)$	$(p_{corrected} = 0.17)$
		Dual-task condition	1	1	1
RPE and total number of answers	$r_s = -0.23$	$r_s = -0.43$	$r_s = -0.21$	$r_s = -0.35$	$r_s = -0.21$
	$(p_{corrected} = 0.84)$	$(p_{corrected} = 0.15)$	$(p_{corrected} = 0.33)$	$(p_{corrected} = 0.40)$	$(p_{corrected} = 0.64)$
RPE and number of correct answers	$r_s = -0.22$	$r_s = -0.40$	$r_s = -0.11$	$r_s = -0.22$	$r_s = -0.07$
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.25)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.93)$	$(p_{corrected} = 0.76)$
RPE and accuracy score	$r_s = -0.00$	$r_s = 0.30$	$r_s = 0.13$	$r_s = 0.34$	$r_s = -0.01$
Mean HR and total number of answers	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.53)$	$(p_{corrected} = 1.00)$
	$r_s = 0.37$	$r_s = 0.25$	$r_s = 0.32$	$r_s = 0.31$	$r_s = 0.37$
	$(p_{corrected} = 0.40)$	$(p_{corrected} = 0.25)$	$(p_{corrected} = 0.40)$	$(p_{corrected} = 0.28)$	$(p_{corrected} = 0.32)$
Mean HR and number of correct answers	$r_s = 0.36$	$r_s = 0.16$	$r_s = 0.28$	$r_s = 0.22$	$r_s = 0.23$
	$(p_{corrected} = 0.44)$	$(p_{corrected} = 0.45)$	$(p_{corrected} = 0.76)$	$(p_{corrected} = 0.59)$	$(p_{corrected} = 0.86)$
Mean HR and accuracy score	$r_s = -0.17$	$r_s = -0.23$	$r_s = 0.11$	$r_s = -0.02$	$r_s = -0.08$
sERT and total number of answers	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$ $r_{s} = -0.08$	$(p_{corrected} = 0.92)$	$(p_{corrected} = 1.00)$
SERT and total number of answers	$r_{s} = 0.30$ $(p_{corrected} = 0.80)$	$r_{s} = 0.14$ $(p_{corrected} = 1.00)$	$r_s = -0.08$ $(p_{corrected} = 1.00)$	$r_{s} = 0.01$ $(p_{corrected} = 1.00)$	$r_{s} = 0.01$ $(p_{corrected} = 0.96)$
sERT and number of correct answers	$r_s = 0.23$	$r_s = 0.12$	$r_s = -0.06$	$r_s = -0.06$	$r_s = -0.04$
select and number of correct answers	$(p_{corrected} = 1.00)$	$p_{corrected} = 1.00$	$p_{corrected} = 1.00$	$(p_{corrected} = 1.0)$	$(p_{corrected} = 0.86)$
sERT and accuracy score	$r_s = -0.10$	$r_s = -0.19$	$r_s = -0.08$	$r_s = -0.27$	$r_s = 0.08$
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.73)$
RTS and total number of answers	$r_s = 0.34$	$r_s = 0.22$	$r_s = 0.08$	$r_s = 0.02$	$r_s = 0.18$
	$(p_{corrected} = 0.50)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.91)$	$(p_{corrected} = 1.00)$
RTS and number of correct answers	$r_s = 0.29$	$r_s = 0.20$	$r_s = 0.08$	$r_s = -0.03$	r _s = 0.11
it is and number of correct answers	$(p_{corrected} = 0.85)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.87)$	$(p_{corrected} = 1.00)$
RTS and accuracy score	$r_s = -0.16$	$r_s = -0.20$	$r_s = -0.07$	$r_s = -0.05$	r _s = 0.15
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.82)$	$(p_{corrected} = 1.00)$
Reps and total number of answers	r _s = 0.43	$r_{s} = 0.28$	r _s = 0.51	r _s = 0.23	$r_s = 0.34$
	$(p_{corrected} = 0.14)$	$(p_{corr} = 0.37)$	$(p_{corrected} = 0.05)$	$(p_{corrected} = 0.28)$	$(p_{corrected} = 0.30)$
Reps and number of correct answers	$r_{s} = 0.44^{*}$	$r_{s} = 0.30$	r _s = 0.55	r _s = 0.27	r _s = 0.24
	$(p_{corrected} = 0.03)$	$(p_{corrected} = 0.12)$	$(p_{corrected} = 0.48)$	$(p_{corrected} = 0.40)$	$(p_{corrected} = 0.26)$
Reps and accuracy score	r _s = -0.09	r _s = 0.14	r _s = 0.32	r _s = 0.15	r _s = 0.06
	$(p_{corrected} = 1.00)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.66)$	$(p_{corrected} = 1.00)$	$(p_{corrected} = 0.77)$

assumption is, at least partly, supported by the absence of statistically significant correlations between RPE scores or mean HR and measures of cognitive performance. Moreover, the absence of significant associations between resistance training experience and cognitive performance suggests that the observed dual-task effect is relatively independent of an individual's expertise level in our study performing low-load barbell back squats on a Smith machine. This finding is in accordance with results of a previous study which did not report an effect of expertise level on cognitive performance changes in the dual-task conditions [48]. However, it is also reported that experts outperform novices in challenging dual-task conditions [49]. Hence, in order to rule out whether this finding is generalizable, further research should directly compare groups with different levels of expertise in resistance training and with different levels of load.

The observation that the number of total responses was significantly higher in the fifth set as compared to the first set indicates that some learning might have occurred. The appearance of a learning effect in DT conditions (e.g., with regard to cognitive measures) is in line with previous findings [50,51] and could be attributed to the automatization of task execution which leads to the freeing of cognitive resources [50]. However, given that the differences between ST and DT remained significant even in the fifth set, the presence of a supposed emerged learning effect would not argue against the assumption that higher cognitive resources are needed to perform low-load barbell back squats on a Smith machine. To further strengthen the assumption that higher cognitive resources are required to perform resistance exercises, more research is needed that investigates whether (i) dual-task effects emerge with other cognitive tasks that target other cognitive domains (e.g., working memory by n-back task), (ii) dual-task effects occur during the performance of other resistance exercises (e.g., seated rowing), or (iii) how dual-task effects are influenced by other exercise variables (e.g., load, rest phases, movement velocity). Additionally, as older adults require more generic resources to perform a motor task (e.g., postural tasks) [13], it seems promising to clarify in future research whether the observed dual-task costs in response to resistance exercises (i.e., barbell back squat) are more pronounced in the elderly.

Limitations

While our results suggest that higher cognitive resources are necessary to perform barbell back squats, the findings need to be interpreted in light of some limitations. A drawback of this study is the abdication of kinematic analyses (e.g. by using motion capture systems) or muscle functional analyses (e.g., by using electromyography) of the barbell back squats. Such kinematic analyses or electromyographic analyses could be helpful to assess the motor-related dual-task costs and their application is recommended in further studies.

Conclusion

In conclusion, our results suggest that in our cohort the execution of low-load barbell back squats requires the recruitment of higher cognitive resources. However, since our results are neither transferable to other cognitive tasks nor other cohorts, further studies which utilize other cognitive tasks (e.g., n-back task), conduct other resistance exercises (e.g., seated row-ing), investigate a potential dose-response relationship (e.g., different loads), and/or recruit further cohorts (e.g., older adults, experts in resistance training) are necessary to confirm and generalize our findings.

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10 Physiological differences between advanced CrossFit athletes, recreational CrossFit participants, and physically-active adults

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Abstract

This investigation examined anthropometric, hormonal, and physiological differences between advanced (ADV; n = 8, 27.8 ± 4.2 years, 170 ± 11 cm, 79.8 ± 13.3 kg) and recreational (REC; n = 8, 33.5 ± 8.1 years, 172 ± 14 cm, 76.3 ± 19.5 kg) CrossFit (CF) trained participants in comparison to physically-active controls (CON; $n = 7, 27.5 \pm 6.7$ years, 171 ± 14 cm, 74.5 ± 14.3 kg). ADV and REC were distinguished by their past competitive success. REC and CON were resistance-trained (>2 years) and exercised on 3–5 days wk⁻¹ for the past year, but CON utilized traditional resistance and cardiovascular exercise. All participants provided a fasted, resting blood sample and completed assessments of resting metabolic rate, body composition, muscle morphology, isometric mid-thigh pull strength, peak aerobic capacity, and a 3-minute maximal cycle ergometer sprint across two separate occasions (separated by 3-7 days). Blood samples were analyzed for testosterone, cortisol, and insulin-like growth factor-1. Compared to both REC and CON, one-way analysis of variance revealed ADV to possess lower body fat percentage (6.7–8.3%, p = 0.007), greater bone and non-bone lean mass (12.5–26.8%, p < 0.028), muscle morphology characteristics (14.2–59.9%, p < 0.05), isometric strength characteristics (15.4–41.8%, p < 0.05), peak aerobic capacity (18.8–19.1%, p = 0.002), and 3-minute cycling performance (15.4–51.1%, $p \le$ 0.023). No differences were seen between REC and CON, or between all groups for resting metabolic rate or hormone concentrations. These data suggest ADV possess several physiological advantages over REC and CON, whereas similar physiological characteristics were present in individuals who have been regularly participating in either CF or resistance and cardiovascular training for the past year.

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Introduction

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CrossFit[®] (CF) is a form of high-intensity functional training that combines resistance exercises, gymnastics, and traditional aerobic modalities (e.g., cycling, rowing, running) into single workouts that vary by day to elicit general physical preparedness [1, 2]. This training form is

enjoyed recreationally by participants of varying levels of fitness, training experience, age, and lifestyles [3] and also exists as its own sport. The primary CF competition is the Reebok Cross-Fit Games[™] (the Games) which awards individual winners the title of "Fittest on Earth[™]". Historically, this competition has consisted of several stages designed to narrow the initial participant pool down to the top athletes. Although the competition's structure has changed over time [4, 5], the presence of an initial online qualifying round (e.g., the CrossFit OpenTM) has remained. This round typically involves multiple workout challenges that are completed over the course of several weeks. Competitors who complete all workouts and rank high enough will progress to the next stage of the competition. Regardless of which stage, it is expected that each workout will consist of a set of challenges that will require some combination of strength, power, endurance, and/or sport-specific skill [1]. However, little is known about which physiological characteristics of competitors who progress beyond the opening round of the competition.

Body mass [6], strength and anaerobic power [6-10], aerobic capacity [9], sport-specific skill [8, 10], and experience [9] have all been associated with either CF workout performance or competitive ranking. Collectively, these data imply that athletes must train to be proficient in each to perform well in competition. However, several limitations exist among these studies that prevent making such a conclusion. For instance, Serafini et al. (2018) reported that higher ranking competitors of the 2016 Open were stronger, more powerful, and more proficient at short-duration, sprint-type CF workouts. Among regional competitors, final ranking was positively related to 400-m sprint time and time-to-completion in longer, benchmark workouts (i.e., Filthy-50) (r = 0.69 - 0.77), and negatively related to maximal weight lifted in the Olympic lifts (r = -0.39 to -0.42) [10]. Although these studies involved participants who have successful competitive records, the measures used to distinguish rank were all self-reported. As such, the authenticity and actual data of measurement (self-reported data were obtained from an online resource) cannot be verified. In contrast, others have measured a variety of physical parameters and related them to CF-style workouts performed in a controlled, laboratory setting [6, 7, 9]. While these studies have also included successful CF athletes, laboratory workouts do not adequately emulate the competitive setting and may influence the physiological response to CF training [11–14]. Thus, questions remain about the distinguishing characteristics of successful CF athletes.

In more traditional sports (e.g., football, baseball, basketball, etc.), identifying the key physiological and athletic characteristics that distinguish performance is common [15-18]. The practice enables strength and conditioning professionals to develop sport-specific training programs that are more effective in translating adaptations to in-game performance. However, CF is unique in that typical training session workouts mirror those that appear in competition. Moreover and consistent with its primary purpose [1, 2], chronic participation in CF training has been documented to improve a variety of fitness parameters [19]. Though it might be assumed that CF training represents an ideal training strategy for developing the physiological characteristics present in successful competitors, such a conclusion would be premature based on the available data.

Evidence of CF training being more advantageous towards developing a variety of fitness outcomes in comparison to alternative training strategies (e.g., resistance training, high-intensity interval training) is equivocal [19–25]. This is likely because most comparative training studies have utilized untrained or novice (to CF) participants, which is problematic because they do not require a very specific or intense training stimulus to elicit adaptations compared to experienced trainees [26]. It is possible that either a longer training duration or more advanced participants are necessary to observe the advantages or disadvantages of the CF strategy. Unfortunately, elite competitors rarely share their training strategies and anecdotal

evidence suggests that they incorporate more than what commonly occurs during a typical CF training session. To the best of our knowledge, only one well-controlled study exists where a variety of physiological parameters were examined between CF-trained participants and those trained in more traditional exercise modalities (e.g., resistance training) [27]. In that cross-sectional investigation, men with at least on year of CF training experience outperformed their resistance-trained (> 1 year) counterparts in a multi-stage shuttle run test and possessed a higher aerobic capacity; all other measures were statistically similar. While this study provides evidence in favor of CF training, there was no aerobic training requirement for the resistancetrained group, and the actual experience of the CF group was unclear beyond their having participated in the strategy for at least one year. It is possible that multiple physiological differences exist when experience is considered. Therefore, the purpose of this study was to examine anthropometric, hormonal, and physiological differences between advanced CF athletes, recreational CF practitioners, and physically-active adults who regularly participate in both resistance and cardiovascular training. Since adaptations are specific to the training modality and effort [26], we hypothesized that body composition, muscle morphology, aerobic and anaerobic performance, and strength would be different between groups. Specifically, the advanced CF athletes would outperform the other groups whereas recreational CF practitioners and physically-active adults would be similar. However, because resting hormonal concentrations do not typically change through training [14], it was hypothesized that these would be similar between groups.

Materials and methods

Experimental design

For this cross-sectional study, physically-active adults were recruited and assigned into groups based on their experience with CF training and performance during specific CF competitions. Participants who possessed CF training experience (> 2 years) were classified as advanced (ADV) if they had previously qualified for the regional round of the Games competition. Otherwise, they were classified as recreational (REC) because they had never progressed beyond the opening round of the competition (i.e., The Open) but still trained on 3-5 days per week for at least the previous year. Individuals who did not possess CF training experience but possessed resistance training experience (> 2 years) and participated in both resistance and cardiovascular training on 3-5 days per week for at least the previous year, were assigned to the physically-active control (CON) group. All participants reported to the Exercise Physiology Laboratory on two separate occasions, within one month of the onset of the Open, to complete all testing. During the first visit, each participant provided a fasted blood sample before completing assessments of muscle morphology and then a graded exercise test to measure peak aerobic capacity. Participants returned to the Exercise Physiology Lab for the second visit (within 3-7 days of the first visit) to complete assessments of resting metabolic rate, body composition, and strength before finishing the study with a 3-minute all-out cycling test. All testing sessions occurred in the morning (~6:00-10:00 a.m.) with the participants having abstained from unaccustomed physical activity and alcohol for 24 hours, caffeine for 12 hours, and fasted for 8 hours. Participants completed all measurements while wearing comfortable athletic clothing and were able to consume a light snack prior to performance testing (i.e., peak aerobic capacity, strength, and 3-minute cycling performance). Prior to leaving the laboratory on the first visit, participants were asked to complete a 24-hour dietary recall, retain a copy, and follow a similar diet prior to their second visit. Comparisons were made between groups for all anthropometric, biochemical, and physiological measures.

Participants

A priori analysis was based on published [8, 28] and related unpublished data collected by our laboratory where comparisons were made between competitive levels and ranks for selfreported measures of strength and power in CF athletes. The effect sizes produced from group comparisons (partial eta squared > 0.485), standard alpha (p = 0.05), and minimum beta ($\beta =$ 0.80) were input into statistical software (G*Power, v. 3.1.9.4, Heinrich-Heine-Universität, Germany). It was determined that a minimum of 20 participants was needed to obtain sufficient power to observe differences between sexes and groups. Consequently, twenty-three physically-active adults (29.7 \pm 6.8 years, 171 \pm 12 cm, 76.9 \pm 15.4 kg) agreed to participate in this study. All participants were free of any physical limitations (determined by medical and physical-activity history questionnaire and PAR-Q+) and had been regularly participating (at the time of recruitment) in their chosen exercise form (i.e., CrossFit training or Resistance/ Cardiovascular training) for a minimum of 2 years. Participants in ADV (n = 8 [men = 4, women = 4], 27.8 ± 4.2 years, 170 ± 11 cm, 79.8 ± 13.3 kg) reported having regularly participated in resistance training for 11.5 ± 5.8 years and CF training for 6.4 ± 5.6 years (6–7 sessions week⁻¹). As individual competitors, the highest rank these participants ever achieved in the Open was $659^{\text{th}} \pm 991^{\text{st}}$ (range: $19^{\text{th}} - 3,052^{\text{nd}}$) within their respective divisions worldwide. While each of these athletes qualified for this study by having competed as members of a team in regional (highest average rank = $11^{th} \pm 13^{th}$) and Games competition (highest average rank = $20^{\text{th}} \pm 9^{\text{th}}$), three competed individually in their respective regions with one having progressed to the Games on multiple occasions. REC participants (n = 8 [men = 4, women = 4], 33.5 ± 8.1 years, 172 ± 14 cm, 76.3 ± 19.5 kg) reported having regularly participated in resistance training for 8.1 \pm 7.9 years and CF training for 3.3 \pm 1.7 years (4–5 sessions week⁻¹). The highest rank these participants had ever achieved in the Open was 22,306th $\pm 14,028$ th (range: 5,466th-44,315th) within their respective divisions worldwide. Participants in CON (n = 7 $[men = 4, women = 3], 27.5 \pm 6.7 \text{ years}, 171 \pm 14 \text{ cm}, 74.5 \pm 14.3 \text{ kg})$ reported having 7.6 ± 4.8 years of regular resistance training experience and incorporated 3.7 ± 1.3 sessions and $3.6 \pm$ 1.0 sessions of resistance and cardiovascular training per week. Although two participants in CON reported having previously participated in CF-style workouts, these did not occur with regularity (< 3 sessions week⁻¹) or for an extended duration (< 1 year) and they had never competed in the Open at the time of data collection. Following an explanation of all procedures, risks and benefits, each participant provided his or her written informed consent to participate in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Kennesaw State University Institutional Review Board (#17-501).

Blood sampling and biochemical analysis

Blood samples were obtained on the first visit prior to any physical activity. All samples were obtained from an antecubital vein using a needle by a research team member who was trained and experienced in phlebotomy. Approximately 15 mL of blood was drawn into SST tubes (for serum collection) and EDTA-treated Vacutainer® tubes (for plasma). SST tubes were allowed to clot for 10 minutes prior to centrifugation, while EDTA treated tubes were centrifuged immediately for 10 minutes at 3600 rpms at 4 °C. The resulting serum and plasma were ali-quoted and stored at -80°C until analysis.

Circulating concentrations of testosterone (T; in $ng \cdot dL^{-1}$), cortisol (C; in $\mu g \cdot dL^{-1}$), and insulin-like growth factor (IGF-1; in $ng \cdot mL^{-1}$) were assessed via enzyme-linked immunosorbent assays (ELISA) via a 96-well spectrophotometer (BioTek, Winooski, VT) using commercially available kits. To eliminate inter-assay variance, all samples for each assay were thawed once

and analyzed in duplicate in the same assay run by a single technician. Samples were analyzed in duplicate, with an average coefficient of variation of 1.63% for T, 6.88% for C, and 2.00% IGF-1.

Muscle morphology

Non-invasive skeletal muscle ultrasound images were collected from the right thigh and arm locations of all participants. Prior to image collection, all anatomical locations of interest were identified using standardized landmarks for the rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), biceps brachii (BB), and triceps brachii (TB) muscles. The landmarks for the thigh musculature were identified along the longitudinal distance over the femur. The RF and VM were respectively assessed at 50% and 20% of the distance from the proximal border of the patella to the anterior, inferior suprailiac crest. The VL was assessed at 50% of the distance from the lateral condyle of the tibia to the most prominent point of the greater trochanter of the femur. VL measurement required the participant to lay on their side. Landmark identification of the BB and TB required the participant to sit upright on the examination table and extend their arm to rest upon the shoulder of the researcher. Both muscles were assessed along the humerus at a position equal to 40% of the distance from the lateral epicondyle to the acromion process of the scapula [29]. Subsequently, the participant resumed laying supine on the examination table for a minimum of 5-10 minutes to allow fluid shifts to occur before images were collected [30]. The same investigator performed all landmark measurements for each participant.

A 12 MHz linear probe scanning head (General Electric LOGIQ S7 Expert, Wauwatosa, WI, USA) was coated with water soluble transmission gel to optimize spatial resolution and used to collect all ultrasound images. Collection of each image began with the probe being positioned on (and perpendicular to) the surface of the skin to provide acoustic contact without depressing the dermal layer. Subsequently, two consecutive images were collected in the extended field of view mode (Gain = 50 dB; Image Depth = 5-6 cm) using a cross-sectional sweep in the axial plane to capture panoramic images of each muscle. At the same sites, two consecutive images were collected with the probe oriented longitudinal to the muscle tissue interface using Brightness Mode (B-mode) ultrasound [31]. Each of these images included a horizontal line (approximately 1 cm), located below the image, which was used for calibration purposes when analyzing the images offline [32]. To capture images of the RF and VM, the participant remained in the supine position, with their legs extended but relaxed. A rolled towel was placed beneath the popliteal fossa of the dominant leg, allowing for a 10° bend in the knee as measured by a goniometer, and the dominant foot secured [33]. For the VL, the participant was placed on their side with their legs together and the rolled towel between their needs. Once again, the legs were positioned to allow a 10° bend in the knees, as measured by a goniometer [33]. Measurement of the BB and TB required the participant to sit upright with their arm extended, resting on the shoulder of the researcher. The same investigator positioned each participant and collected all images.

After all images were collected, the ultrasound data were transferred to a personal computer for analysis via Image J (National Institutes of Health, Bethesda, MD, USA, version 1.45s) by the same technician. All panoramic images were used to measure cross-sectional area (CSA) and echo intensity. For these measures, the polygon tracking tool in the ImageJ software was used to isolate as much lean muscle as possible without any surrounding bone or fascia [31]. Subsequently, Image J calculated the area contained within the traced muscular image and reported this value in centimeters squared ($\pm 0.1 \text{cm}^2$). Concurrently, echo intensity was determined by grayscale analysis using the standard histogram function in ImageJ [31] and expressed as an arbitrary unit (au) value between 0–255 (0: black; 255: white) with lower values reflecting more contractile tissue within each muscle [31, 34]. Mean echo intensity values were then corrected for subcutaneous fat thickness (SFT; averaged from the SFT values obtained at the medial, midline, and lateral sites of each muscle) using Eq 1 [35]. All B-mode images were used to measure muscle thickness (\pm 0.01 cm; perpendicular distance between the superficial and deep aponeuroses) and pennation angle (\pm 0.1°; intersection of the fascicles with the deep aponeurosis). Fascicle length (\pm 0.1 cm) across the deep and superficial aponeuroses was estimated from muscle thickness and pennation angle using Eq 2. Intraclass correlation coefficients (ICC_{3,k} = 0.77–0.99) for determining muscle thickness, pennation angle, CSA and echo intensity was previously determined in ten active, resistance-trained men (25.3 \pm 2.0 years, 180 \pm 7 cm, 90.8 \pm 6.8 kg) using the methodology described above. The methodology for determination of FL has a reported estimated coefficient of variation of 4.7% [36].

Corrected echo intensity (EI) = Raw echo intensity $+ (SFT \times 40.5278)$ Eq 1

Fascicle length = Muscle thickness
$$\cdot \sin (\text{pennation angle})^{-1}$$
 Eq 2

Graded exercise testing

Peak aerobic capacity (VO₂peak; ml·kg⁻¹·min⁻¹), respiratory compensation threshold (RCT; ml·kg⁻¹·min⁻¹), and gas exchange threshold (GET; ml·kg⁻¹·min⁻¹) were assessed using a continuous, ramp exercise protocol performed on an electromagnetic-braked cycle ergometer (Lode Excalibur Sport, Lode., B.V., Groningen, The Netherlands). Prior to testing, each participant completed a standardized warm-up that consisted of riding a cycle ergometer for 5 minutes at the participant's preferred resistance and cadence followed by 10 body weight squats, 10 alternating lunges, 10 walking knee hugs and 10 walking butt kicks. Participants were then permitted to continue their warm-up with any additional practices that would help them feel comfortable entering the test. Participants were fitted with a heart rate (HR) monitor (Team², Polar, Lake Success, NY), a nose clip, and a 2-way valve mask connected to a metabolic measurement system (True One 2400, ParvoMedics Inc., Salt Lake City, UT) to measure expired gases. The cycle ergometer seat height and handlebar distance were adjusted to the participant's comfort. The participants initially completed a 3-minute warm-up period with the resistance set at 50 W before starting the test at 75 W. During testing, the participants were asked to maintain a self-selected pedaling rate (> 50 rpm's) while power output was increased by 25 W every minute until volitional fatigue or pedaling rate dropped below 50 rpm's for longer than 15 seconds. Upon completion of the test, each participant immediately progressed to a 3-minute active recovery period where they continued to pedal at their own cadence against a 50 W load. HR was assessed on each minute of the 3-minute recovery period. Participants were then removed from the cycle ergometer and asked to rest in a chair for an additional two minutes.

Relative oxygen consumption values (i.e., $VO_2 \cdot kg^{-1}$) collected on each breath were averaged using the 11-breath averaging technique [37] and used to determine the highest value achieved during the test (i.e., VO_2 peak). RCT, also known as the second ventilatory threshold, was identified as the VO_2 value at which the increase in ventilation- VO_2 relationship was accompanied by an increase in the ventilation- VCO_2 relationships [38]. The GET was determined using the V-slope method described by Beaver et al. [39]. The GET was defined as the VO_2 value corresponding to the intersection of two linear regression lines derived separately from the data points below and above the breakpoint in the CO_2 produced (VCO_2) versus the VO_2 relationship [40].

Dietary recall

Participant's dietary intake was tracked for the 24-hour period preceding each visit via a paper dietary food recall form. All participants were instructed on how to properly log their food, snacks and drinks via the paper form. Specifically, following their enrollment on their first visit, participants were asked to record their food intake (breakfast, lunch, dinner, drinks and snacks) for the previous 24 hours prior. Prior to leaving the laboratory on the first visit, the participants were given a copy of their food recall form and asked to consume a similar diet during the 24 hours prior to their second visit. Each form was visually inspected to confirm dietary compliance.

Resting metabolic rate assessment

Resting metabolic rate (RMR, kcals·day⁻¹) assessment was completed in a quiet room with minimal lighting (e.g., only light from the RMR machine) located within the Exercise Physiology Laboratory. Prior to their arrival, participants were informed of all pre-test guidelines as outlined by Compher et al. [41]. These included: 1) avoiding alcohol consumption 24 hours prior to testing, 2) no food or caffeine ingestion 8 and 12 hours prior to testing, respectively, and 3) discontinuing unaccustomed physical activity 24 hours prior to testing. Resting metabolic rate was measured via a metabolic measurement system (Parvo Medics TrueOne 2400, ParvoMedics Inc., Salt Lake City, UT) utilizing a ventilated hood. Participants were asked to rest in the supine position with the ventilated hood placed over their face and neck for a maximum of 30 minutes. RMR determination was based on a 5-minute interval of measured volume of oxygen consumption (VO₂) with a coefficient of variation less than 10% [41]. The average coefficient of variation was 6.36%.

Body composition assessments

Initially, height (± 0.1 cm) and body mass (± 0.1 kg) were determined using a stadiometer (WB-3000, TANITA Corporation, Tokyo, Japan) with the participants standing barefoot, with feet together, in their normal daily attire. Subsequently, body composition was assessed by three common methods (i.e., dual energy X-ray absorptiometry [iDXA, Lunar Corporation, Madison, WI], air displacement plethysmography [BodPod, COSMED USA Inc., Chicago, IL], and bioelectrical impedance analysis [770 Body Composition and Body Water Analyzer, InBody, Seoul, South Korea]) using standardized procedures. Briefly, iDXA scanning required participants to remove any metal or jewelry and lay supine on the iDXA table prior to an entire body scan in "standard" mode using the company's recommended procedures and supplied algorithms. Quality assurance was assessed by daily calibrations performed prior to all scans using a calibration block provided by the manufacturer. All iDXA measurements were performed by the same researcher using standardized subject positioning procedures. For air displacement plethysmography, the device and associated scale were calibrated daily using a known volume and mass provided by the manufacturer. During testing, participants were asked to wear a tight-fitting bathing suit or compression shorts and swim cap before entering the device. Two trials were performed for each participant to obtain two measurements of body volume within 150 mL. A third trial was performed if body volume estimates from the first two trials were not within 150 mL, and values from the two closest trials were averaged. Thoracic lung volume was estimated [42]. Bioelectrical impedance analysis required participants to stand barefoot on two metal sensors located at the base of the device and hold two hand grips for approximately 30-60 seconds. Prior to stepping onto the device, participants cleaned the soles of their feet with alcohol wipes provided by the manufacturer.

Following testing, body mass, bone mineral content (BMC; from iDXA), body volume (from BodPod), and total body water (from bioelectrical impedance analysis) were entered into a 4-compartment model, Eq.3 to estimate body fat percentage (BF%) [43], fat mass (\pm 0.1 kg), and fat-free mass (\pm 0.1 kg). These values, along with regional (arms [sum of each arm], legs [sum of each leg], and trunk [sum of spine and pelvis]) estimates of bone mineral content (\pm 0.1 kg) and non-bone lean mass (\pm 0.1 kg) obtained from iDXA following manual demarcation of these regions of interest were used for all group comparisons. Intraclass correlation coefficients (ICC_{3,1} = 0.74–0.99) for manually determining regional estimates of bone mineral content and non-bone lean mass had been previously found in 10 healthy, physically-active adults (25.1 \pm 2.4 years; 176 \pm 7 cm, 81.1 \pm 18.5 kg).

 $BF\% = \frac{(2.748 \ x \ volume) - (0.699 \ x \ water) + (1.129 \ x \ BMC) - (2.051 \ x \ Body \ Mass)}{Body \ Mass} x \ 100 \ \text{ Eq 3}$

Strength assessment

Following RMR and body composition assessments, strength was assessed by an isometric mid-thigh pull test. Prior to testing, each participant completed the same standardized warmup described for the first visit (i.e., 5 minutes of cycling, dynamic stretching, additional selfselected warm-up practices) followed by a protocol specific to the isometric mid-thigh pull test. The specific component included three isometric efforts on an immobilized barbell positioned at approximately the mid-thigh using a perceived intensity of 50, 70, and 90% of maximum effort, interspersed with a one-minute recovery. The specific warm-up and isometric mid-thigh pull test were completed within a power rack (Rogue Fitness, Columbus, OH) while standing upon a portable force plate (Accupower, AMTI, Watertown, MA). While standing on the force plate, the mid-thigh position was determined for each participant before testing by marking the midpoint distance between the knee and hip joints. Each participant was instructed to assume their preferred second pull power-clean position by self-selecting their hip and knee angles. The height of the barbell was adjusted to a position approximately equal $(\pm 2.54 \text{ cm})$ to the mid-thigh. The participants were then asked to use an overhand, hooked grip on the barbell. The hook grip was selected for this test because all participants reported having had experience with the technique and it is commonly used among CF athletes during competition. Participants were also allowed to wrap their thumbs with athletic training tape and use chalk. Upon the researcher's "3, 2, 1, Go!" command, the participants were instructed to pull upwards on the barbell as hard and as fast as possible and to continue their maximal effort for 6 seconds. All participants were instructed to relax before the command "GO!" to avoid precontraction and were allotted three maximal attempts. The portable force plate measured the ground reaction forces, imposed onto the plate by the participant, as he/she pulled upon the bar. Peak force (F; in N) production, peak and average rate of force development $(RFD_{PEAK}, RFD_{AVG}; in N \cdot s^{-1})$, and F and RFD across specific time bands (i.e., 0–30, 0–50, 0-90, 0-100, 0-150, 0-200, and 0-250 milliseconds) were subsequently calculated, as previously described [44].

3-minute all-out cycling test

Following the strength assessment, performance was assessed during a 3-minute maximal sprint on an electromagnetic-braked cycle ergometer (Lode Excalibur Sport, Lode., B.V., Groningen, The Netherlands). Prior to the test, seat height and handlebar positions were adjusted to mirror their positions during the peak aerobic capacity test, and participants were provided with time (~3–5 minutes) to acclimate to the cycle ergometer. A 5-minute rest period was then allotted before initiating the testing protocol, which has been previously described in detail elsewhere [45]. Briefly, the test began with a 1-minute baseline period that involved 55 seconds of unloaded cycling at 90 rpm and then accelerating up to approximately 110 rpm over the last 5 seconds of the minute. The protocol immediately transitioned to the 3-minute testing period where the participants attempted to maintain cadence as high as possible throughout its entirety. Resistance for the test was set using the linear mode of the cycle ergometer (linear factor = power / [preferred cadence]²). That is, the linear factor was calculated as the power output halfway between the VO₂peak and GET, divided by the preferred cadence of untrained cyclists (70 rpm²) [46–48]. To prevent pacing and ensure an all-out effort, participants were not informed of the elapsed time and strong verbal encouragement was provided. After 3 minutes, the participants progressed to a 3-minute recovery stage at 50 Watts at their preferred cadence. Peak power (\pm 1 W), critical power (CP; average power over the final 30 seconds of the test; \pm 1 W) [47], and anaerobic work capacity (AWC; work done above CP; (\pm 0.1 kJ) (48) were calculated based upon performance during the 3-minute sprint test.

Statistical analysis

Data were modeled using both a frequentist and Bayesian approach. The frequentist approach involved a two-tailed, two-way (Group x Sex) analysis of variance (ANOVA) for each dependent variable. Since no between-group differences were observed, age was not included in the model as an additional factor or covariate. Assumptions of normality and equal variance were verified by Shapiro-Wilk and Levene's tests, respectively. Significant interactions and main effects were further examined using Tukey's post-hoc analysis. Criterion alpha was set at $p \leq 0.05$. To further assess the likelihood (or the effect of group and/or sex) of the data under the alternative hypothesis compared to the null hypothesis, a two-way Bayesian ANOVA was performed with default prior scales [49]. Likelihood was represented in the form of Bayes factors (i.e., BF₁₀) and were interpreted according to the recommendations of Wagenmakers et al. [50]. That is, data were interpreted as evidence in favor of the null hypothesis when BF₁₀ < 1. Otherwise, it was interpreted as "anecdotally" ($1 < BF_{10} < 3$), "moderately" ($3 < BF_{10} > 10$), "strongly" ($10 < BF_{10} < 30$), "very strongly" ($30 < BF_{10} < 100$), or "extremely" ($BF_{10} > 100$) in favor of the alternative hypothesis. All statistical analyses were performed using JASP 0.10.2 (Amsterdam, the Netherlands). All data are reported as mean \pm standard deviation.

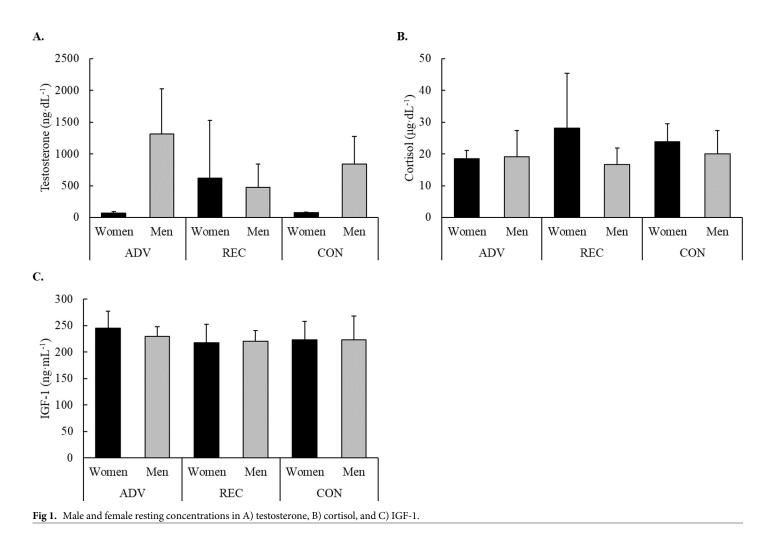
Results

Resting hormone concentrations

No interactions were observed for T, C, IGF-1. However, a trend for an interaction (F = 2.87, p = 0.090) driven by a main sex effect was seen for T (F = 6.11, p = 0.027) with *anecdotal* differences between sexes being 2.058 times likely compared to the null hypothesis. Specifically, women in ADV tended to exhibit lower T concentrations (p = 0.083) than ADV men. Male and female hormone concentrations are illustrated in Fig 1.

Muscle morphology

Measures of muscle morphology for each group and sex are presented in Table 1. Significant (p < 0.05) group x sex interactions were observed for BB fascicle length and EI for each muscle, though the likelihood of these interactions favored the null hypotheses (BF₁₀ < 1). Rather, the observed interactions were primarily driven by *anecdotal*-to-*strong* evidence ($1.7 < BF_{10} < 30.0$) of main effects for sex and group. The observed interaction for BB fascicle length was primarily driven by a main effect for sex where women were 8.8 times more likely to possess shorter fascicles than men, specifically REC women compared to the men of REC (p = 0.029)



and CON (p = 0.012). Though the underlying causes for the interactions seen for EI varied with each muscle, *anecdotal*-to-*moderate* evidence indicated that men were 1.7–5.5 times more likely to possess a lower EI than women. Specifically, women in REC possessed higher EI (p < 0.05) than men in ADV (RF, VL, and TB; a trend [p = 0.056] for VM) and REC (RF, VM, VL, and TB; a trend [p = 0.087] was noted for BB), and tended (p < 0.10) to be higher than men in CON (RF, VL, and TB). Even though a main effect was not seen, the effect of group was 2.4–30.0 times likely to influence EI. Specifically, post-hoc analysis of the interaction showed that women in REC possessed higher EI than their counterparts in ADV (RF, VM, VL, and TB).

Significant group effects were found for muscle thickness (VL and BB), pennation angle (BB and TB), fascicle length of TB, and CSA (VM and VL). Compared to CON, ADV possessed greater muscle thickness in VL (p = 0.013, BF₁₀ = 3.0) and in BB (p = 0.012, BF₁₀ = 2.2), larger BB pennation angle (p = 0.007, BF₁₀ = 21.9), and greater CSA in VM (p = 0.050, BF₁₀ = 2.1) and VL (p = 0.009, BF₁₀ = 0.7). Compared to REC, ADV possessed greater muscle thickness in VL (p = 0.026, BF₁₀ = 1.9), larger pennation angle in TB (p = 0.009, BF₁₀ = 3.2), longer fascicles in TB (p = 0.019, BF₁₀ = 3.9), and greater CSA in VL (p = 0.009, BF₁₀ = 3.2), longer fascicles in TB (p = 0.019, BF₁₀ = 3.9), and greater CSA in VL (p = 0.009, BF₁₀ = 0.8); a tendency for greater muscle thickness in BB was also noted for ADV compared to REC (p = 0.086, BF₁₀ = 0.9). No differences were seen between REC and CON. Morphological comparisons are presented in Table 1.

		ADV		REC		CON		Gr	Group		Sex		x Sex
		Women	Men	Women	Men	Women	Men	p	BF ₁₀	p	BF ₁₀	p	BF ₁₀
Muscle thickness (cm)	Rectus femoris	2.48 ± 0.36	3.28 ± 0.50	2.32 ± 0.27	2.86 ± 0.20	2.46 ± 0.25	2.61 ± 0.43	0.155	8.2	0.004	6.7	0.248	0.5
	Vastus medialis	3.44 ± 0.84	4.35 ± 0.58	2.77 ± 0.08	4.28 ± 0.61	3.41 ± 0.62	4.26 ± 0.22	0.439	>100	0.001	39.8	0.502	0.3
	Vastus lateralis	1.92 ± 0.49	2.47 ± 0.39	1.49 ± 0.25	1.97 ± 0.19	1.63 ± 0.19	1.67 ± 0.28	0.009	11.9	0.017	7.9	0.288	1.7
	Biceps brachii	3.32 ± 0.60	4.29 ± 0.87	2.62 ± 0.19	3.74 ± 0.71	2.43 ± 0.08	3.31 ± 0.17	0.013	>100	0.001	40.6	0.910	1.3
	Triceps brachii	2.51 ± 0.45	3.05 ± 0.76	2.44 ± 0.64	2.91 ± 0.58	1.98 ± 0.24	3.05 ± 0.43	0.674	7.1	0.008	2.1	0.541	0.3
Pennation angle (°)	Rectus femoris	12.7 ± 3.5	15.5 ± 0.5	10.0 ± 3.0	14.5 ± 1.2	13.3 ± 5.6	16.4 ± 5.5	0.375	2.9	0.036	1.4	0.894	0.5
	Vastus medialis	19.2 ± 3.9	26.2 ± 9.3	17.2 ± 3.4	24.9 ± 6.0	27.7 ± 10.8	24.0 ± 3.5	0.417	0.7	0.216	0.4	0.229	0.2
	Vastus lateralis	14.3 ± 3.1	14.8 ± 2.9	10.7 ± 3.4	12.4 ± 5.8	12.3 ± 0.4	13.2 ± 3.5	0.286	0.6	0.502	0.5	0.947	0.1
	Biceps brachii	13.6 ± 3.1	17.5 ± 2.2	12.8 ± 2.1	12.3 ± 5.0	10.5 ± 2.2	9.7 ± 1.5	0.009	7.0	0.489	3.2	0.249	0.4
	Triceps brachii	17.9 ± 5.1	26.8 ± 7.8	11.1 ± 3.2	16.8 ± 4.5	14.2 ± 2.6	20.3 ± 4.1	0.012	34.5	0.004	13.8	0.791	2.4
Fascicle length (cm)	Rectus femoris	12.2 ± 5.1	12.3 ± 2.2	14.1 ± 3.8	11.5 ± 0.8	12.8 ± 7.6	10.3 ± 4.5	0.852	0.6	0.365	0.3	0.786	0.1
	Vastus medialis	10.6 ± 2.4	11.2 ± 4.8	9.6 ± 1.8	10.6 ± 2.7	7.9 ± 2.4	10.6 ± 1.1	0.552	0.6	0.280	0.3	0.758	0.1
	Vastus lateralis	7.7 ± 0.4	9.9 ± 2.1	8.3 ± 1.5	10.5 ± 4.6	7.6 ± 0.6	7.7 ± 2.2	0.399	0.8	0.163	0.4	0.643	0.2
	Biceps brachii	14.5 ± 2.8	14.2 ± 1.3	$12.0\pm1.1^{\rm df}$	19.0 ± 4.8^{c}	13.8 ± 2.8	19.9 ± 2.7^{c}	0.271	9.8	0.002	8.8	0.043	0.5
	Triceps brachii	8.7 ± 2.8	7.3 ± 2.9	12.8 ± 1.7	10.5 ± 3.1	8.2 ± 0.9	9.1 ± 2.2	0.018	4.3	0.370	2.4	0.448	0.5
Cross-sectional area (cm ²)	Rectus femoris	10.8 ± 2.4	17.8 ± 3.3	8.8 ± 1.5	15.6 ± 1.8	11.2 ± 1.3	15.0 ± 2.8	0.216	>100	0.000	>100	0.364	0.4
	Vastus medialis	24.3 ± 6.6	29.8 ± 4	17.0 ± 3.9	27.8 ± 6.4	18.2 ± 2.1	23.7 ± 1.2	0.046	22.3	0.002	12.0	0.441	0.8
	Vastus lateralis	29.8 ± 4.1	44.9 ± 4.1	24.4 ± 2.6	38.2 ± 4.5	24.1 ± 1.7	37.9 ± 3.0	0.004	>100	0.001	>100	0.912	0.5
	Biceps brachii	8.4 ± 1.7	17.7 ± 9.2	7.4 ± 2.0	14.9 ± 2.5	7.9 ± 0.2	12.9 ± 1.0	0.464	77.5	0.001	32.6	0.622	0.3
	Triceps brachii	10.5 ± 1.2	18 ± 4.3	7.0 ± 1.4	17.0 ± 5.7	8.9 ± 1.4	14.0 ± 3.6	0.273	>100	0.000	>100	0.417	0.3
Echo intensity (au)	Rectus femoris	116 ± 26^{c}	113 ± 11^{c}	174 ± 33^{abd}	97 ± 14^{ce}	151 ± 16^{d}	129 ± 14	0.061	30.0	0.001	5.5	0.008	0.6
	Vastus medialis	105 ± 16^{c}	108 ± 11	153 ± 39^{ad}	104 ± 12^{c}	116 ± 11	134 ± 13	0.093	2.4	0.289	0.8	0.012	0.4
	Vastus lateralis	111 ± 20^{c}	113 ± 15^{c}	171 ± 42^{abd}	107 ± 19^{c}	134 ± 16	123 ± 10	0.087	4.3	0.023	1.7	0.028	0.7
	Biceps brachii	123 ± 26	140 ± 9	170 ± 45	115 ± 29	148 ± 9	142 ± 17	0.585	0.6	0.206	0.5	0.044	0.2
	Triceps brachii	83 ± 17^{c}	89 ± 18^{c}	145 ± 43^{abd}	78 ± 13^{c}	114 ± 16	100 ± 6	0.079	7.2	0.014	1.8	0.012	0.6

Table 1. Measures of muscle morphology by group and sex.

 $^{\rm a}$ = Significantly (p < 0.05) different from ADV women

 $^{\rm b}$ = Significantly (p < 0.05) different from ADV men

^c = Significantly (p < 0.05) different from REC women

^d = Significantly (p < 0.05) different from REC men

^e = Significantly (p < 0.05) different from CON women

 $^{\rm f}$ = Significantly (p < 0.05) different from CON men.

Graded exercise test

No significant group x sex interactions were observed for VO₂peak (F = 1.09, p = 0.358, BF₁₀ = 10.1), RCT (F = 0.32, p = 0.730, BF₁₀ = 1.7), or GET (F = 0.05, p = 0.949, BF₁₀ = 1.1). However, *moderate*-to-*strong* evidence were found in favor of main group effects for each variable. VO₂peak (F = 9.10, p = 0.002, BF₁₀ = 17.0) and RCT (F = 5.56, p = 0.014, BF₁₀ = 4.5) were significantly greater in ADV compared to REC ($p \le 0.039$) and CON ($p \le 0.020$), while GET (F = 5.29, p = 0.016, BF₁₀ = 5.7) was significantly greater in ADV compared to CON (p = 0.016) and tended to be greater compared to REC (p = 0.087). No differences were seen between REC and CON. Further, the percentage of VO₂peak for GET and RCT were similar between ADV (GET = 55.2 ± 11.2%; RCT = 71.7 ± 7.5%), REC (GET = 55.9 ± 6.8%; RCT = 73.5 ± 5.9%), and CON (GET = 53.9 ± 4.3%; RCT = 74.6 ± 7.7%). Group differences in measures of aerobic performance are illustrated in Fig 2.

Resting metabolic rate

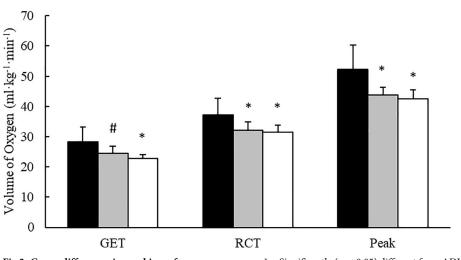
Neither a group x sex interaction (F = 0.21, p = 0.817, BF₁₀ = 0.2) or main group effect (F = 1.67, p = 0.220, BF₁₀ = 0.1) was observed for RMR recordings in ADV (1788 ± 232 kcal·day⁻¹), REC (1768 ± 407 kcal·day⁻¹), and CON (1572 ± 356 kcal·day⁻¹).

Body composition

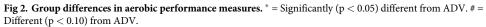
No significant group x sex interactions were observed for any measure of body composition (presented in Table 2). However, the evidence was *strongly*-to-*extremely* in favor of main group effects for body density, regional and total BMC, regional and total lean mass, and BF%. Compared to the REC, ADV possessed greater body density (p = 0.004), greater BMC of the arms (p = 0.009), greater lean mass (i.e., total and regional; $p \le 0.035$), lower BF% (p = 0.009), and tended to possess more BMC (total-body: p = 0.066; legs: p = 0.060) and less fat mass (p = 0.064). Compared to CON, ADV possessed greater body density (p = 0.006), greater BMC throughout the body ($p \le 0.024$), lean mass throughout the body ($p \le 0.009$), and lower BF% (p = 0.023). No differences were observed between REC and CON.

Strength

No significant group x sex interactions were observed for variables obtained from the isometric mid-thigh pull assessment. *Extreme* evidence suggested significant main group effects for F (F = 3.89, p = 0.042, BF₁₀ = 667,577) and RFD at 200 ms (F = 3.67, p = 0.049, BF₁₀ = 12,676), as well as tendencies for group differences in F at 150 ms (F = 2.80, p = 0.091, BF₁₀ = 1,898), F at 200 ms (F = 3.50, p = 0.055, BF₁₀ = 17,296), F at 250 ms (F = 3.14, p = 0.071, BF₁₀ = 21524), RFD at 150 ms (F = 2.94, p = 0.082, BF₁₀ = 1,868), and RFD at 250 ms (F = 3.37, p = 0.060, BF₁₀ = 20,187). According to post-hoc analysis, ADV produced a higher peak F than CON (p = 0.036) and expressed greater RFD at 200 ms (p = 0.097) compared to REC. No other specific differences were seen between groups. Group differences in F and RFD production across time are illustrated in Fig 3.







	ADV			REC				Group		Group x Sex			
	Women	Men	Total	Women	Men	Total	Women	Men	Total	p	BF ₁₀	p	BF ₁₀
Anthropometric													
Height (cm)	160 ± 13	177 ± 3	170 ± 11	161 ± 4	183 ± 8	172 ± 14	158 ± 4	180 ± 9	171 ± 14	0.785	>100	0.526	0.3
Weight (kg)	68.3 ± 5.0	91.5 ± 5.1	79.8 ± 13.3	59.0 ± 2.0	93.5 ± 9.5	76.3 ± 19.5	60.8 ± 6.3	84.9 ± 7.3	74.5 ± 14.3	0.127	>100	0.169	0.3
BMI (kg⋅m ⁻²)	26.0 ± 3.5	29.2 ± 1.9	27.6 ± 3.1	22.9 ± 1.2	28.0 ± 3.4	25.5 ± 3.6	24.4 ± 3.4	26.1 ± 0.7	25.4 ± 2.2	0.163	6.6	0.456	0.6
Density (kg·L ⁻¹)	1.07 ± 0.01	1.07 ± 0.01	1.07 ± 0.01	1.05 ± 0.01	1.06 ± 0.01	$1.05 \pm 0.01^{*}$	1.04 ± 0.02	1.06 ± 0.01	$1.05 \pm 0.02^{*}$	0.002	13.8	0.159	1.1
Bone Mineral Content (kg)													
Total	3.05 ± 0.38	3.75 ± 0.13	3.45 ± 0.44	2.42 ± 0.16	3.62 ± 0.41	3.02 ± 0.70#	2.43 ± 0.14	3.29 ± 0.41	$2.92\pm0.55^*$	0.012	>100	0.299	0.7
Arms	0.45 ± 0.07	0.62 ± 0.05	0.55 ± 0.11	0.32 ± 0.03	0.57 ± 0.05	$0.44 \pm 0.14^{*}$	0.30 ± 0.01	0.48 ± 0.06	$0.40\pm0.11^*$	0.001	>100	0.266	1.2
Legs	1.12 ± 0.13	1.44 ± 0.11	1.30 ± 0.20	0.82 ± 0.05	1.38 ± 0.17	1.10 ± 0.32#	0.81 ± 0.03	1.31 ± 0.22	$1.09\pm0.31^*$	0.022	>100	0.255	0.5
Trunk	0.95 ± 0.11	1.16 ± 0.03	1.07 ± 0.13	0.79 ± 0.11	1.11 ± 0.14	0.95 ± 0.21	0.82 ± 0.08	0.97 ± 0.09	$0.90 \pm 0.11^{*}$	0.028	>100	0.271	0.8
Non-bone fat-free mass (kg)													
Arms	7.15 ± 0.89	11.12 ± 1.22	9.42 ± 2.35	4.87 ± 0.49	10.02 ± 0.56	$7.45 \pm 2.79^{*}$	4.83 ± 0.42	9.04 ± 1.14	$7.24 \pm 2.40^{*}$	0.001	>100	0.400	0.6
Legs	18.4 ± 1.4	25.4 ± 1.6	22.4 ± 4.0	14.3 ± 1.0	24.4 ± 1.1	$19.3 \pm 5.4^{*}$	14.7 ± 0.7	22.5 ± 3.2	$19.2 \pm 4.7^{*}$	0.008	>100	0.252	0.5
Trunk	27.7 ± 2.9	35.2 ± 2.0	32.0 ± 4.5	20.3 ± 1.2	33.5 ± 1.4	$26.9 \pm 7.2^{*}$	21.4 ± 2.2	30.1 ± 3.7	$26.4 \pm 5.5^{*}$	0.001	>100	0.073	0.8
4-compartment model													
Body fat percentage (%)	11.9 ± 2.4	11.0 ± 2.6	11.4 ± 2.3	23.3 ± 2.4	16.1 ± 6.2	19.7 ± 5.8*	23.9 ± 8.4	13.7 ± 3.2	$18.1 \pm 7.6^{*}$	0.007	16.1	0.183	2.7
Fat-free mass (kg)	60.2 ± 3.5	81.3 ± 3.4	72.3 ± 11.7	45.2 ± 2.3	78.1 ± 5.3	61.7 ± 18.0*	45.9 ± 1.6	73.3 ± 8.4	61.6 ± 15.9*	0.001	>100	0.097	0.5
Fat mass (kg)	8.2 ± 2.1	10.2 ± 2.7	9.3 ± 2.5	13.8 ± 1.4	15.4 ± 7.0	14.6 ± 4.7#	14.9 ± 6.7	11.5 ± 2.2	13.0 ± 4.5	0.069	1.5	0.436	0.3

Table 2. Group differences in measures of body composition.

* = Significantly (p < 0.05) different from ADV

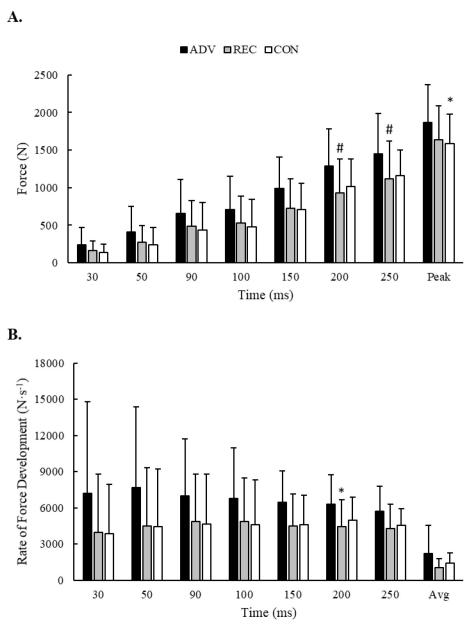
= Different (p < 0.10) from ADV.

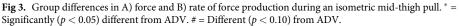
3-minute all-out cycling test

No significant group x sex interactions were observed for measures collected from the 3-minute all-out cycling test. *Extreme* evidence in favor of a significant group main effect for CP (F = 7.56, p = 0.005, BF₁₀ = 267) indicated that ADV possessed a higher CP than REC (p = 0.029) and CON (p = 0.005). Although extreme evidence was also seen for AWC (F = 4.79, p = 0.023, BF₁₀ = 247), post-hoc analysis did not reveal specific group differences. No other differences were observed. Group differences in measures of performance during the 3-minute all-out cycling test are illustrated in Fig 4.

Discussion

The primary objectives of this study were to examine anthropometric, hormonal, and physiological differences between advanced CF athletes, recreational CF participants, and resistance and cardiovascular trained adults. Previously, only one other cross-sectional investigation has made physiological comparisons between individuals with at least one year of CF or resistance-training experience [27]. The authors reported no differences between the groups except for the CF-trained group possessing greater aerobic ability. This outcome, however, is not surprising considering that the resistance-trained group was not required to also have been performing aerobic exercise. Typical CF training workouts will concurrently incorporate strength and conditioning elements into training [1, 2, 51]. Although the conditioning component





varies in intensity and duration for each workout, it is important that alternative exercise strategies include both elements to make a fair comparison. The present study builds upon this limitation by having required participants in the CON group to have been participating in both resistance and cardiovascular training on at least 3 days per week each; a similar training frequency was expected of the recreational CF group (i.e., training on at least 3 days per week). Another important aspect of CF training worth consideration is that it includes a wide variety of traditional resistance and aerobic training exercises, along with simple-to-complex gymnastic movements. Proficiency in these movements cannot be assumed after only a year of training and would likely necessitate frequent workout modification. Recently, our group has reported different physiological responses and recovery rates to CF workouts that are

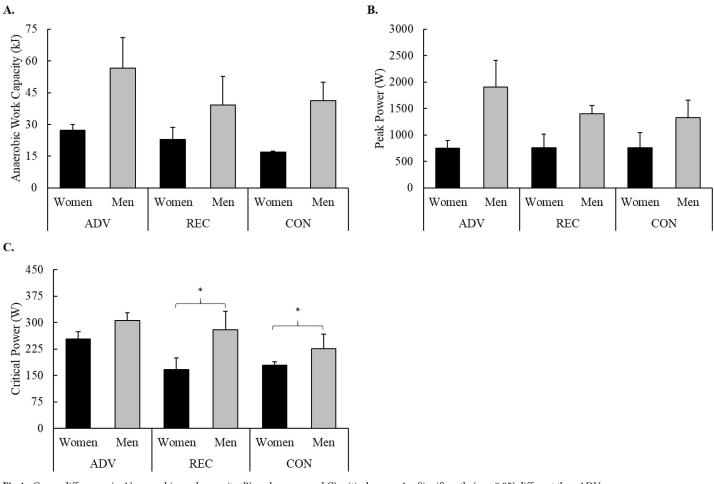


Fig 4. Group differences in A) anaerobic work capacity, B) peak power, and C) critical power. * = Significantly (p < 0.05) different than ADV.

completed as prescribed versus those that are modified (i.e., scaled) [11]. Thus, CF-trained participants were required to possess at least two years of experience and they were further divided into ADV and REC based upon evidence of their skill as CF athletes (i.e., their previous success in CF competition). Within these contexts, advanced CF athletes were observed to have a more favorable body composition and muscular morphological characteristics, as well as greater aerobic capacity, strength, and ability to sustain high-intensity effort compared to recreational CF participants and physically-active adults. In contrast, no differences were observed between recreational CF participants and physically-active adults in any measure and no differences were seen in resting hormone concentrations or metabolic rate across all groups. This is the first investigation to make comparisons among CF practitioners based on their competitive rank and relative to resistance- and cardiovascular-trained, active adults.

Most competitive CF workouts require athletes to perform 2 or more exercises in a circuit or listwise fashion for several repetitions and rounds, and to do so as quickly as possible or to complete as much work as possible within a given time limit [1, 2, 51]. Athletes who can maintain a faster pace or rapidly recover between minimal rest periods would appear to be best positioned to excel in this sport. A recent study in advanced CF athletes, as determined by their performance in a common benchmark workout (i.e., "Fran"), supports this idea [52]. Feito et al. (2018) found that the best predictor of repetitions completed during a 15-minute CF workout was the amount of work the athletes could perform on the final trial of four

maximal Wingate sprints separated by 90 seconds of rest. In the present study, the ADV group possessed a lower percentage of body fat and greater non-bone fat-free mass compared to the REC and CON groups. In sports, possessing an ideal ratio of skeletal muscle to fat mass may offer a competitive advantage by improving efficiency, thermoregulation, and the ability to sustain effort [53]. Aside from their historical success in CF competition, the ADV group's performance during testing provide evidence of this ability. ADV participants possessed a higher VO₂peak than the other groups, which would imply that they were able to perform aerobic work throughout a greater range of workloads [54, 55] but it does not completely explain their ability to sustain effort at higher intensities [56]. As the oxygen requirements of a workload exceed an athlete's capacity to efficiently deliver oxygen, the ability to sustain effort may be further explained by measures of anerobic performance and specific threshold points indicative of the onset of fatigue (i.e., GET, RCT, and CP) [48, 56, 57]. Participants in the ADV group were also found to possess a higher GET, RCT, and CP, which are all strongly correlated [57] and thought (specifically RCT and CP) to demarcate the point in which exercise transitions from 'heavy' to 'severe' [57, 58]. Together, these data suggest that the ADV athletes in this study had a greater capacity to produce energy aerobically, and that they were better equipped to maintain efforts at higher absolute workloads and thus, be successful in their sport.

Skeletal mass and the morphological characteristics of muscle are suggestive of a greater ability to produce force [59-62]. That is, the size, architecture and quality of skeletal muscle reflect the capability of activated muscle to produce force, whereas bone mass provides the structural support and stability needed to effectively translate force production into human movement. In the present study, ADV athletes possessed greater bone and muscle mass/size, larger pennation angles, shorter fascicles, and better quality in the arm and quadriceps musculature compared to the other groups. However, these only partially translated to greater force production by ADV group participants during the IMTP test. IMTP performance was highly variable until 0 to 200-250 ms, upon which ADV clearly produced greater force and at a faster rate. The lack of uniformity across all strength measures might be explained by testing specificity and the skillset of our sample. The importance of being able to rapidly activate muscle (i.e., higher RFD) and the magnitude of IMTP force production varies across sports and athletic activities. In weightlifters, significant relationships have been reported between one-repetition maximums in the Olympic lifts and IMTP force (peak and from 0 to 100–250 ms) [63] but relationships to RFD have either been limited to specific time bands (from 0 to 200-250 ms) [63] or remain unclear in other athletes [64, 65]. Although maximal strength in the Olympic and power lifts can distinguish competitive ranking in CF athletes [8, 10], it is not a common requisite of CF competition to maximally perform these lifts. Rather, most competitive workouts either utilize submaximal loads that are performed for several repetitions or they require the athlete to perform maximal (or near maximal) lifts after a fatiguing task (i.e., not a true measure of maximal strength) [51]. It is also possible that the composition of the ADV group may help explain the variability observed prior to 200 ms. While all ADV group participants ranked higher than REC in the Open, their participation in later rounds of the Games competition had primarily occurred as part of a team. Within this capacity, team members may be included based on their skill set (e.g., strong/powerful athletes, gymnastically-skilled athletes, endurance athletes) to minimize team weaknesses. This differs from individual competitors who must be proficient in a broader set of skills to be competitive [8, 10]. Currently, evidence documenting the physiological differences between high-ranking individual and team competitors does not exist.

There is little evidence to suggest that consistent alterations will occur to resting concentrations in T, C, or IGF-1 as a result of chronic training [14]. Rather, their concentrations generally reflect the current status of muscle tissue in response to the demands of training. Transient changes in T, IGF-1, and C may occur following acute and prolonged overreaching (or overstress) periods that could negatively impact anabolic status [14]. CF training is characterized by an effort to maximize training density (i.e., complete a set amount of work as quickly as possible, or maximize work completed within given time frame) within an unplanned (i.e., non-periodized) training structure to promote general physical preparedness [1, 2]. Further, the 5-week Open is the most common avenue used by athletes to qualify for the Games [4, 5]. Prior to an important competitive event, athletes may elevate training intensity to promote peak performance [66]. Thus, the combination of the CF training strategy and the approach of an important, extended competitive event could increase the likelihood of a prolonged period of overstress. The occurrence of which might be identified by changes in resting hormonal concentrations, resting metabolic rate, performance, as well as a variety of other factors [14, 67, 68]. However, the present investigation did not reveal any evidence of prolonged stress or negative adaptations to training. Resting hormone concentrations and metabolic rates were similar between groups and the physiological advantages demonstrated by the ADV group appeared to reflect their reported training habits over the past six months (via medical and physical activity history questionnaire). Excluding the conditioning component typically present in CF workouts, members from each group reported using a similar number of sets per muscle group (3-6), repetitions (3-12), and rest intervals (60-90 seconds) during the strength component of their workouts. Only training frequency was reported to be different with the ADV group utilizing a form of resistance exercise on approximately 5.3 days per week whereas the REC and CON groups averaged 4.6 days per week and 3.7 days per week, respectively. Although the greater training frequency seen in ADV would have theoretically provided more of an opportunity to accumulate training volume and promote adaptations, it could have also interfered with their recovery. Nevertheless, ADV possessed a more favorable body composition and generally outperformed the other groups in each performance measure. Therefore, as of one-month prior to competition, adequate recovery appeared to be present in this group. Likewise, the lack of differences seen between REC and CON, who were not actively training for the Open, also provides evidence of adequate recovery. Future investigations can expand on this by more closely monitoring training and performance surrounding the extended Open competition.

The findings of this study suggest that advanced CF athletes possess a more favorable body composition, greater bone and muscle mass, greater muscle quality and strength, greater aerobic capacity, and a greater ability to sustain effort than recreational CF participants and physically-active adults. The reasons for these differences remain unclear due to the cross-sectional design of this study but may be related to differences in training experience and recent training habits. Although all participants in this study could be considered well-trained [69], ADV group participants reported having more resistance training experience and having been training more frequently over the past 6 months than the other groups. It is possible that their advantages are simply the result of training for a longer amount of time or creating more opportunities to increase their volume load throughout the week. Without documentation (i.e., extensive, detailed training logs), however, it is only possible to speculate upon their potential influence as unknown factors (e.g., training quality, genetic predisposition) would certainly modulate resultant adaptations. It may be worthwhile for future investigations to make comparisons between advanced CF athletes and non-CF individuals with comparable training experience and habits to better determine whether an advantage exists.

It is also interesting to note that despite the superficial differences in each strategy (i.e., CF versus traditional resistance and cardiovascular training), REC and CON were found to possess similar physiological characteristics. It is possible that this was the consequence of our sample size being sufficient to observe the large differences that existed between ADV and the

other groups but not for the smaller differences that existed between REC and CON. However, it may also be the consequence of effort and volume load during training being similar between these groups. To be included in the study, both REC and CON had to have been regularly participating in their chosen training strategy on 3–5 days per week for at least the past year. Beyond this requirement, however, our ability to quantify training volume load was limited to the participants' recall over the past 6 months. Future longitudinal investigations that document both the quality and quantity of these training forms may help to provide insight into whether an advantage exists between these strategies or if they promote comparable adaptations among recreationally-active adults. Nevertheless, the present findings represent a starting point for future comparisons between experienced CF participants (athletes and recreational) and resistance-trained and cardiovascular-trained adults, as well as between sexes across these populations.

Supporting information

S1 Data. (CSV)

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11 The effects of a 6-week core exercises on swimming performance of national level swimmers

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Abstract

The aim of this study was to assess the impact of a 6-week specialized training program aimed at strengthening core muscles to improve the effectiveness of selected elements of a swimming race on a group of Polish swimmers. Sixteen male national level swimmers (21.6 ± 2.2 years) participated in the research. The competitors were randomly assigned to 1 of 2 groups before the data collection process: an experimental (EG, n = 8) and a control (CG, n = 8) group. Both groups of swimmers underwent the same training program in the water environment (volume and intensity), while swimmers from the EG additionally performed specific core muscle training. The task of the swimmers was an individual front crawl swim of 50 m, during which the kinematic parameters of the start jump, turn and swimming techniques were recorded using a video camera system. In both groups, a minor increase in the flight phase was observed at the start (EG = 0.06 m, 1.8%; p = 0.088; CG = 0.08 m, 2.7%; p = 0.013). The time to cover a distance of 5 m after the turn and the recorded average speed in swimming this distance for the EG statistically significantly improved by 0.1 s (-28.6%; p < 0.001) and 3.56 m·s⁻¹ (23.2%; p = 0.001), respectively. In the EG, a statistically significant improvement in 50 m front crawl swimming performance of 0.3 s (-1.2%, p = 0.001) was observed. The results of the research show that the implementation of isolated strengthening of the stabilizing muscles seems to be a valuable addition to the standard training of swimmers.

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Introduction

Strength and muscular power are significant determinants of success in swimming-related sports. Appropriate training of the abdominal muscles and torso seems to be one of the key elements determining the effectiveness of the training process [1]. The main goal of swimming competition is to overcome the given distance in the shortest possible time, which is achieved mainly by proper body positioning in the water and minimizing resistance [2–5]. Numerous publications show that exercises strengthening the core muscles are an integral part of many swimming training

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programs [4,6,7]. Increased work of the stabilizing muscles can form the basis for generating more strength through the limbs [8,9]. According to many sources, the concept of core muscles is expanding, including the rectus abdomen, latissimus dorsi, gluteus maximus or trapezius [6,10]. Proper control of the body position while swimming at a distance, as well as during the start jump and turn, increases the efficiency and thus reduces the distance traveled [11].

Appropriate strengthening of the muscles responsible for the correct positioning of the body is fundamental to the swimming technique [12]. This involves correct positioning of individual body segments, i.e., the head, shoulder girdle, torso, pelvis girdle and legs. Efficiency in swimming can be achieved if these muscles follow a nearly linear arrangement, thereby minimizing the resistance applied by the water to the body [11,13,14]. The unstable background against which the body of the swimmer is located requires exemplary core muscle work, and a lack of stable support implies a deficit of one or several muscles, which can cause significant time losses. In addition to minimizing resistance, an appropriate high and stable body position allows one to optimize the power of his or her upper and lower limbs [4,15,16].

There is much evidence in the literature on the effectiveness of dry-land training in improving the results achieved in swimming [17,18]. In the research of Patil et al. [4], in accordance with the authors' expectations, the proposed specialized core muscle strengthening training improved the performance of this area (functional core muscle strength test) and led to significant improvement during a 50 m front crawl swim.

Additionally, Gencer's [19] experiment aimed to review the effects of an 8-week core training program to see how certain physical and motor attributes change, as well as measure the difference in performance in front crawl swimming by female athletes. The results showed that the experimental group significantly improved their performance in the 50-meter front crawl time trial. The authors also noted an improvement in horizontal jump, vertical jump, and push-ups after the prescribed 8-week training block. Similar conclusions were drawn by Gönener et al. [20], who stated that training with the use of Thera-Band tapes (including their use to engage core muscles) improves the performance of swimmers. Core muscle training has been widely researched in the recent years, and even though it seems like there is an universal conviction that the correct strength of stabilizing muscles improves the athletic level of competitors, some papers show only marginal impact of this type of training on the final sporting success [7,10,19]. Many studies do not show a direct relationship between improving muscular strength on land and improving the results achieved by swimmers in the water [21,22]. Inconsistent findings of the previous research encouraged us to take a different approach and study the effects of dry-land training (with emphasis on strengthening core muscles) on selected kinematic parameters and performance in 50 m front crawl swimming.

We hypothesize that strengthening the core muscles will positively impact the effectiveness of the studied elements of the swimming start, turn technique and other aspects of a swimming race over a distance of 50 m. We also assume that as a result of the experiment, this strengthening will improve the completion time for a 50 m front crawl swim.

Thus, the present study aimed to investigate the effect of strengthening core muscles as a result of dry-land training in a 50 m front crawl performance of national level male swimmers. This study will also examine the effect of strengthening core muscles on a number of kinematic variables in front crawl swimming.

Methods

Participants

Sixteen male national level swimmers who are members of the Polish National Swimming Team (seniors and youth) were involved in this research. The competitors had a minimum of 10 years of training experience, and their best results were at least 800 points according to the FINA classification. The participants in the experiment were in the same period of preparation for competition, i.e., in the subperiod of specific preparation. The competitors were randomly placed into either an experimental group (EG) of 8 swimmers or a control group (CG) of 8 swimmers. Both groups of swimmers carried out the same training program (10 training sessions in water and 2 training sessions in the gym per week), while swimmers in the EG additionally carried out specialized core muscle training (SCMT), which took place 3 times a week for 6 weeks. For both groups, water training took place from 6:00-8:00 a.m. and 5:00-7:00 p. m., and the strength training occurred on the days when the swimmers did not perform SCMT. Both strength training and experimental training took place after the water training. The training performed in the experiment did not disturb the preparation of the swimmers to start in competitions. All participants had up-to-date medical examinations, and any contraindications to participate in the studies were excluded. None of the swimmers were taking drugs, medication, or dietary supplements known to influence physical performance. During the experiment, the subjects were tested on an equal and balanced diet. The calorific value was selected individually based on the measurement of body mass composition and the volume and intensity of the training program. The swimmers also offered written consent to participate in the experiment. All the swimmers participating in this study were informed about the procedures, goals, and expected duration of the experiment. They were also informed that they were free to withdraw from the research at any stage. The research project was approved by the University Bioethics Committee for Research at The Jerzy Kukuczka Academy of Physical Education in Katowice (No. 8/2018). Anthropometric data of the competitors are presented in Table 1. Body height was assessed using a stadiometer (Seca 213, Seca GmbH & Co, Hamburg, Germany) with a precision of 0.5 cm, while the body mass and its composition were determined by the method of electrical impedance using the InBody 220 device (Biospace Co. Japan).

Procedures

The training program, which lasted six weeks, consisted of 18 units of targeted dry-land training. The duration of the main unit did not exceed 25 minutes. According to the purpose of the research, the developed training program included exercises involving the core muscles. In the general sense, we referred to them as torso muscles or, less recently, used the term "body core". Comparing with other definitions of this term, we find a common denominator, i.e., the deep muscles that provide stabilization of the whole body and the basis for functional stability of the lumbar, sacral and iliac areas [6,10,12,14]. SCMT consists of four exercises: flutter kicks (scissors), single leg V-ups, prone physio ball trunk extension, and Russian twists. Progression consisted of changing the position of the body, adding a motion element, adding an unstable ground and increasing the resistance. The same training units were carried out three times a

Variable	Experimental group (n = 8)	Control group (n = 8)	p-value
Age (year)	20.2 ± 1.17	20.0 ± 1.9	0.606
Body mass (kg)	74.9 ± 10.67	75.4± 6.27	0.926
Height (cm)	183.0 ± 6.57	182.1 ± 3.18	0.761
Fat mass (%)	6.52 ± 3.22	8.09 ± 2.23	0.140
Lean body mass (kg)	30.5 ± 5.46	29.4 ± 1.31	0.101
Fat mass of trunk (%)	5.75 ± 3.01	7.96 ± 2.30	0.124

Table 1. Physical characteristics of participants (mean ± SD).

week. Depending on the exercise, the level of difficulty progressed in weekly or biweekly cycles. If a swimmer was unable to complete the task with a certain resistance, he returned to the load from the previous microcycle until the end of the duration of the given exercise. All exercises were performed in 4 series, with a 40-second work schedule and a 20-second break between sets. The duration of the training and the number of series were based on the coaching experience of the authors, but they are also justified by the literature. Many authors [11,23] suggest a temporary dosage of exercises in core muscle training and a certain number of series. Based on these, our research protocol was established. The details of the training program are presented in Table 2.

The tests consisted of two stages: one preceding the experiment and one performed after the experiment. During the research, the same procedure was carried out at the same time of day and with the same order of athletes. The measurements were carried out in a 25 m swimming pool (The Jerzy Kukuczka Academy of Physical Education in Katowice) three days before and after the core muscle training was completed. During the tests, the air temperature was \sim 25°C, the water temperature was \sim 27°C, the water pH was \sim 6.93, and the relative air humidity was ~60%. The task of the swimmers was to swim 50 m front crawl technique from the starting block under race conditions. To accurately measure the times achieved by the participants, the Omega electronic time measurement system was used (OMEGA S.A., Switzerland). The swimming race was recorded using two digital video cameras (JVC GC-PX100BE, Japan) with a rapid shutter speed (1/1000 s) operating at a sampling rate of 50 Hz. One of the cameras was set 1.5 m above the water at a distance of 2 m from the starting wall perpendicular to the direction of the road traveled by the swimmer to register the dive start and the entrance of the swimmer into the water. The second camera was placed 1.5 m above the water exactly in the middle of the swimming pool lengthwise (12.5 m from the starting wall) to capture the distance swum. Both cameras were mounted on tripods positioned at poolside 0.5 m from the edge of the pool perpendicular to lane 2. To register the glide after the turn, a third camera (Sony FDR-X3000, Japan) was placed underwater at a distance of 2 m from the turning wall at a depth of 1.0 m at the sidewall of the pool basin; the lens of this camera covered both the turning wall and a mark located 5 m from the turning wall. These cameras were calibrated using a series of poles of known lengths positioned at specifically known positions throughout the length of the area that the swimmers traveled during each trial. The following parameters of

Week of training	Flutter kicks (scissors)	Single leg V-ups	Prone physio ball trunk extension	Russian twists
1	Arms crossed on the chest	No extra load	Arms crossed on the chest	No extra load
2	Streamlined position No extra load		Arms crossed on the chest	No extra load
3	Arms crossed on the chest + weights on Dumbbells in hands the ankles		Holding medicine ball	Holding kettlebell
4	Streamlined position + weights on the ankles	Dumbbells in hands	Holding medicine ball	Holding kettlebell
5	Arms crossed on the chest + weights on the ankles (swimmer performs this progression on a wiggle cushion)	Dumbbells in hands + weights on the ankles	Medicine ball trunk extension throw	Holding kettlebell (swimmer performs this progression while sitting on a wiggle cushion)
6	Streamlined position + weights on the ankles (swimmer performs this progression on a wiggle cushion)	Dumbbells in hands + weights on the ankles	Medicine ball trunk extension throw	Holding kettlebell (swimmer performs this progression while sitting on a wiggle cushion)

Table 2. A brief description of the exercises of SCMT and their progression over a 6-week training program.

the dive start were analyzed: entry distance (cm), time in the air with take-off (s), reaction time (s), time in the air (s), entry velocity $(m \cdot s^{-1})$, and dive angle (°). The time was measured when a swimmer reached a distance of 5 m after the turn, and then the speed of swimmer after the turn after completion of the first 5 m was calculated. Additionally, based on the swimming velocity data and the duration of three complete stroke cycles, the stroke rate (SR) (cycles $\cdot s^{-1}$) and the stroke length (SL) (m) were determined (a detailed description of all measured parameters is provided in Table 3). All video files were analyzed by 2 different researchers with experience in digitization management via the Kinovea software (v. 0.8.26, Kinovea, Paris, France), which allowed time-motion analysis of the registered elements. To assess the reliability of the digitizing process (interobserver), 6 trials were quantified using intraclass correlation coefficients (ICCs). The ICCs ranged from 0.979 (95% CI, 0.972–0.984) to 0.994 (95% CI, 0.983–0.997).

Statistical analysis

Means and standard deviations were used to represent the average and typical spread of values of all performance variables of the swimmers. The normal Gaussian distribution of the data was verified by the Shapiro-Wilk's test. Levene's test for the equality of means showed no significant differences in the group variances. A two-way analysis of variance with repeated measures and a Bonferroni post hoc test were used to investigate the main effects and the

Entry distance (cm)	Distance from the starting wall to the head entry point. It is considered as the length of the flight and is measured parallel (horizontal) to the water surface.
Entry velocity (m·s ⁻¹)	The horizontal velocity of the swimmer traveling through the air during the flight phase before entry into the water (based on the length of the flight phase and the time in the air).
Time in the air with take-off (s)	This is the sum of the "flight phase" and the "reaction time".
Time in the air (s) (flight phase)	The time from when the swimmer leaves the block to when the swimmer's head enters the water. It is also known as the flight phase.
Dive angle (degrees)	The angle at which the swimmer enters the water. It is the angle between the water surface and the central axis of the body at the time when the head touches the water surface.
Reaction time (s)	The time needed by the swimmer to leave the block following the starting signal. It is considered as the reaction time.
Time 5 m after the flip turn (s)	The time needed by the swimmer to reach the 5 m line after the turn. It covers the period between when the swimmer pushes off the wall and when the swimmer's head crosses the 5 m line.
Average velocity after the flip turn $(m \cdot s^{-1})$	The horizontal velocity, which the swimmer reaches 5 m after pushing off the wall.
Swimming velocity (m·s ⁻¹)	The horizontal velocity, which the swimmer obtains after swimming a distance of 5 m. It was measured between 12.5 and 17.5 m during the first and second 25 m.
Duration of 3 cycles (s)	The time needed by the swimmer to perform 3 strokes. It was measured for the first and second 25 m.
Stroke rate (cycles·s ⁻¹)	The time required to perform 3 stroke cycles was measured (in the middle section of the first and the second lap) and then used to calculate the stroke rate; $SR = 60 \times 3/tSR$ (SR: stroke rate, tSR: duration of 3 cycles).
Stroke length (m)	The distance covered in one stroke. It was calculated by dividing the swimmer's distance by the stroke rate. The SL calculation was based on the data gathered in 9 m sectors of the 50 m distance during both laps (for the first lap, between 15 and 24 m, and for the second lap, between 40 and 49 m).
Total time to complete the 50 m (s)	The total time needed to cover the distance of 50 m from the starting signal until the wall is touched by hand of the swimmer at the end.

Table 3. Detailed description of the parameters measured by using the Kinovea software while swimming 50 m front crawl.

interaction between the group factor (experimental vs. control) and time factor (pretraining vs. post-training), as well as the existence of differences between groups in the initial and final data of all variables.

The magnitudes of the differences between the results of the pretest and posttest were expressed as relative differences in percentages and as standardized mean differences (Cohen effect sizes). The criteria to interpret the magnitude of the effect sizes were as follows: <0.2, trivial; 0.2–0.6, small; 0.6–1.2, moderate; 1.2–2.0, large; and >2.0, very large. Additionally, the absolute and percentage change from pre- to post-test was calculated for all variables for each group.

Statistical power equations were used to determine the minimum study population at the p < 0.05 level with a power of 0.8 and revealed a sample of a minimum of 6 subjects in each group. Statistical significance was set at p < 0.05. All statistical analysis were conducted using Statistica 13.3 (TIBCO Software Inc.).

Results

Table 4 shows the results of all measurements before (pretest) and after (posttest) training.

In both the EG and CG, after the end of the 6-week training, we could observe an increase in the entry distance during take-off. In the EG, the improvement was 0.06 m (1.8%, p = 0.088, ES = *Moderate*), while in the CG, the improvement was 0.08 m (2.7%, p = 0.013, ES = *Moder-ate*). With the elongation of the flight phase in the EG, a statistically significant increase in the

Performance variable	PretrainingPost-trainingChange Δ (%)Mean \pm SDMean \pm SD[\pm 95% CI]		p	ES / rating	ANOVA (F, p)						
						Time effect		Group effect		Group	
						F	p	F	p	F	p
Entry distance (m)	3.11 ± 0.09	3.16 ± 0.08	0.06 (1.8%) [-0.01; 0.13]	.088	0.66 / Moderate	13.39	.003	6.75	.021	0.39	.545
	2.96 ± 0.13	3.04 ± 0.12	0.08 (2.7%) [0.02; 0.14]	.013	0.65 / Moderate						
Entry velocity (m·s ⁻¹)	12.77 ± 1.65	13.34 ± 1.47	0.57 (4.3%) [0.11; 1.02]	.021	0.36 / Small	0.03	.860	0.41	.533	3.01	.105
	13.99 ± 2.87	13.53 ± 2.81	-0.46 (-3.4%) [-1.79; 0.87]	.438	0.16 / Trivial						
Time in the air with take-off (s)	1.05 ± 0.03	0.95 ± 0.05	-0.09 (-9.7%) [-0.13; -0.06]	< .001	2.14 / V. large	34.91 < .001	1.48	.243	10.24	.006	
	1.05 ± 0.10	1.03 ± 0.08	-0.03 (-2.7%) [-0.06; 0.01]	.092	0.32 / Small						
Time in air (s)	0.25 ± 0.04	0.24 ± 0.03	-0.01 (-3.1%) [-0.02; 0.01]	.285	0.22 / Small	0.04	.846	0.76	.397	1.92	.188
	0.22 ± 0.05	0.23 ± 0.06	0.01 (4.4%) [-0.02; 0.04]	.388	0.19 / Trivial						
Dive angle (⁰)	40.13 ± 4.36	39.75 ± 4.23	-0.38 (-0.9%) [-3.53; 2.78]	.787	0.09 / Trivial	0.01	.929	1.76	.206	0.41	.535
	37.38 ± 3.25	37.88 ± 2.95	0.50 (1.3%) [-0.27; 1.27]	.170	0.16 / Trivial						
Reaction time (s)	0.80 ± 0.03	0.71 ± 0.03	-0.09 (-11.9%) [-0.12; -0.05]	.001	2.87 / V. large	33.73	< .001	11.53	.004	2.70	.123
	0.83 ± 0.05	0.79 ± 0.04	-0.05 (-6.1%) [-0.09; -0.01]	.025	1.02 / Moderate						
Time 5 m after the turn (s)	0.43 ± 0.06	0.34 ± 0.06	-0.10 (-28.6%) [-0.12; -0.07]	< .001	1.51 / Large	41.10	.10 < .001	4.98	.043	1.83	.194
	0.50 ± 0.11	0.44 ± 0.08	-0.06 (-14.2%) [-0.11; -0.01]	.026	0.65 / Moderate						
Average velocity 5 m after the turn $(m \cdot s^{-1})$	11.77 ± 1.68	15.34 ± 2.80	3.56 (23.2%) [2.16; 4.97]	.001	1.54 / Large	39.58	< .001	6.13	.027	9.55	.008
	10.37 ± 2.14	11.58 ± 2.11	1.22 (10.5%) [0.1; 2.33]	.037	0.57 / Small						
Stroke rate (cycles·s ⁻¹)	1.02 ± 0.08	1.03 ± 0.08	0.02 (1.5%) [-0.01; 0.04]	.242	0.19 / Trivial	1.80	.201	3.36	.088	0.63	.441
	0.97 ± 0.04	0.97 ± 0.05	0.00 (0.4%) [-0.01; 0.02]	.633	0.09 / Trivial						
Stroke length (m)	1.63 ± 0.15	1.58 ± 0.16	-0.05 (-3.5%) [-0.12; 0.01]	.091	0.36 / Small	3.50	.083	0.06	.805	3.24	.094
	1.59 ± 0.06	1.59 ± 0.08	0.00 (-0.1%) [-0.03; 0.02]	.924	Trivial						
Total time to cover 50 m (s)	25.24 ± 0.35	24.94 ± 0.49	-0.3 (-1.2%) [-0.43; -0.16]	.001	0.71 / Moderate	8.89	.010	15.13	.002	0.58	.458
	26.82 ± 1.09	26.64 ± 1.19	-0.18 (-0.7%) [-0.53; 0.18]	.274	0.16 / Trivial						

Table 4. Pre- and post-training values of the performance variables for swimmers. In each data block, the upper row is for the EG, and the lower row is for the CG.

CI-confidence interval; ES-effect size: <0.2, trivial; 0.2–0.6, small; 0.6–1.2, moderate; 1.2–2.0, large; >2.0, very large; Δ (%)–Absolute and percentage of change from pre- to posttest; *p*–*p*-value.

entry velocity was noted at 0.57 m·s⁻¹ (4.3%, p = 0.021, ES = *Small*), accompanied by a statistically significant reduction in the time in the air with take-off by 0.09 s (-9.7%, p < 0.001, ES = *Very large*). ANOVA revealed a significant interaction (training group × test time point) for the time in the air with take-off ($F_{(1,14)} = 10.242$, p = 0.006). In both the EG and CG, a statistically significant reduction in reaction time on the starting platform of 0.09 s (-11.9%, p = 0.001, ES = *Very large*) and 0.05 s (-6.1%, p = 0.025, ES = *Moderate*), respectively, was recorded.

At the end of the experiment, the time after covering a distance of 5 m after the turn and the recorded average speed in swimming this distance in both the EG and CG improved. In the EG, the abovementioned elements of the swimming race were significantly improved by 0.1 s (-28.6%, p < 0.001, ES = *Large*) and 3.56 m·s⁻¹ (23.2%, p = 0.001, ES = *Large*), respectively, while in the CG, these parameters improved by 0.06 s (-14.2%, p = 0.026, ES = *Moderate*) and 1.22 m·s⁻¹ (10.5%, p = 0.037, ES = *Small*), respectively. ANOVA revealed a significant interaction for the average velocity 5 m after the turn ($F_{(1,14)} = 9.547$, p = 0.008).

The result of all observed changes was the value of the last of the tested parameters–the time required to cover the distance of 50 m via front crawl swimming. In the EG, a statistically significant improvement in athletic performance of 0.3 s (-1.2%, p = 0.001, ES = *Moderate*) was observed, while swimmers in the CG had a statistically insignificant improvement in athletic performance of 0.18 s (-0.7%, p = 0.274, ES = *Trivial*).

Discussion

In this article, it was hypothesized that in a selected group of swimmers of either senior or adolescent age, strengthening of the core muscles will positively impact the effectiveness of the studied elements of a 50 m swimming race, which may lead to an improvement in sports results.

Both in the EG and CG, the parameter of the entry distance improved, which may indicate a positive aspect of the training carried out by the competitors in a given period. Notably, the specialized core muscle training did not change the value of this parameter, further reducing the start jump time (parameter improvement of 0.09 s, ES = *Very large*), which is the result of the reaction time and flight phase time measured until the swimmer touches the water surface with the head. The value of this parameter among EG athletes was statistically significantly lower (p < 0.001), while the improvement that followed was higher than in the CG. In the EG, there was a statistically significant increase in the speed of entry of the swimmer into the water (4.3%, ES = *Small*), in contrast to the CG, in which regression of the analyzed velocity (-3.4%) occurred.

As published research results show, the start jump in swimming directly affects the competitive level, depending on the type of competition, and especially the distance covered, as it accounts for 0.8% of the time needed to complete 1500 m and 26.1% of the time required to complete 50 m (front crawl) [24,25]. According to the assumptions of this experiment, one of the analyzed parameters was the start jump, which can be divided into three stages: on the starting block, flight and the underwater phase [25]. In this work, the first two were analyzed, and it is worth noting that under the influence of SCMT, the studied swimmers improved their reaction times. However, these studies did not measure swimmers' reaction time (the time taken by the swimmers to leave the starting block following starting signal) [26,27], which is a neuromuscular skill playing a very important role especially in short distance swimming. Specialized core muscle training in this study significantly (p = 0.001, ES = *Very large*) improved the reaction time of the swimmers.

These results are consistent with the work of Rejman et al. [26], in which the time on the starting block was shortened due to a six-week plyometric training, and the speed of a

swimmer achieved during the flight phase increased $(0.71 \text{ m} \cdot \text{s}^{-1})$, which may be related to an improvement in lower limb power [26]. Although there was no statistically significant increase in swimmer entry velocity into the water, in the EG, there was an improvement in this parameter, in contrast to the CG, in which there was a statistically insignificant regression of the analyzed velocity. It seems that under the influence of core muscle training, the integration of the muscles of the lower and upper limbs and torso improved, which translated into a more efficient transfer of energy from the lower limbs to the body and further to the arms and thus a more efficient (faster) torpedo (starting) position [8,15].

Many studies show the importance of the flight phase, the maximization of which, combined with the appropriate entry into the water, allows a swimmer to achieve higher speeds during the underwater phase [28,29]. The distance of the flight phase is a very important parameter of the effectiveness of a swimmer during a race because the body travels much faster in the air than in the water [30]. In the study of Breed and Young [24], dry-land resistance training did not affect the distance of the flight phase during the starting jump, which may be related to its specificity. In the studies conducted by the authors, in both the EG and CG, there was a statistically significant improvement in the length of the flight phase parameter, which may indicate a positive aspect of the training carried out by the swimmers in the given start-up preparation period. Notably, specialized core muscle training did not affect the value of this parameter and shortened the start-up time, which is the resultant of the reaction time and flight phase time measured until the swimmer touches the water surface with the head. The glide speed after the start jump is highly dependent on the time of entry into the water, swimmers position, direction and depth of entry [31,32]. In studies based on a correlation analysis, it was determined that there is a strong relationship between "take-off horizontal velocity and time on block" and the time obtained by competitors after an initial distance of 15 meters [28,33]. Increasing the take-off horizontal velocity should cause the swimmer to enter the water at a smaller angle. In the conducted studies, in the EG group, an improvement in the flight phase velocity and a decrease in the swimmer entry angle (statistically insignificant) were observed, while in the CG group, both parameters did not improve. According to other studies, it can be presumed that an incorrect position upon entry into the water, despite the appropriate speed of the starting jump, will not translate into the speed that the swimmer will reach during the underwater phase [27].

In both analyzed groups, a decrease in swimming time in the first 5 m after the turn was observed, and the decrease in this value was statistically significant in the EG (an improvement of 28.6%, p < 0.001, ES = *Large*). Moreover, it significantly influenced the next analyzed parameter, i.e., the speed of the swimmer 5 m after the return from the turn wall; this value improved by 23.2% (p = 0.001, ES = Large). There are very few studies in the literature investigating the effectiveness of swimming turns, especially the tumble turns, due to a lack of appropriate technologies and other factors. The swimming turn is a complicated technical element due to the environment in which it takes place, multilevel and multiaxis movement, and the number of involved body segments [34]. It is undeniable that a properly performed turn can improve the total swimming duration [34]. It is known that a slight improvement in the components of the turn can improve the effectiveness of swimming over the total distance. One of the elements of the turn is the glide, which may depend on the push-off and the proper position of the swimmer's body [4,11]. Seemingly, the decrease in the time required to cross the first few meters after the turn may significantly affect the final time measured at the end of the race. In the EG, an increase in the SR of 1.5% was observed (ES = *Trivial*), as well as shortening of the swimming SL, which, for competitors performing core muscle training, decreased by 3.5% (p = 0.091, ES = Small). There were no significant changes among CG competitors. The increase in the speed of swimming may be caused by an increase in stroke length with a

simultaneous drop in the stroke rate, but it can also be achieved only by extending the swim stroke length [35]. In the work of Patil et al. [4], there were no statistically significant changes in the stroke rate or stroke length under the influence of core muscle training. The lack of similar results of various studies can be explained by another preparatory period in which the experiments were carried out. In addition, many authors have determined the SR and SL to be factors of swimming performance, which is associated mainly with strength and muscular power [36]. The competition over the mentioned distances researched by the authors for the needs of this study is characterized by high dynamics. The desired effect of directed stabilization muscle training will seemingly be observed at longer distances, e.g., 200 m, where the correct position of the swimmer's body seems to be crucial, and thus, the stroke length may be longer.

The result of all the observed changes was the value of the last tested parameter-the time required to cover a distance of 50 m. In the EG, a statistically significant improvement in athletic performance of 1.2% (p = 0.001, ES = Moderate) was observed. During the final test, swimmers in the CG also achieved a better result, but this improvement was not statistically significant. On the basis of the available literature, a rational explanation for this issue may be the increased core muscle activity, which allows for a more effective transfer of strength between the limbs and for maintaining the body in a streamlined position [11,37]. There are many papers on the impact of dry-land training on performance in swimming sports; however, the results of these studies are not consistent. For example, Tanaka et al. [37] suggest that the increase in strength achieved through resistance land training does not affect the swimmer's driving force in the water and therefore does not improve swimming performance [37-39]. A large improvement was observed in the study of Weston et al. [10]; however, it may be caused by the much longer period of the specialized training program, as well as the younger research group. Another study found an improvement in central stabilization that did not translate into swimming efficiency [4]. However, there are numerous studies proving the positive impact of dry-land training on the results of swimming, and the recorded progression of results oscillates between 1.3% and 4.4% [12,40]. The results obtained by the authors of the above studies are similar to the results of the work of Weston et al. [10], in which, as a result of twelve weeks of training involving core muscles, a 2% improvement in sprinting distance was observed. Patil et al. [4] also noted a statistical progression of the results achieved in competition after a six-week training intended to strengthen the stabilizing muscles. In this study, the improvement in the efficiency of individual swimming elements translated into better final competition results, i.e., shorter times required to cover a distance of 50 m. In the present study, it is likely that SCMT causes improvement of a number of swimming variables, which together result an overall increase in 50 m front crawl swimming performance by 1.2%, whereas the CG swimmers improved their performance just by 0.7%.

Conclusions

The present study involved a group of selected swimmers who completed a specially designed training program aimed at improving the strength and endurance of their core muscles. The research results suggest that the implementation of isolated training to strengthen the stabilizing muscles seems to be a valuable addition to a standard swimming training. Based on the conducted experiment, it can be concluded that the described training affects the efficiency of swimming over a short distance. It is especially notable that in this study, the improvement in the efficiency of individual swimming elements did translate into better final sports results, i.e., shorter times required to swim a distance of 50 m. The authors observed a statistically significant progression of the results, which seems to be fundamental for the sprinting distance.

In direct sports competition, even a slight improvement in time may guarantee final success. The novelty of this work is a detailed analysis of many parameters related to the techniques of swimming, including the start jump and turn. However, the similarity between the results of this experiment and those of other experiments indicates the need to continue research in the field of dry-land training for swimmers, especially the need to strengthen the core muscles. Future experiments should also be enriched with EMG tests showing proper and conscious tensioning of the stabilizing muscles.

Author Contributions

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12 Effect of caffeine on neuromuscular function following eccentric-based exercise

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Abstract

This study investigated the effect of caffeine on neuromuscular function, power and sprint performance during the days following an eccentric-based exercise. Using a randomly counterbalanced, crossover and double-blinded design, eleven male jumpers and sprinters (age: 18.7 \pm 2.7 years) performed a half-squat exercise (4 x 12 repetitions at 70% of 1 RM), with eccentric action emphasized by using a flexible strip attached to their knees (Tirante Musculador[®]). They ingested either a capsule of placebo or caffeine (5 mg.kg⁻¹ body mass) 24. 48 and 72 h after. Neuromuscular function and muscle power (vertical countermovement-jump test) were assessed before and after the half-squat exercise and 50 min after the placebo or caffeine ingestion at each time-point post-exercise. Sprint performance was measured at pre-test and 75 min after the placebo or caffeine ingestion at each time-point post-exercise. Maximal voluntary contraction (overall fatigue) and twitch torque (peripheral fatigue) reduced after the half-squat exercise (-11 and -28%, respectively, P < 0.05) but returned to baseline 24 h post-exercise (P > 0.05) and were not affected by caffeine ingestion (P > 0.05) 0.05). The voluntary activation (central fatigue) and sprint performance were not altered throughout the experiment and were not different between caffeine and placebo. However, caffeine increased height and power during the vertical countermovement-jump test at 48 and 72 h post half-squat exercise, when compared to the placebo (P < 0.05). In conclusion, caffeine improves muscle power 48 and 72 h after an eccentric-based exercise, but it has no effect on neuromuscular function and sprint performance.

Introduction

Strength training with eccentric emphasis is used by sprinters and jumpers to provoke inseries sarcomere hypertrophy, which could increase the velocity of muscle contraction [1].

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However, exercise with eccentric emphasis also increases muscle damage via increased sarcoplasmic rupture, dilation of the transverse tubule system, myofibrillar component distortion and sarcoplasmic reticulum fragmentation [2,3]. Due these structural changes, there is a late increase in creatine kinase (CK), a marker of muscle damage, with a concomitant increase in muscle pain after an eccentric exercise (24 to 72h post-exercise), the so-called delayed onset muscle soreness (DOMS). Increased muscle damage and DOMS might carry over into exacerbated neuromuscular fatigue, which can reduce performance during the subsequent training sessions even in individuals accustomed to this mode of training [4–6].

Only a few studies have investigated the time course of neuromuscular fatigue after eccentric exercise [4–8]. While it has consistently been demonstrated that DOMS, measured with a 10-point Pain Intensity Scale [9], peaks 48 h after an eccentric exercise, the time course of neuromuscular fatigue after an eccentric exercise is more controversial. Maximal voluntary contraction (MVC), an indicator of overall fatigue, can remain reduced from 24 h to 8 days or more after an eccentric exercise [4,8]. Central fatigue, measured by voluntary activation (VA) via the twitch interpolated technique is restored within 24 h to 72 h [4,6,10], while twitch torque (an indicator of peripheral fatigue) is restored in 24 h [10], 48 h [8] or sometimes more than 4 days [4,6]. Part of these inconsistencies might be due to different protocols inducing muscle damage such as elbow flexion [6], backward downhill walking [10] or heavy-resistance (strength) and jump training [8]. It is noteworthy that only one study recruited athletes as volunteers and used exercises that are part of the training routine of sprinters and jumpers [8]. Therefore, the time course of central and peripheral fatigue after an exercise with eccentric emphasis typically used in athlete's training routine is underexplored in well-trained athletes and deserves further investigation.

Because athletes train almost every day, it is important to maintain performance during subsequent training sessions [11]. In this sense, caffeine (1,3,7-trimethylxanthine) seems to be a promising alternative. Following its removal from the World Anti-Doping Agency (WADA) Prohibited List in 2004, the use of caffeine for track and field athletes increased substantially [12]. The use of caffeine has been reported to be large in national/international level athletes (~48%) and in power athletes (~57%) (12). Because caffeine acts on the central nervous system (CNS) as an adenosine receptor antagonist, caffeine maintains neuronal excitability and counteracts central fatigue. Caffeine has also analgesic properties that could reduce the perception of pain during exercise [13]. In addition, caffeine improves excitation-contraction coupling by increasing calcium release/reuptake and Na⁺-K⁺⁻ATPase pump activity [14]. Caffeine also increases peak isokinetic torque after activities that resulted in exercise-induced muscle damage [15]. Considering these effects, caffeine might be an effective alternative to counteract the negative effect of DOMS provoked by a prior exercise with eccentric emphasis on power, sprint performance and central and peripheral fatigue.

Therefore, the first aim of the present study was to investigate the time course of CK, DOMS, jump and sprint performance and central and peripheral components of neuromuscular fatigue after a half-squat exercise with eccentric emphasis. The second aim was to investigate whether caffeine would influence central and peripheral components of neuromuscular fatigue and jump and sprint performance during the following days after the half-squat exercise with eccentric emphasis. We hypothesized that acute caffeine ingestion would improve central and peripheral components of neuromuscular fatigue and jump and sprint performance of neuromuscular fatigue and jump and sprint performance in the following days after a half-squat exercise with eccentric emphasis.

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

Materials and methods

Participants

Eleven well-trained young males' sprinters and jumpers (age: 18.7 ± 2.7 years old; weight: 69.9 ± 6.4 kg; height: 180.7 ± 7.7 cm), accustomed to eccentric training and with low-to-moderate habitual caffeine consumption (< 80 mg.day⁻¹) [16] participated in this study. The inclusion criteria were: (a) to be free from neuromuscular and musculoskeletal disorders; (b) to have had at least one year of experience with athletic training; (c) to have been training 5 to 6 times per week (12 to 15 hours per week) and; (d) to have been involved in national or international competitions in the year prior to the study. The participants and their guardians were informed about the procedures prior to the beginning of the study and signed a written informed consent. The research was conducted according to the Declaration of Helsinki and was approved by the Research Ethics Committee of the Federal University of Pernambuco.

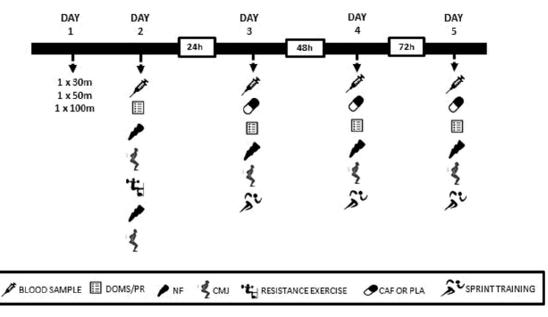
Experimental design

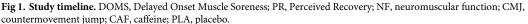
The study was carried out within a 3-week period of a preparatory phase of an annual training periodization. In this phase, training sessions were designed to improve general resistance and not focused in eccentric training. Two preliminary sessions were designed to assess one-repetition maximal strength in a half-squat exercise (1RM) using a *Tirante Musculador*^(R) (see one-repetition maximal strength test section for details) and to familiarize the participants with the assessment of neuromuscular function. In the first preliminary session, anthropometric measurements were taken, and a full familiarization with the assessment of neuromuscular function and 1RM was performed. Forty-eight hours later, in the second preliminary session, 1RM was determined and participants had another opportunity to be familiarized with the assessment of neuromuscular function.

After two days of low-intensity training, premeasurements were performed over the next two days. On day 1, after a 20 min warm-up (10 minutes running + 10 minutes of dynamic stretching), pre-test sprints (one sprint of 30 m following by 5 min rest, one sprint of 50 m following by 7 min rest and one sprint of 100 m) were performed in an outdoor athletic track (Fig 1). On Day 2, baseline blood sample, DOMS, perceived recovery, neuromuscular function was preceded by a warm-up (see neuromuscular function assessment section for details). Five minutes later, 4 sets of 12 repetitions at 70% of 1RM (2-min rest between sets) of a half-squat exercise with emphasis on eccentric action were performed using the *Tirante Musculador*^(B). After exercise, the neuromuscular function and CMJ performance were reassessed. During the next three days (days 3, 4 and 5), a blood sample was collected and participants ingested a gelatin capsule containing either 5 mg.kg⁻¹ body mass of anhydrous caffeine (CAF) or cellulose (PLA) 50 min before the assessment of DOMS, perceived recovery, neuromuscular function and CMJ. Participants also performed a sprint training session 75 min after the supplement ingestion.

After a 7-day washout period performing a low-intensity training program, participants repeated the procedures described above, but those who had ingested caffeine in the first moment ingested a placebo and those who had ingested a placebo ingested caffeine (crossover design). The order of CAF and PLA ingestion were randomly counterbalanced and supplements offered in a double-blind manner. All tests were performed at the same time of day to avoid any effect of the circadian variation. Participants refrained from alcohol and caffeinated beverages throughout the study. Participants were also instructed to record their food and drink intake starting 48 h before the Day 1 until the Day 5 and then to replicate this intake in

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the second phase of the experiment. Participants were asked not to eat anything two hours before each test session.

One-repetition maximal strength test

The 1RM test was performed during a half-squat exercise with participants coupled to a *Tir-ante Musculador*[®] (TMR-World, Barcelona, Spain). This accessory is a simple belt that allows anchor the calf and leave the body hanging with the gravity center far from the fulcrum of the knee and thus increasing the eccentric overload (Fig 2). The two flexible strips of the belt were attached below participants' knees and fixed to a column. Participants performed the flexion and extension of the knees (90° amplitude). This flexible strip has been demonstrated suitable to increase eccentric action during a half-squat exercise [17]. Participants were familiar with this accessory as they had used it in their training routine as a form to increase eccentric action without using an excessive external load, which may reduce the risk of injuries associated with eccentric-based training, mainly in a preparatory phase of an annual training periodization [17]. The participants performed a warm up consisting of 15 repetitions at ~ 50% of the estimated 1RM [18]. After that, participants rested for 2 min and the load was increased to the expected 1 RM. Five attempts were necessaries to achieve the 1RM load (i.e., the maximum load that could be lifted once using the proper technique), with a 2 min interval between the attempts, as previously suggested [18]. The 1RM load was 130.2 \pm 18.6 kg.

Half-squat exercise with eccentric emphasis

Participants performed 15 repetitions of half-squat exercise at 50% of 1RM as warm up and after a 2 min rest, performed 4 sets of 12 repetitions at 70% of 1RM, with 2 min rest between sets. Each repetition took 3 s (2 seconds for the eccentric phase and 1 second for the concentric phase) and was controlled using a metronome. The eccentric action was emphasized by participants performing the half-squat using the *Tirante Musculador*[®], as detailed above. We ascertain this protocol with athetes's coach to simulate their training routine. We chose a protocol

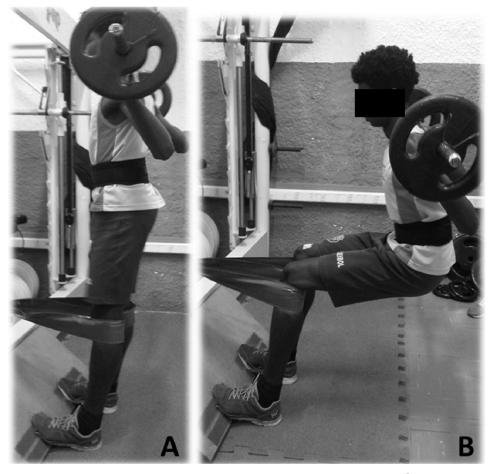


Fig 2. Half-squat exercise with eccentric emphasis performed with the *Tirante Musculador*[®]**.** A: Initial position of the movement; B: Final position of the movement.

able to provoke moderate rather than a more intense DOMS because athletes were in a preparatory phase of an annual training periodization; therefore, protocol to induced more intense DOMS would make athletes more susceptive to muscle injuries. The load at 70% of 1RM was 91.1 ± 13.9 kg.

Neuromuscular function assessment

The neuromuscular function was assessed with the participants seated on a custom-made bench with their hip and knees angles set at 120° and 90°, respectively. A non-compliant cuff attached to a calibrated linear strain gauge was fixed to the right ankle superior to the malleoli for force measurement (EMG System of Brazil, São Jose dos Campos, Brazil). A monopolar 0.5 cm diameter cathode electrode was positioned at the right femoral nerve for electrical stimulation (Ambu[®] Neuroline 715, Ballerup, Denmark). The anode was positioned on the gluteal fold opposite to the cathode. The position of the electrodes was marked with indelible ink to ensure identical placement in subsequent visits.

The optimal intensity of stimulation was determined by percutaneous electrical nerve stimuli (1 Hz and 80 µs duration) applied to the femoral nerve using an electrical stimulator (Neuro-TES, Neurosoft, Ivanovo, Russia). The intensity of stimulation began with 100 volts and increased 30 volts every 30 s until the occurrence of a plateau in the muscle membrane excitability (M_{wave}) and quadriceps twitch torque (Q_{tw}). To ensure a supramaximal stimulus, the intensity of stimulation that M_{wave} and Q_{tw} plateaued was further increased by 20%. The optimal intensity of stimulation was determined in the first visit and checked before every subsequent visit (301 ± 49 volts).

The neuromuscular function assessment (except immediately after the half-squat exercise) was preceded by a warm up composed of a 5 min jogging and 4 x 5 s isometric contractions of knee extension at ~60% of maximal subjective isometric force (30 s rest between contractions). The protocol of neuromuscular function assessment consisted of 6 x 5 s MVC of knee extensors, interspaced by 60-s recovery, as previously suggested [19]. Stimulus of 1 Hz (80 μ s) was applied during each MVC (superimposed twitch) at the plateau of isometric force. Potentiated quadriceps twitch torque evoked by 1 Hz (Q_{twpot}) and paired pulses at 10 Hz (Q_{tw10}) and 100 Hz (Q_{tw100}) was measured 2, 4 and 6 s after each MVC, respectively. The M_{wave} peak-to-peak amplitude was calculated for each 1 Hz stimulus. A Q_{tw10}·Q_{tw100}⁻¹ ratio was calculated to identify low-frequency fatigue [20]. The VA was measured by the following equation [21]:

$$\% VA = (1 - \text{superimposed} \div Qtwpot) \times 100 \tag{1}$$

where superimposed is the difference between the force before the stimulus and the peak of force induced by the stimulus.

The average of the last four MVC for each time point was used for further statistical analysis of the neuromuscular function [19].

Countermovement jump test

Participants were familiar with CMJ in their training routine. The participants started in a standing position, dropped to a squatting position and jump upwards as high as possible. The jumps were performed with hands on the hip. The flying time during the CMJ was measured using a contact mat (Jump System Pro, Cefise, São Paulo, Brazil). The CMJ height was calculated from flying time, while CMJ power calculated from flying time and body mass, using a commercial software (Jump System 1.0, Cefise, São Paulo, Brazil). The jumps were performed three times (10 s interval) and the mean height and power used for further statistical analysis.

Sprint training

Sprint training was composed by a series of sprints similar to that performed in training routine. The sprint training session started with a 20 min warm-up (10 minutes running + 10 minutes of dynamic stretching). Then, participants performed: 1) 3 sets of 30-m sprints, with a 5 min rest between sets; 2) 3 sets of 50-m sprints, with a 7 min rest between sets and; 3) 3 sets of 100-m sprints, with a 10 min rest between sets. They were instructed to run as fast they could and wore the same footwear. Participants performed 10 min of running and stretching after training to cool down. The timing of the sprints was monitored using photocells (TC-System, Brower Timing System, US). Participants positioned immediately before the first photocell to start. The mean velocity in each distance was calculated and used for further analysis.

Blood sample

Blood samples (8 ml) were drawn by a professional phlebotomist from the antecubital vein by venipuncture and transferred to tubes containing Clot activator and gel for serum separation (SST II Plus, BD Vacutainer[®], USA). The serum CK concentration was determined by an enzymatic method using commercial kits (Labtest Diagnostica S.A., Minas Gerais, Brazil), with the resultant reaction reading in a spectrophotometer (Genesys 10 S UV-vis, Thermo Electron Scientific Instruments, Madison, WI, United States).

Delayed onset muscle soreness and perceived recovery

The DOMS was measured with the Pain Intensity Scale ranging from 0 (no pain) to 10 (very intense pain, almost unbearable) [9]. The perception of recovery was measured on the Total Quality of Recovery Scale ranging from 6 to 20, where 6 is "nothing recovered" and 20 is "fully recovered" [22]. Participants were familiar with these scales as they had used them during their training routine.

Statistical analyses

Because of the lack of data regarding the caffeine effects on neuromuscular function post an eccentric-based exercise in athletes, the required sample size was estimated using an effect size (ES = 0.37) reported in a meta-analysis investigating the effect of caffeine ingestion on muscular strength [23]. With an alpha of 0.05 and a desired power of 0.80, the total sample size necessary to achieve statistical significance was estimated to be 10 participants. However, the starting sample size was increased to 11 participants, assuming that 10% might drop out during the data collection. The sample size calculation was performed using G*Power software (version 3.1.9.2, Kiel University, Germany).

The Shapiro-Wilk test was performed to determine the normality of the data. The neuromuscular function parameters (MVC, VA, $M_{wave ampl}$, Q_{tw10} , Q_{tw10} , Q_{tw100} and $Q_{tw10} \cdot Q_{tw100}^{-1}$), CMJ, CK and perceived recovery were analyzed using a two-way, repeated-measures ANOVA [supplement (PLA and CAF) x time (baseline, post-exercise and 24, 48 and 72 h post exercise)], with a Duncan post-hoc test being utilized when ANOVA detected significant main effects and/or interaction. The pre-test sprint performance before the first and second experimental blocks were compared using a paired t test in order to check if athletes had fully recovered from the previous tests and training sessions. The performance during the sprint training was then analyzed separately using a two-way, repeated-measures ANOVA [supplement (PLA and CAF) x time (24, 48 and 72 h post exercise). The DOMS was analyzed using Friedman ANOVA followed by the Wilcoxon test because the DOMS had not shown a normal distribution. Partial eta squared (η_p^2) was also calculated as a measure of the effect size and classified as small ($\eta_p^2 < .06$), moderate ($.06 \le \eta_p^2 < .15$) or large ($\eta_p^2 \ge .15$). The statistical significance was accepted at P < 0.05. Data are reported as mean \pm SD, unless otherwise stated. Statistical analyses were performed using statistics package for Windows (version 10, StatSoft, Tulsa, OK, USA).

Results

Muscle damage, DOMS and perceived recovery

The CK increased above baseline levels 48 and 72 h post-exercise (main effect of time, $F_{(4,32)} = 10.810$, P = 0.001, $\eta_p^2 = 0.57$, Table 1). The DOMS increased from baseline to 24 h post-exercise, remaining above the baseline values until 72 h post-exercise ($\chi^2_{(10)} = 25.312$, P = 0.001). Compared to baseline, the perceived recovery was always incomplete at 24, 48 and 72 h post-exercise (main effect of time, $F_{(3, 27)} = 12.429$, P = 0.001, $\eta_p^2 = 0.58$, Table 1). There was no effect of supplement for CK, DOMS and perceived recovery (P > 0.05).

Neuromuscular function

The MVC decreased significantly from baseline to immediate post-exercise (-11%) but recovered fully 24 h later (main effect of time, $F_{(4,32)} = 6.560$, P = 0.001, $\eta_p^2 = 0.45$, Fig 3A). Similarly, markers of peripheral fatigue (Q_{twpot} , Q_{tw10} and Q_{tw100}) decreased significantly with the exercise (-30%, -35% and -12%, respectively) and recovered fully 24 h later (main effect of time,

		PLA	CEBO		CAFFEINE						
	Baseline	24 h	48 h	72 h	Baseline	24 h	48 h	72 h	F	р	η_p^2
CK (U/L)	356.6 ± 220.1	381.9 ± 136.3	$481.4 \pm 66.3^{*}$	$491.6 \pm 209.8^{*}$	320.5 ± 148.5	337.9 ± 114.1	496.8 ±173.0*	$497.3 \pm 163.6^{*}$	1.07	0.38	0.11
	[199.1-514.1]	[284.3-479.4]	[331.3-631.4]	[326.1-657.1]	[214.2-426.7]	[256.2-419.5]	[373.0-620.5]	[371.5-623.1]			
DOMS (score)	0.5 ± 1.0	$2.3 \pm 1.8^{*}$	$1.9 \pm 1.4^{*}$	$3.0 \pm 1.7^{*}$	0.9 ± 1.0	$2.1 \pm 1.8^{*}$	$2.0 \pm 1.5^{*}$	$2.2 \pm 1.4^{*}$		< 0.01	
PR (score)	17.4 ± 1.75	$15.6 \pm 1.74^{*}$	$15.2 \pm 2.49^{*}$	$14.8 \pm 2.63^{*}$	17.8 ± 1.66	$16.2 \pm 1.90^{*}$	$14.7 \pm 1.95^{*}$	$15.0 \pm 2.05^{*}$	1.02	0.39	0.10
	[16.2–18.6]	[14.4–16.8]	[13.5–16.9]	[13.0-16.5]	[16.7–18.9]	[14.9–17.5]	[13.4–16.0]	[13.5–16.4]			

Table 1. Creatine kinase, delayed onset muscle soreness and perceived recovery at baseline, and at 24, 48 and 72 h later a half-squat exercise with placebo or caffeine ingestion.

Values are expressed as mean ± SD [95% confidence interval], except for DOMS that values are expressed as median ± interquartile distance (non-normally distributed). CK, creatine kinase; DOMS, delayed onset muscle soreness; PR, perceived recovery.

*Significantly different from pre-exercise in both conditions (P<0.05).

$$\begin{split} F_{(4,32)} &= 21.502, P = 0.001, \eta_p^{-2} = 0.72, F_{(4,32)} = 37.562, P = 0.001, \eta_p^{-2} = 0.82 \text{ and } F_{(4,32)} = 17.928, \\ P &= 0.001, \eta_p^{-2} = 0.69, Fig 3B, 3C \text{ and } 3D, \text{ respectively}). However, Qt_{w100} was slightly increased 48 and 72 h after the exercise in comparison to baseline (P = 0.001). The Q_{tw100} -1^{-1} decreased significantly from baseline to immediate post-exercise (-25%) and recovered fully 24 h later (main effect of time, F_{(4,32)} = 17.794, P = 0.001, \eta_p^{-2} = 0.68, Fig 3E). The VA did not change throughout the experiment (main effect of time, F_{(4,24)} = 2.297, P = 0.088, \eta_p^{-2} = 0.27, Fig 3F). The M_{wave}$$
 amplitude increased significantly from baseline to post-exercise and returned to baseline values 24 h later (main effect of time, F_{(4,32)} = 3.081, P = 0.029, \eta_p^{-2} = 0.27, Fig 3G). Caffeine had no effect on any neuromuscular function parameters (P > 0.05).

Countermovement jump and performance during the sprint training

There was a significant supplement *vs.* time interaction for CMJ performance. The height $(F_{(4,28)} = 3.299, P = 0.024, \eta_p^2 = 0.32, Fig 4A)$ and power $(F_{(4,28)} = 2.885, P = 0.040, \eta_p^2 = 0.29, Fig 4B)$ during the CMJ were higher 24 h after exercise compared to baseline and post-exercise in both supplements (P = 0.011 and P = 0.005, respectively). However, height and power during the CMJ 48 and 72 h after exercise remained above baseline values only when caffeine was ingested (P = 0.001). The height and power during the CMJ 48 and 72 h after exercise was also higher in caffeine compared to the placebo (P = 0.010).

Pre-test sprint performance did not differ between placebo and caffeine (30 m: 6.78 ± 0.24 vs. 6.72 ± 0.22 m.s⁻¹, P = 0.372; 50 m: 7.48 ± 0.10 vs. 7.58 ± 0.06 m.s⁻¹, P = 0.632; 100 m: 7.89 ± 0.15 vs. 7.98 ± 0.10 m.s⁻¹, P = 0.392). In addition, sprint performance for all distances (30m, 50m and 100m) was not affected by supplement ($F_{(1,9)} = 0.083$, P = 0.779, $\eta_p^2 = 0.009$; $F_{(1,9)} = 0.203$, P = 0.663, $\eta_p^2 = 0.022$ and $F_{(1,9)} = 0.085$, P = 0.776, $\eta_p^2 = 0.009$, Table 2). However, for both conditions, sprint performance in 50 m was faster at 72 h compared to 24 h and 48 h after exercise ($F_{(2,18)} = 5.395$, P = 0.014, $\eta_p^2 = 0.374$) and faster in 100 m at 72 h compared to 48 h after exercise ($F_{(2,18)} = 6.367$, P = 0.008, $\eta_p^2 = 0.414$). There was no time effect for 30 m sprint ($F_{(2,18)} = 3.172$, P = 0.066, $\eta_p^2 = 0.260$).

Discussion

The results of the present study indicate that although markers of muscle damage and DOMS (CK and pain feeling) remained elevated over 72 h after an exercise with eccentric emphasis, markers of central and peripheral fatigue returned to baseline values within 24 h. Caffeine did not affect neuromuscular function, but jump performance was improved with caffeine 48 and 72 h after an exercise with eccentric emphasis.

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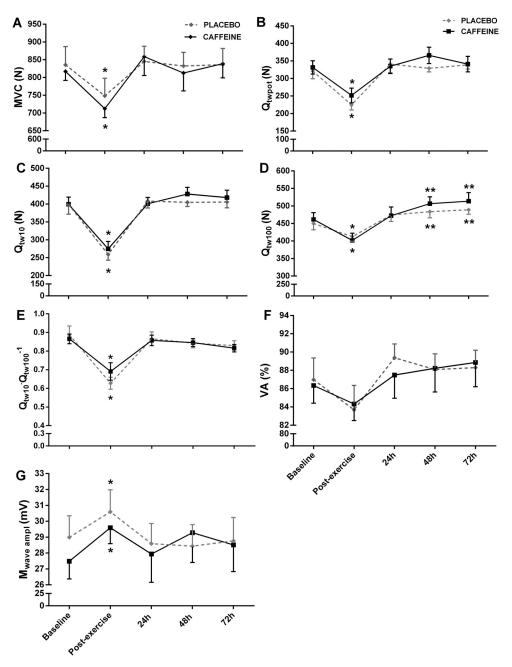


Fig 3. Neuromuscular function assessed before and after a half-squat exercise, and at 24, 48 and 72 h after placebo or caffeine ingestion. Values are expressed as mean \pm SEM. A: MVC, maximal voluntary contraction; B: VA, voluntary activation; C: $M_{wave ampl}$ maximal M-wave amplitude; D: Q_{twpot} single stimulation (potentiated twitch); E: Q_{tw10} paired stimulations of 10 Hz; F: Q_{tw100} paired stimulations of 100Hz. G: $Q_{tw10} \cdot Q_{tw100}^{-1}$ ratio. *Significantly lower than pre, 24, 48 and 72 h post half-squat exercise under both conditions; **Significantly higher than pre- and post half-squat exercise under both conditions.

The CK serum concentration increased 48 h after exercise with eccentric emphasis and remained elevated until 72 h. However, DOMS increased 24 h after exercise with eccentric emphasis and remained above baseline levels until 72 h, which coincided with a reduction in the perception of recovery. The CK after an eccentric exercise peaks 1–4 days and can remain elevated for several days [3,24,25]. An important issue is that daily training results in persistent

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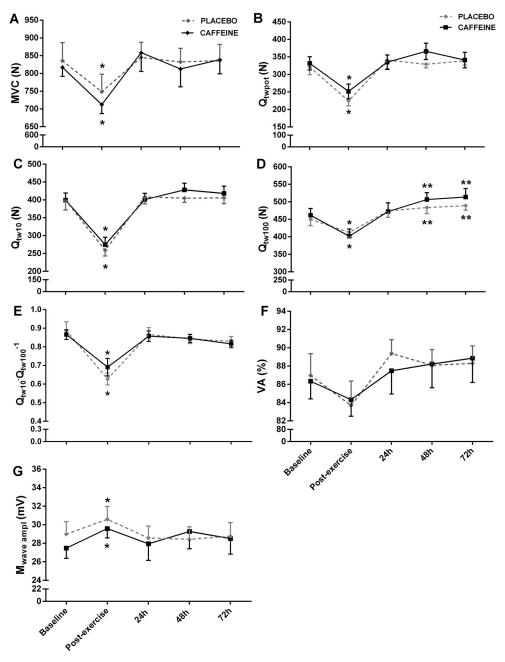


Fig 4. Countermovement jump test performed before and after a half-squat exercise and at 24, 48 and 72 h after placebo or caffeine ingestion. Values are expressed as mean ± SEM. A: Height; B: Power (relative to body mass). *Significantly higher than pre- and post half-squat exercise in both conditions; [#]Significantly higher than pre- and post half-squat exercise only in caffeine condition; [†]Significantly higher than placebo at the same time point.

CK elevation in athletes, with resting values being higher than in non-athletes [25]. In the present study, even providing two days of low-intensity training before taking blood sample, we found baseline CK values ranging from 199 to 514 U/L, which are slightly above the upper reference limit established to general population (174 U/L) [26]. Because it is not possible to maintain athletes without any kind of training for days, a "true" CK baseline value is hard to find in athletes. However, the values found in the present study are in agreement with

	PLACEBO				CAFFEINE				
	24h	48h	72h	24h	48h	72h	F	р	η_p^2
30m (m.s ⁻¹)	6.56 ± 0.45	6.75 ± 0.39	6.65 ± 0.27	6.70 ± 0.42	6.78 ± 0.32	6.55 ± 0.37	1.29	0.29	0.12
	[6.25-6.86]	[6.48–7.01]	[6.46-6.83]	[6.41-6.97]	[6.56–7.00]	[6.28-6.82]			
50m (m.s ⁻¹)	7.49 ± 0.43	7.54 ± 0.55	$7.26 \pm 0.33^{*}$	7.42 ± 0.36	7.49 ± 0.38	$7.27 \pm 0.34^{*}$	0.36	0.70	0.03
	[7.19–7.78]	[7.16–7.91]	[7.04-7.48]	[7.17–7.67]	[7.23-7.74]	[7.02-7.51]			
100m (m.s ⁻¹)	7.89 ± 0.35	7.88 ± 0.38	7.81 ± 0.39**	7.85 ± 0.30	8.05 ± 0.30	7.80 ± 0.33**	1.71	0.20	0.15
	[7.64-8.12]	[7.61-8.14]	[7.55-8.07]	[7.56-8.14]	[7.75-8.34]	[7.44-8.14]]		

Table 2. Sprint performance at 24, 48 and 72 h later a half-squat exercise with placebo or caffeine ingestion.

Values are expressed as mean \pm SD [95% confidence interval].

*Significantly different from 24 h and 48 h after exercise in both conditions (P<0.05).

**Significantly different from 48 h after exercise in both conditions (P<0.05).

reference values proposed for male athletes (82–1083 U/L) [25]. We also found an increase in CK levels 48 h after exercise, regardless of the supplement ingested. This finding is in agreement with a study showing that CK peaks within 48 h after a marathon [24]. However, our CK level 48 h after the half-squat exercise was much lower than those reported 24 h after a marathon (434–844 U/L) [24]. This is expected because CK levels after prolonged exercise such as marathon can reach up to 50 times the rest values due to the greater muscle damage caused by this kind of activity [24].

The exercise did not influence central fatigue (VA), but induced to a peripheral fatigue, as evidenced by the large reduction in Q_{twpot} , Q_{tw10} and Q_{tw100} immediately post-exercise (-30, -35 and -12%, respectively) and a low-frequency fatigue, as evidenced by a reduction in $Q_{tw100} \cdot Q_{tw100}^{-1}$ (-25%). However, peripheral and low-frequency fatigue returned to baseline levels 24 h after the exercise. The time course of neuromuscular fatigue after a eccentric-based exercise is largely variable, with studies reporting that central fatigue returned to baseline within 24 to 96 h, while peripheral fatigue within 24 to 192 h [4–8,10]. Different protocols of eccentric exercise may take in account for these inconsistencies. In the present study, we optioned for an eccentric exercise nearer to that used in the athlete's training routine. In addition, to avoid injuries, eccentric exercises used in regular training program are not designed to generate an elevated degree of muscle damage and DOMS. Our results suggest that although the considerable degree of peripheral fatigue after an exercise with eccentric emphasis (~ 30%), well-trained athletes accustomed to eccentric training can quickly restore their capacity to produce force (~24 h).

Caffeine had no influence on CK, DOMS or neuromuscular function. However, the height and power during the CMJ returned to baseline levels 48 h after the exercise with the placebo, while the height and power was maintained above baseline levels until 72 h after the exercise with caffeine. Muscle power was also higher in caffeine than in placebo at 48 and 72 h postexercise. Previous studies have shown improved jump performance after the ingestion of 3 to 6 mg.kg⁻¹ of caffeine [27–32]. Recent reviews concluded that caffeine promotes an ergogenic effect on muscle strength and power [23,33,34]. However, no study to date has demonstrated this improvement even after an exercise with eccentric emphasis using high performance sprinters and jumpers. Nevertheless, the improved power was not translated to an improved sprint performance in the present study. Similar findings have been reported showing no improvement in repeated-sprint ability with caffeine ingestion [30]. The shorter duration of the contraction and the simplicity of the technique may explain why performance was improved in the CMJ but not in the sprint with caffeine [30]. Our study demonstrated that a half-squat exercise session with an eccentric emphasis induces peripheral but not central fatigue, which is restored within 24 hours in well-trained jumpers and sprinters. Anhydrous caffeine (5 mg.kg⁻¹) improved jump performance 48 and 72 h after a half-squat exercise with an emphasis on eccentric action. However, neuromuscular function and sprint performance were not influenced by caffeine intake. Our results has considerable practical relevance as they are indicating that caffeine can optimize jumping performance in well-trained athletes even when a certain degree of muscle damage and DOMS are present. Thus, ingestion of anhydrous caffeine in some jump training sessions may be an interesting strategy to improve training quality and performance of these athletes.

There are some limitations in the present study that should be mentioned. Our resistance training protocol generated mild to moderate muscle pain, resulting in no impairment of subsequent training capacity. Thus, whether caffeine would be useful when greater muscle pain is present deserves further investigation. Another potential limitation is that experimental conditions were performed only once each (one for placebo and another one to caffeine). Repetitions of the experimental blocks may have provided additional information regarding the reproducibility of our findings.

Conclusions

In conclusion, our study demonstrates that even with a certain low degree of muscle damage and DOMS over 72 h after an exercise with eccentric emphasis, neuromuscular function, muscle strength and sprint performance are preserved in well-trained sprinters and jumpers. Caffeine ingestion (5 mg.kg⁻¹) improves muscle power 48 and 72 h after an exercise with eccentric emphasis, but it has no effect on neuromuscular function and sprint performance.

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13 Electromyographic activity in deadlift exercise and its variants. A systematic review

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Abstract

The main purpose of this review was to systematically analyze the literature concerning studies which have investigated muscle activation when performing the Deadlift exercise and its variants. This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA). Original studies from inception until March 2019 were sourced from four electronic databases including PubMed. OVID, Scopus and Web of Science. Inclusion criteria were as follows: (a) a cross-sectional or longitudinal study design; (b) evaluation of neuromuscular activation during Deadlift exercise or variants; (c) inclusion of healthy and trained participants, with no injury issues at least for six months before measurements; and (d) analyzed "sEMG amplitude", "muscle activation" or "muscular activity" with surface electromyography (sEMG) devices. Major findings indicate that the biceps femoris is the most studied muscle, followed by gluteus maximus, vastus lateralis and erector spinae. Erector spinae and guadriceps muscles reported greater activation than gluteus maximus and biceps femoris muscles during Deadlift exercise and its variants. However, the Romanian Deadlift is associated with lower activation for erector spinae than for biceps femoris and semitendinosus. Deadlift also showed greater activation of the quadriceps muscles than the gluteus maximus and hamstring muscles. In general, semitendinosus muscle activation predominates over that of biceps femoris within hamstring muscles complex. In conclusion 1) Biceps femoris is the most evaluated muscle, followed by gluteus maximus, vastus lateralis and erector spinae during Deadlift exercises; 2) Erector spinae and quadriceps muscles are more activated than gluteus maximus and biceps femoris muscles within Deadlift exercises; 3) Within the hamstring muscles complex, semitendinosus elicits slightly greater muscle activation than biceps femoris during Deadlift exercises; and 4) A unified criterion upon methodology is necessary in order to report reliable outcomes when using surface electromyography recordings.

Introduction

Resistance training provides several health benefits related to enhancing muscle strength, reversing muscle loss, reducing body fat, improving cardiovascular health, enhancing mental

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Abbreviations:

Exercises abbreviations: BallPro, walk-in machine deadlift with feet ball-hand; DL, deadlift; EB, elastic bands; FW, free weights; ToePro, walk-in machine deadlift with toes-hand; 1RM, 1 repetition maximum.

Other abbreviations: Conc, concentric phase; eccen, eccentric phase; ISOpull, isometric pulls; mV, microvolts; RM, repetition maximum; RMS, root mean square; ROM, range of motion; sEMG, surface electromyography. health and increasing bone mineral density [1-6]. Accordingly, resistance training should be considered essential for the whole population, but it is even more relevant when the target is the transference into some specific activity or daily life tasks [7, 8], injury prevention [9] or maximizing sports performance [10].

Free-weight resistance training is already well known as a key point in every strength training program [11–13]. In categories of creating diverse stimulus for muscle groups, different modalities such as barbell, kettlebells, hexagonal bars or dumbbells devices are typical recurring resources for coaches and trainers [14, 15]. Besides, other implements which can considerably modify the exercise load profile are elastic bands [2, 25], chains [29] or Fat Gripz devices [24].

It is essential to be acquainted with which muscles are activated during certain exercises and to compare different movement patterns when choosing exercises for a concrete objective [16]. Surface electromyography (sEMG) is one of the main tools used to measure muscle activation, and it can be defined as an electrophysiological recording technology used for the detection of the electric potential crossing muscle fiber membranes [17]. Thereby, task-specific data regarding motor unit recruitment patterns are reported through sEMG. For instances, athletes have the possibility to perform a concrete exercise when targeting a particular muscle [18, 19].

Deadlift, *Squat* and *Bench Press* are basic resistance exercises performed in several training programs for improving physical fitness in athletes [20]. This explains the great interest in studying muscle activation, which also translates these movements into some of the most investigated exercises in the current literature using sEMG [14, 21, 22]. *Deadlift* is frequently performed primarily when the goal is the strengthening of thigh and posterior chain muscles; specifically gluteus, hamstrings, erector spinae and quadriceps [23, 24]. Thus, *Deadlift* is classified as one of the most typical resistance exercise for posterior lower limb strengthening, as well as its variants [25]. Moreover, *Deadlift* has been mentioned in numerous studies comparing this exercise with other variants such as *Stiff Leg Deadlift* [26], *Hexagonal Bar Deadlift* [22] or *Romanian Deadlift* [27]. It has also been contrasted with other less popular variants such as *Sumo Deadlift* [13], unstable devices [28] and elastic bands *Deadlift* [8], among others.

To the best of our knowledge, there is no comprehensive review of the current literature concerning *Deadlift* movement pattern, and there is significant controversy when determining which muscles are involved within each *Deadlift* variants. For instance, the greatest muscle activation has been reported for the biceps femoris compared with the erector spinae and gluteus maximus during *Deadlift* [8], whereas Snyder et al. (2017) found greater erector spinae activation in comparison with gluteus maximus and biceps femoris. In contrast, Andersen et al. (2018) reported maximal activation for biceps femoris versus gluteus maximus and erector spinae for the same tested movement.

Thus, the main purpose of this manuscript was to systematically review the current literature investigating muscle activation measured with sEMG of muscles recruited when performing the *Deadlift* exercise and all its best-known variants. An increased understanding of the muscle activation that occur during these exercises will provide the researcher, clinician and athletes with relevant information about the use of the best exercise to activate a specific muscle or group of muscles associated with the *Deadlift* and its variants.

Methods

This systematic review was reported and developed following the Preferred Reporting of Systematic Reviews and Meta-Analysis (PRISMA) guidelines [29, 30]. The protocol for this systematic review was registered on PROSPERO (CRD42019138026) and is available in full on

the National Institute for Health Research (https://www.crd.york.ac.uk/prospero/display_ record.php?ID=CRD42019138026). The quality of included studies was assessed by two reviewers using the PEDro quality scale, which consists on eleven questions and distributes the score proportionally to the total amount of questions included. However, due to the inability to blind researchers and trainees, three of eleven questions were excluded from the scale resulting in a maximum of eight [17].

A literature search of PubMed, OVID, Scopus & Web of Science electronic databases was performed from March–April 2019. Reviews included publications from inception until March 2019.

The search strategy conducted in the different databases, along with Medical Subject Heading (MeSH) descriptors, related terms and keywords used were as follows; (a) PubMed & OVID: (deadlift OR "dead-lift" OR "romanian deadlift" OR "stiff-leg deadlift" OR "barbell deadlift" OR "hexagonal bar deadlift" OR "hip hinge" OR "hip extension") AND ("resistance training" OR "strength training" OR "resistance exercise" OR "weight lifting" OR "weight bearing") AND ("muscular activity" OR "muscle activation" OR electromyography OR electromyographical OR electromyographic OR electromyogram OR "surface electromyography" OR semg OR EMG) (b) Scopus: (TITLE("deadlift" OR "dead-lift" OR "romanian deadlift" OR "stiff-leg deadlift" OR "barbell deadlift" OR "hexagonal bar deadlift" OR "hip hinge" OR "hip extension") AND ("resistance training" OR "strength training" OR "resistance exercise" OR "weight lifting" OR "weight bearing") AND ("muscular activity" OR "muscle activation" OR "electromyography" OR "electromyograpical" OR "electromyographic" OR "electromyogram" OR "surface electromyography" OR "sEMG" OR "EMG")); (c) Web of Science: ALL = (((deadlift* OR "dead-lift"* OR "romanian deadlift"* OR "stiff-leg deadlift"* OR "barbell deadlift"* OR "hexagonal bar deadlift"* OR "hip hinge"* OR "hip extension"*) AND ("resistance training"* OR "strength training"* OR "resistance exercise"* OR "weight lifting"* OR "weight bearing"*) AND ("muscular activity"* OR "muscle activation"* OR electromyograpical* OR electromyographic* OR electromyogram* OR "surface electromyography"* OR semg* OR EMG*))). Studies were included if they met the following criteria:

- i. cross-sectional or longitudinal (experimental or cohorts) study design;
- ii. evaluated neuromuscular activation during *Deadlift* exercise or variants;
- iii. included healthy and trained participants, with no injuries for at least six months before measurements;
- iv. analyzed "sEMG amplitude", "muscle activation" or "muscular activity" with surface electromyography devices (sEMG);

Most articles found were written in English, but there were no language restrictions. Reviews, congress publications, theses, books, books chapters, abstracts, and studies with poor protocol description or insufficient data were not included. Studies whose participants did not have at least six months of resistance training experience were excluded. We also excluded all studies in which participants were under eighteen years old due to underdevelopment of strength and coordination [31]. Studies reporting muscle activation only from upper limbs during *Deadlift* exercise were also considered.

As different terms are related to the same concept, in categories of unifying criteria, the "muscle activation" term will be used when referring to "sEMG amplitude", "muscle excitation", "muscle activity", "neuromuscular activity" or similar.

Articles were selected by two independent reviewers according to inclusion and exclusion criteria. After eliminating duplicates, the titles and abstracts were analyzed and if there was not

enough information, the full text was evaluated. All studies identified from the database searches were downloaded into the software EndNote version X9 (Clarivate Analytics, New York, NY, USA).

Every decision was approved by both reviewers. However, a third reviewer was consulted in case of disagreement. The whole search process took two weeks. All steps taken are thoroughly described in the flow chart (Fig 1).

During the data extraction process, the following information was collected from every study: reference, exercise-movements measured, sample size (n), gender, age (years), experience (years), evaluated muscles, electrodes location, limb tested (non-dominant/dominant), sEMG collection method, sEMG normalization method, outcomes, percentage maximal voluntary isometric contraction (% MVIC), and main findings.

Muscle activation was the main data gathered, dividing eccentric and concentric sEMG activity data when reported. All studies finally selected reported muscle activation of every muscle and exercise separately. Furthermore, data related to exercise loading and exercise description details were collected.

Data collected in this review could not be analyzed as a meta-analysis since there was not enough homogeneity in terms of the type of analysis and methods carried out amongst studies. Therefore, a qualitative review of the results was conducted.

Results

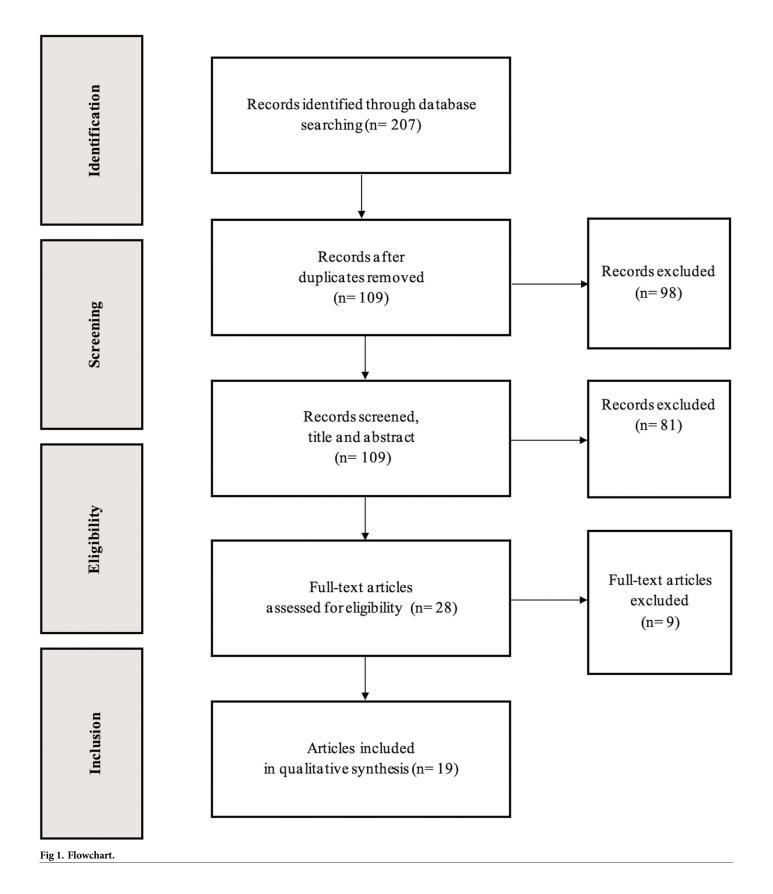
Search results

A total of 207 articles were identified from an initial survey executed by two independent reviewers. 98 of these articles were duplicated, which led to a remaining amount of 109 in the process. The next step involved reading the title and abstract with the purpose of eliminating all those not meeting the inclusion criteria. Finally, twenty-eight articles were fully read, and nineteen of these were eventually selected for the review (Fig 1). The publication date of all selected articles ranged from 2002 to January 2019. Additionally, all studies were categorized as having a good/excellent quality in the methodological process based on the PEDro quality scale.

All selected articles presented a cross-sectional design. In fact, most experimental studies found used an untrained participant sample, so they were excluded. Regarding experience time, all participants had at least six months of previous resistance training experience, although some studies did not report the exact experience time of participants (Table 1).

No common criteria were followed when referring to the exercise loading at which exercises were evaluated during sEMG recordings. As a matter of fact, only two studies used a similar method, assessing one repetition maximum intensity (1RM) [22, 32]. Some studies measured a number of repetitions of xRM, whereas others measured a number of repetitions of a range between 65–85% of 1RM (Table 1), which could be considered in all cases as a submaximal load intensity [33].

Data regarding the studies' general description and main findings are presented in Table 1, while Tables 2–5 contain data referring to muscle activation during *Deadlift* exercise and/or its variants. We found no unified criteria for the sEMG normalization method. Out of all included studies, seven reported data description regarding muscle activation in relation to exercise type and normalized sEMG activity as a percentage of maximal voluntary isometric contraction (% MVIC) (Table 2); three of them as percentage of peak root mean square (% peak RMS) (Table 3); two studies reported data expressed as absolute RMS values in microvolts (mV) (Table 4); and three studies expressed data as a percentage of 1 repetition maximum (% 1RM) (Table 5). In addition, there were four studies which were not included in the



Reference	Exercises tested	Sample	Age (years)	Experience (years)	sEMG collection method	Activity sEMG recorded of muscles:	Main findings
Krings et al. (2019) [<u>32</u>]	Deadlift versus fat gripz deadlift	15 Men	22.4 ± 2.4	Not indicated	1 rep 1RM	Biceps brachialis, triceps braquialis and forearm muscles	Greater forearm activation and significant decrease in 1RM during fat gripz deadlift
Andersen et al. (2019) [8]	Deadlift versus FW-2EB and FW-4EB	15 Men	23.3 ± 2.2	3.9 ± 1.9	2 reps 2RM	Gluteus maximus, vastus lateralis, biceps femoris, semitendinosus and erector spinae	Greater erector spinae activation when more elastic bands added
McCurdy et al. (2018) [40]	Stiff leg deadlift versus back squat and modified single leg squat	18 Women	20.9 ± 1.1	1–5 years	3 rep with 8RM	Gluteus maximus and hamstrings	Greater gluteus maximus activation than hamstrings for all exercises. Modified single leg squat elicited the greatest activation
Lee et al. (2018) [27]	Deadlift versus Romanian deadlift	21 Men	22.4 ± 2.2	> 3 years	5 rep 70% of RD 1RM	Gluteus maximus, rectus femoris and biceps femoris	Greater gluteus maximus and rectus femoris activation for deadlift
Korak et al. (2018) [<u>39</u>]	Deadlift versus paralell back squat and paralell front squat	13 Women	22.8 ± 3.1	> 1 year	3 reps 75% 1RM	Gluteus maximus, biceps femoris, vastus medialis, vastus lateralis and rectus femoris	Greater gluteus maximus activation during front and back squat in comparison to deadlift
Edington et al. (2018) [<u>35]</u>	ISOMETRIC: close-bar deadlift versus far-bar deadlift	5 men & 5 women	32 ± 10	6.05 ± 3.35	3 trials in both starting positions. ISOpull	Gluteus maximus, biceps femoris, vastus lateralis, erector spinae and latissimus dorsi	Greater erector spinae and biceps femoris activation than the rest of muscles for both exercises. Greater vastus lateralis activation during far-bar deadlift
Andersen et al. (2018) [22]	Deadlift versus hexagonal bar deadlift and hip thrust	13 Men	21.9 ± 1.6	4.5 ± 1.9	1 rep 1RM	Gluteus maximus, biceps femoris and erector spinae	Greater biceps femoris activation during deadlift. Greater gluteus maximus activation during hip thrust. Erector spinae activation showed no differences among exercises
Snyder et al. (2017) [<u>36</u>]	Deadlift versus walk-in deadlift machine (2 different feet positions)	2 women & 13 men	18–24	Not indicated	3 reps 80% 3RM	Gluteus maximus, biceps femoris, vastus lateralis and erector spinae	Greater erector spinae activation during deadlift. Greater gluteus maximus and lower vastus lateralis activation during deadlift compared to walk-in machine deadlifts
Iversen et al. (2017) [41]	Stiff leg deadlift versus stiff leg deadlift with elastic bands	17 men & 12 women	25 ± 3 men 25 ± 2 women	Not indicated	3 reps 10RM	Gluteus maximus, biceps femoris, semitendinosus, vastus medialis, vastus lateralis, rectus femoris, erector spinae and external oblique	Greater activation for all muscles during conventional resistance exercises compared to elastic band deadlifts. Rectus femoris showed no differences activation among exercises
Bourne et al. (2017) [16]	Stiff leg deadlift versus unilateral stiff leg deadlift, hip hinge, 45° hip extension and nordic hamstring exercise	18/10 Men	23.9 ± 3.1	Not indicated	6 reps 12RM	Biceps femoris and semitendinosus	Greater semitendinosus concentric activation during unilateral stiff leg deadlift versus remaining exercises. Similar biceps femoris and semitendinosus activation during both deadlift exercises
Nijem et al. (2016) [<u>37</u>]	Deadlift versus deadlift with chains	13 Men	24.0 ± 2.1	Not indicated	3 reps 85% 1RM	Gluteus maximus, vastus lateralis and erector spinae	Greater gluteus maximus activation during deadlift. Greater erector spinae activation during the beginning of the movement for both exercises

Table 1. Data gathered from selected articles regarding intervention, sample size, gender, training experience, age, sEMG collection method, outcomes and main findings.

(Continued)

Table 1. (Continued)

Reference	Exercises tested	Sample	Age (years)	Experience (years)	sEMG collection method	Activity sEMG recorded of muscles:	Main findings
Camara et al. (2016) [<u>38]</u>	Deadlift versus hexagonal bar deadlift	20 Men	23.3 ± 2.1	> 1 year	3 reps 65% 1RM & 3 reps 85% 1RM	Biceps femoris, vastus lateralis and erector spinae	Greater vastus lateralis activation; and lower biceps femoris and erector spinae activation during hexagonal bar deadlift
Schoenfeld et al. (2015) [42]	Stiff leg deadlift versus prone lying leg curl in machine	10 Men	23.5 ± 3.1	4.6 ± 2.2	1 set 8RM	Biceps femoris and semitendinosus	Greater upper biceps femoris and upper semitendinosus activation during stiff leg deadlift
McAllister et al. (2014) [44]	Romanian deadlift versus glute ham-raise, good morning and prone leg curl	12 Men	27.1 ± 7.7	8.6 ± 5.5	85% 1RM	Gluteus medius, biceps femoris, semitendinosus, erector spinae and medial gastrocnemius	Greater semitendinosus activation than biceps femoris and erector spinae activation for all exercises
Bezerra et al. (2013) [<u>26</u>]	Deadlift versus stiff leg deadlift	14 Men	26.7 ± 4.9	> 2 years	3 reps 70% 1RM	Biceps femoris, vastus lateralis, lumbar multifidus, anterior tibialis and medial gastrocnemius	Greater vastus lateralis activation during deadlift. Greater medial gastrocnemius activation during stiff leg deadlift
Chulvi- Medrano et al. (2010) [28]	Deadlift versus Bosu deadlift and T-Bow device deadlift	31	24.2 ± 0.4	> 1 year	Dinamic effort, 6 reps 70% of MVIC	Lumbar multifidus, thoracic multifidus, lumbar spinae and thoracic spinae	Greater overall activation during deadlift versus Bosu and T-Bow device deadlifts
Ebben (2009) [43]	Stiff leg deadlift versus unilateral stiff leg deadlift, good morning, seated leg curl, nordic hamstring exercise and squat	21 men & 13 women	20.3 ± 1.7	Not indicated	2 reps 6RM	Rectus femoris and hamstrings	Greater biceps femoris activation during seated leg curl and nordic hamstring than remaining exercises. Greater rectus femoris activation during squat
Hamlyn et al. (2007) [<u>34</u>]	Deadlift versus paralell squat	8 men & 8 women	24.1 ± 6.8	Not indicated	6 reps 80% 1RM	Lower abdominal, external oblique, lumbar-sacral erector spinae and upper lumbar erector spinae	Greater upper lumbar erector spinae activation during deadlift
Escamilla et al. (2002) [<u>13</u>]	Deadlift versus sumo deadlift (both with/ without belt)	13 Men	20.1 ± 1.3	Not indicated	4 reps 12 RM	Gluteus maximus, biceps femoris, vastus medialis, vastus lateralis, rectus femoris, lateral and medial gastrocnemius, tibialis anterior, L3, T12, medial and upper trapezius, rectus abdominis and external oblique	Greater vastus medialis, vastus lateralis and tibialis anterior activation during sumo deadlift. Greater medialis gastrocnemius activation during deadlift. Greater rectus abdominis activation during belt deadlift and belt sumo deadlift

Exercises abbreviations: EB, elastic bands; FW, free weights.

Other abbreviations: ISOpull, isometric pulls; MVIC, maximal voluntary isometric contraction; reps, repetitions; RM, repetition maximum; ROM, range of motion.

tables because they assessed the sEMG only from the upper limbs or showed the muscle activation in a different measurement unit than that used in our analysis [28, 32, 34, 35].

Most researched *Deadlift* variants include the *Conventional Barbell Deadlift* (10/19 studies) [8, 13, 22, 27, 28, 32, 36–39] and the *Stiff Leg Deadlift* (6/19 studies) [16, 35, 40–43], which are followed by *Unilateral Stiff Leg Deadlift* (2/19 studies) [16, 43], *Romanian Deadlift* (2/19 studies) [27, 44] and *Hexagonal Bar Deadlift* (2/19 studies) [22, 38] (Table 1).

It is also important to clarify that exercises such as "Olympic Barbell Deadlift", "Straight Bar Deadlift", "Barbell Deadlift", "No Chains Deadlift" and "Conventional Barbell Deadlift" all refer to the same exercise, so "Deadlift" will be used for all cases.

Reference	Exercise	Gluteus Maximus	Biceps Femoris	Semitendinosus	Hamstrings	Vastus Lateralis	Vastus Medialis	Rectus Femoris	Erector Spinae
McCurdy et al. (2018) [40]	Stiff leg deadlift	51.1 ± 22.1% mean conc 29.9 ± 16.2% mean eccen	n/a	n/a	39.8 ± 16.6% mean conc 19.9 ± 11.3% mean eccen	n/a	n/a	n/a	n/a
Andersen et al. (2018)	Deadlift	~95% mean	~108% mean	n/a	n/a	n/a	n/a	n/a	~86% mean
[22]	Hexagonal bar deadlift (HBDL)	~88% mean	~83% mean	n/a	n/a	n/a	n/a	n/a	~82% mean
Iversen et al. (2017) [<u>41</u>]	Stiff leg deadlift	~42% peak conc ~17% peak eccent	~38% peak conc ~17% peak eccent	~44% peak conc ~22% peak eccent	n/a	~13% peak conc ~14% peak eccent	~10% peak conc ~9% peak eccent	~6% peak conc ~7% peak eccent	~69% peak conc ~38% peak eccent
	Stiff leg deadlift with elastic bands	~27% peak conc ~17% peak eccent	~20% peak conc ~17% peak eccent	~23% peak conc ~21% peak eccent	n/a	~12% peak conc ~14% peak eccent	~9% peak conc ~8% peak eccent	~5% peak conc ~6% peak eccent	~57% peak conc ~36% peak eccent
Bourne et al. (2017) [<u>16</u>]	Stiff leg deadlift	n/a	~55% mean conc ~23% mean eccen	~50% mean conc ~18% mean eccen	n/a	n/a	n/a	n/a	n/a
	Unilateral stiff leg deadlift	n/a	~50% mean conc ~26% mean eccen	~62% mean conc ~27% mean eccen	n/a	n/a	n/a	n/a	n/a
Schoenfeld et al. (2015) [42]	Stiff leg deadlift	n/a	~40% mean lower ~73% mean upper	~47% mean lower ~125% mean upper	n/a	n/a	n/a	n/a	n/a
Ebben (2009) [43]	Stiff leg deadlift	n/a	n/a	n/a	49±27% mean	n/a	n/a	n/a	n/a
	Unilateral stiff leg deadlift	n/a	n/a	n/a	48±39% mean	n/a	n/a	n/a	n/a
	Good morning	n/a	n/a	n/a	43±16% mean	n/a	n/a	n/a	n/a
Escamilla et al. (2002)	Deadlift	35±27% mean	28±19% mean	27±23% mean	n/a	40±22% mean	36±25% mean	19±16% mean	n/a
[13]	Sumo deadlift	37±28% mean	29±19% mean	31±23% mean	n/a	48±24% mean	44±27% mean	18±13% mean	n/a

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Table 2. Data description regarding	ig service activity in each stud	iy, in relation to exercise ty	pe and normalized service activation e	corressed as mean or peak % MVIC.

Conc, concentric phase; eccen, eccentric phase.

Concentric and eccentric phases

Generally, studies analyzing electromyographical data assess muscle activation on each repetition, treating it as a single unit. Nonetheless, it has been reported that electromyographical activity could differ significantly between concentric and eccentric phases of the movement. Therefore, some authors have already carried out this division in their research [45–47]. Not all studies included in the current review divided sEMG exercises into concentric and eccentric phases. In fact, only seven studies performed such a subdivision [16, 28, 34, 37, 38, 40, 41], in which the concentric phase showed greater muscle activation than the eccentric phase for every single case.

Muscle activation

The biceps femoris has been the most investigated muscle in terms of sEMG for the *Deadlift* exercise and its variants (13/19 studies). Gluteus maximus is the next muscle most evaluated

Reference	Exercise	Gluteus maximus	Biceps Femoris	Vastus Lateralis	Rectus Femoris	Erector Spinae	Lumbar Multifidus
Lee et al. (2018) [27]	Deadlift	51.52 ± 6.0 peak RMS	57.45 ± 6.34% peak RMS	n/a	58.57 ± 13.73% peak RMS	n/a	n/a
	Romanian deadlift	46.88 ± 7.39% peak RMS	56.66 ± 18.56% peak RMS	n/a	25.26 ± 14.21% peak RMS	n/a	n/a
Snyder et al. (2017) [<u>36]</u>	Deadlift	~47% peak RMS	~28% peak RMS	~48% peak RMS	n/a	~73% peak RMS	n/a
	BallPro	~30% peak RMS	~25% peak RMS	~80% peak RMS	n/a	~53% peak RMS	n/a
	ToePro	~30% peak RMS	~31% peak RMS	~63% peak RMS	n/a	~58% peak RMS	n/a
Bezerra et al. (2013) [26]	Deadlift	n/a	100.1 ± 24.7% peak RMS	128.3 ± 33.9% peak RMS	n/a	n/a	112.7 ± 42.7% peak RMS
	Stiff leg deadlift	n/a	98.6 ± 28.5% peak RMS	101.1 ± 14.6% peak RMS	n/a	n/a	106 ± 20.5% peak RMS

Table 3. Data description regarding sEMG activity in each study, in relation to exercise type and normalized sEMG activation expressed as % peak RMS.

BallPro, walk-in machine deadlift with feet ball-hand; RMS, root mean square; ToePro, walk-in machine deadlift with toes-hand.

(10/19) followed by vastus lateralis and erector spinae muscles (9/19). The semitendinosus and rectus femoris are positioned in fourth position (5/19) followed by vastus medialis, external oblique and medial gastrocnemius (3/19) (Table 1).

Due to the diversity regarding methodology, it was considered appropriate to report the results by grouping the studies according to the sEMG normalization process carried out in each study (mean or peak % MVIC, % peak RMS, RMS mV or % 1RM).

Studies in which muscle activation was expressed as a mean or peak % MVIC are shown in Table 2. Erector spinae showed the greatest muscle activation during the *Stiff Leg Deadlift* exercise [41], and also showed a similar muscle activation than the gluteus maximus or biceps femoris during *Deadlift* and *Hexagonal Bar Deadlift* exercises [22]. Except for the *Deadlift* exercise [22], the gluteus maximus showed greater muscle activation than biceps femoris [13,

Table 4. Data description regarding sEMG activity in each study, in relation to exercise type and normalized sEMG activation expressed as absolute RMS values	in
mV.	

Reference	Exercise	Gluteus maximus	Biceps Femoris	Semitendinosus	Vastus Lateralis	Erector Spinae
Andersen et al. (2019) [<u>8]</u>	Deadlift	236 RMS (mV)	312 RMS (mV)	367 RMS (mV)	239 RMS (mV)	341 RMS (mV)
	DL FW-2EB	231 RMS (mV)	313 RMS (mV)	359 RMS (mV)	234 RMS (mV)	330 RMS (mV)
	DL FW-4EB	250 RMS (mV)	326 RMS (mV)	375 RMS (mV)	238 RMS (mV)	357 RMS (mV)
McAllister et al. (2014) [44]	Romanian deadlift	n/a	~360 RMS (mV) conc ~300 RMS (mV) eccen	~810 RMS (mV) conc ~790 RMS (mV) eccen	n/a	~210 RMS (mV) conc
	Glute ham- raise	n/a	~380 RMS (mV) conc ~160 RMS (mV) eccen	~1180 RMS (mV) conc ~490 RMS (mV) eccen	n/a	~430 RMS (mV) conc
	Good morning	n/a	~290 RMS (mV) conc ~210 RMS (mV) eccen	~910 RMS (mV) conc ~590 RMS (mV) eccen	n/a	~205 RMS (mV) conc
	Prone leg curl	n/a	~240 RMS (mV) conc ~85 RMS (mV) eccen	~870 RMS (mV) conc ~330 RMS (mV) eccen	n/a	~255 RMS (mV) conc

Conc, concentric phase; eccen, eccentric phase; EB, elastic bands; FW, free weight; mV, microvolts; RMS, root mean square.

Reference	Exercise	Gluteus maximus	Biceps Femoris	Vastus Lateralis	Vastus Medialis	Rectus Femoris	Erector Spinae
Korak et al.	Deadlift	72% 1RM	82% 1RM	104% 1RM	92%1RM	105%1RM	n/a
(2018) [<u>39</u>]	Parallel back squat	80% 1RM	78% 1RM	97% 1RM	96%1RM	102%1RM	n/a
	Parallel front squat	94% 1RM	81% 1RM	102% 1RM	98%1RM	101%1RM	n/a
Nijem et al. (2016) [37]	Deadlift	82.5 ± 6.9% 1RM	n/a	115.9 ± 30.1% 1RM	n/a	n/a	97.9 ± 8.7% 1RM
	Deadlift with chains	76.8 ± 6.8% 1RM	n/a	123.3 ± 45.1% 1RM	n/a	n/a	93.2 ± 11% 1RM
Camara et al. (2016) [<u>38]</u>	Deadlift	n/a	83.5 ± 19%1RM conc 34.7 ± 11%1RM eccen	96.8 ± 22%1RM conc 55.9 ± 12.6%1RM eccen	n/a	n/a	98.9 ± 26% 1RM conc 75.3 ± 28% 1RM eccen
	Hexagonal bar deadlift	n/a	72.3 ± 20%1RM conc 31.5 ± 10%1RM eccen	119.9 ± 22%1RM conc 87.9 ± 31%1RM eccen	n/a	n/a	88 ± 27% 1RM conc 61.4 ± 21% 1RM eccen

Table 5. Data description regarding muscle activation in mV expressed as a percentage of EMG (mV) during 1RM effort.

1RM, 1 repetition maximum; concentric phase; eccen, eccentric phase; RMS, root mean square.

22, 40, 41]. When comparing muscle activation within the hamstrings, there was a greater activation for the semitendinosus muscle than the biceps femoris during *Stiff Leg Deadlift* [16, 41, 42], which is even more pronounced when performing *Unilateral Stiff Leg Deadlift* [16]. The concentric phase showed a greater activation in the gluteus maximus and hamstring muscles than the eccentric phase for all exercises evaluated [16, 40, 41] (Table 2).

Data regarding muscle activation expressed as percentage peak RMS (% peak RMS) are shown in Table 3. The erector spinae and lumbar multifidus showed greater muscle activation than the gluteus maximus and biceps femoris [26, 36]. However, conflicting results have been reported for the *Deadlift* exercise. Lee et al. [27] reported more activation in the biceps femoris than the gluteus maximus, while Snyder et al. [36] reported more activation in the gluteus maximus than the biceps femoris (Table 3). Whereas the vastus lateralis showed greater muscle activation than the biceps femoris [26, 36], and the rectus femoris showed greater muscle activation than the biceps femoris and gluteus maximus during *Deadlift* exercise [27] (Table 3).

Data regarding muscle activation expressed as RMS in mV are shown in <u>Table 4</u>. Erector spinae and semitendinosus are the most activated muscle in the *Deadlift* exercise [22]. When comparing muscle activation within the hamstrings, there was a greater activation recorded for the semitendinosus muscle in comparison to that for the biceps femoris [22, 44] (Table 4). The concentric phase showed greater activation than the eccentric phase in all muscles and exercises evaluated [44].

Data regarding muscle activation in mV expressed as a percentage of sEMG (mV) during a 1RM effort are shown in Table 5. The Erector spinae presented higher muscle activation than the gluteus maximus [37] and biceps femoris [38]. The vastus lateralis and vastus medialis showed greater muscle activation than the biceps femoris and gluteus maximus during *Deadlift* exercises and its variants [37–39]. The concentric phase showed greater activation in the biceps femoris, vastus lateralis and erector spinae than the eccentric phase during the *Deadlift* exercise as well as during the hexagonal bar *Deadlift* exercise [38].

Discussion

The main aim of the present study was to carry out a comprehensive literature review assessing muscle activation measured with sEMG when performing the *Deadlift* exercise and all its variants.

The most relevant results compiled from the literature review revealed that the biceps femoris is the most evaluated muscle when performing this kind of exercises [8, 13, 16, 22, 26, 27, 36–39, 41, 42, 44, 48], followed immediately by the gluteus maximus [8, 13, 22, 27, 36–41].

Erector spinae presented higher muscle activation than the gluteus maximus and the biceps femoris muscles for all exercises [8, 26, 37, 38, 41]. Only one study presented contrary outcomes, showing lower muscle activation in the erector spinae than the biceps femoris and semitendinosus during the *Romanian Deadlift* exercise [36].

Another important finding in the current review was that muscles from the quadriceps complex appeared to elicit the greatest muscle activation compared to the gluteus maximus and hamstrings muscles for *Deadlift* exercise [26, 27, 36–39]. Furthermore, the semitendinosus generally tended to elicit slightly greater muscle activation than the biceps femoris within the hamstring complex [8, 44].

Methodological issues

One concern about the findings of the review was the lack of unification of collecting data methodological process amongst studies. This includes the kind of muscle contraction evaluated, the number of participants, the participants' resistance training experience, exercise intensity during evaluation, sEMG collection method, electrode location, and number of evaluation days. All studies following a specific methodology process had diverse aims and different outcomes, which made difficult to deliver consistent results. Only one study evaluated just an isometric position of the movement, the preparatory position [35], whereas the rest evaluated exercises from a dynamic perspective.

The included studies were variate in number of participants (8-34) but similar in their sample population ages (18-34), who had a minimum of 6 months resistance training experience. It is important to highlight the impact that training status have upon muscle activation pattern, since familiarization with the movement could substantially modify muscle activation elicited during each exercise [49–51]. Furthermore, twelve of the studies had a male sample, while the rest combined both genders [34–36, 41, 43], and only two studies included exclusively females [39, 40]. This raises the necessity to invest more research into females in this field.

In line with previous reviews, exercise loading for sEMG recordings has been one of the biggest concerns [52–54]. Only two studies performed same 1RM intensity [14, 25], whereas others performed exercises at a predetermined repetition maximum load, and the rest measured a number of repetitions within a range of 65–85% 1RM (Table 1). Differences in the applied methodology should be reduced for future studies, providing an enhanced outcomes reliability [42].

No unified criterion has been followed in categories of time management during exercise phase among study methodologies, which could also be treated as a potential bias risk. For future studies focused on sEMG, it would be of significant interest to report divided electro-myographical data into concentric and eccentric phases, as well as exercise timing. Such information would help coaches and trainers when choosing one or another exercise for a concrete target when prescribing an optimized training [7].

In relation to the electrode location, reports on surface recording of sEMG should include electrode shape and size, interelectrode distance, electrode location and orientation over muscle with respect to tendons and fiber direction among others (Merletti & Di Torino, 1999). It is vital to report in detail the placement of electrodes over the muscle belly when we aim to compare outcomes with other similar studies.

Different protocols for surface electromyography electrode placement have been described in the literature. One of the most popular protocols is the SENIAM Guidelines (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles). Eight studies following the SENIAM Guidelines have been included in our review [8, 16, 22, 35–37, 41, 42]. The rest followed some other Guidelines or a previous reference, and only four studies did not report any protocol for electrode location [26, 28, 32, 34].

In regard to interelectrode distance, five studies reported using the recommended 2 cm center-to-center distance between electrodes according to SENIAM Guidelines [8, 22, 27, 35, 43]. In addition to not following these Guidelines, some other studies also did not report the inter-electrode distance [26, 32, 34, 38–40, 44]. Furthermore, four studies reported to have placed the electrodes with a center-to-center distance ranging between 15–35mm but different from 20 mm [13, 16, 28, 37, 41]. The higher the interelectrode distance, the wider the detection volume and consequently the detected amplitude [55]. Future research should attempt to follow established Guidelines, so they can reach optimum research quality and diminish the risk of data collection bias.

On the other hand, most of the reviewed studies included between 2–4 days/sessions (visits to the laboratory) for the measurement process, normally leaving 2–7 days' rest between each visit. Tasks performed during those days cover anthropometric data gathering, familiarization with exercises, RM testing and sEMG data collection. To ensure reliable sEMG data outcomes, sEMG data must be collected at the same session [8]. Otherwise, some studies collected sEMG data on two different days, which might have entailed electrode location mistakes [32, 40, 41, 44].

In order to avoid fatigue bias risks, a randomized counterbalanced order for exercise testing was followed in all studies but one, which followed a preset exercise order [41]. In addition with the same aim, a minimum break of 2–5 min was considered between exercise testing trials [22, 27, 28, 34, 36, 37, 40–42, 44].

Most studies did not report hand grip and stance position in any depth of detail. Some studies allowed a preferred stance position for each participant but maintained the same for all exercises tested [8, 22], whereas others also indicated a hand grip slightly wider than shoulder width [26, 27, 32, 40–43].

Likewise, information about MVIC should be strictly reported. Our reviewed studies reported a range between 2–3 trials, 3–5 seconds holding and 15–60 seconds rest between trials [13, 16, 22, 32, 40, 43].

Other Deadlift variations

Apart from the above-mentioned *Deadlift* exercises, there are some other studies which focused on less conventional variants of this movement. The *Good Morning* exercise appears to be an appropriate substitute to *Romanian Deadlift* when it is preferable to place the load on the back instead of lifting it from the floor. *Good Morning* provokes a similar muscular pattern activation as *Romanian Deadlift*, but it showed more muscle activation for the semitendinosus and less muscle activation for the biceps femoris than *Romanian Deadlift* [44].

In addition, some authors proposed interesting alternatives for the *Deadlift* exercise with the goal of overcoming the sticking region. This involves a phase during the lift in which there is a mechanical disadvantage that elevates injury risk and leads to a deceleration on the speed lift [56]. In relation to this issue, Nijem et al. (2016) compared *Deadlift* versus *Deadlift with chains* and reported the existence of a lightest load at the sticking point which would allow one to maintain a neutral spine during *Deadlift with chains*. Regarding muscle activation, there were significant differences for the gluteus maximus muscle, which present greater activity during *Deadlift with chains*. Furthermore, Andersen et al. (2019) reported another resource by using the addition of elastic bands attached to the ceiling to displace the

sticking point. This method would reduce the load from lower phases of the lift and increase the resistance as the bar goes up.

Elastic bands have also been used as a tool in *Deadlift* learning processes, when the athlete is not ready to lift high loads with a proper technique or in those cases when some injury prevents the athlete from using conventional resistance equipment. Muscular activation presented during elastic bands *Stiff Leg Deadlift* was lower than that elicited during free weights *Stiff Leg Deadlift*, with significant differences when referring to the gluteus maximus, biceps femoris and semitendinosus muscles [41].

Furthermore, it should be noted that if your aim is to increase muscle activation from forearm musculature during the *Deadlift* exercise, it is recommended to use a Fat Gripz device, a wider grip implement that sticks to the bar. Worth mentioning that a significant reduction in 1RM strength would appear when using this kind of implement [32].

Comparing Deadlift to other exercises

Some studies included in this review also compared muscle activation elicited during *Deadlift* exercises versus other typical weight bearing exercises performed in weight rooms. McCurdy et al. (2018) reported significantly greater muscle activation for the gluteus maximus and hamstring muscles during *Modified Single Leg Squat* in comparison to *Back Squat* and *Stiff Leg Deadlift*. Whereas, Korak et al. (2018) reported the highest muscle activation for the gluteus maximus during *Front Squat* comparing to *Deadlift* exercise, with no differences for this muscle between *Front* and *Back Squat*.

Moreover, the *Hip Thrust* exercise has also been found to elicit greater muscle activation for the gluteus maximus than *Deadlift* and *Hexagonal Bar Deadlift*. Also, lower muscle activation for the biceps femoris muscle was shown during *Hip Thrust* compared to *Deadlift*. No muscle activation differences were presented among those three exercises for the erector spinae muscle. Hence, a greater torque and greater stress in the hip joint during *Deadlift* compared to both other exercises was also reported [22].

Additionally, several authors have compared *Deadlift* exercises to single joint and machinebased exercises in their research. For example, Bourne et al. (2017) reported significantly greater muscle activation during 45° *Hip Extension* and *Nordic Hamstring Exercise* than *Stiff Leg Deadlift* and *Unilateral Stiff Leg Deadlift* for biceps femoris and semitendinosus muscles. Similar results support these findings, showing a greater muscle activation during the *Nordic Hamstring Exercise* and during *Seated Leg Curl* for hamstring muscles in comparison to the muscle activation elicited for hamstring muscles during *Stiff Leg Deadlift* and *Unilateral Stiff Leg Deadlift* [43].

On the other hand, the *Prone Leg Curl* in machine was found to elicit higher muscle activation for both upper and lower sections of the biceps femoris muscle than during *Stiff Leg Deadlift* but showed no significant differences for the semitendinosus muscle (Schoenfeld et al., 2015). On the contrary, McAllister et al. (2014) reported greater biceps femoris muscle activation during *Romanian Deadlift* than during the *Prone Leg Curl*. It would be necessary to unify the muscle activation normalization method and protocol carried out. Likewise, researchers should ascertain a proportional exercise load when comparing bilateral multi joint exercises to single leg and machine-based exercises, in order to obtain consistent outcomes.

Conclusions

After performing the current systematic and comprehensive review, several conclusions have been reached. Main findings outlined that:

1. Biceps femoris is the most studied muscle (13/19), followed by gluteus maximus (10/19), vastus lateralis and erector spinae (9/19) during *Deadlift* exercises.

- 2. Erector spinae and quadriceps muscles are more activated than gluteus maximus and biceps femoris muscles within *Deadlift* exercises (9/19).
- 3. Within the hamstring muscles complex, semitendinosus elicits slightly greater muscle activation than biceps femoris during *Deadlift* exercises (6/19).

Some recommendations for future research involving surface electromyography recordings are:

- 1. Participants training status and participants resistance training experience should be outlined in detail. Only 11/19 studies showed this information.
- 2. Exercise load quantification method during sEMG recordings must be standardized, so exercises could be comparable among them.
- 3. Taking into consideration the different muscle activation pattern reported during concentric and eccentric exercises phases, it is highly recommended to perform such subdivision for future studies.
- 4. A unified criterion upon methodology protocol is necessary in order to avoid several bias risks and report reliable outcomes when using surface electromyography recordings. Information regarding electrode location, number of testing days and sEMG normalization method should be strictly reported.

Practical applications

Currently, *Deadlift* is an exercise frequently performed to improve the lower limb muscles, mainly biceps femoris and semitendinosus (hamstrings), and gluteus maximus. Based on this systematic review about the sEMG activity in the *Deadlift* exercise and its variants, it has been demonstrated that other muscles such as erector spinae and quadriceps are more activated than hamstrings and gluteus maximus, although some studies found conflicting results.

Deadlift exercise comprises a movement which could have a transference into daily life activities; also considered as one of the greatest compound lifts, as it involves several muscles groups coordination. A broad spectrum of *Deadlift* variants has been reported, so diverse applications for these exercises could merge, covering health, rehabilitation and performance environments.

Therefore, it must be considered that muscle activation would depend on the *Deadlift* variant performed. For instances, posterior thigh muscles would show greater muscle activity when performing exercises that holds the knees on a fixed and extended position (e.g. *Romanian Deadlift* or *Straight Leg Deadlift*). On the contrary, whether your goal is to maximize anterior thigh and lower back muscle activity, *Deadlift* would be the exercise of choice. *Hexagonal Bar Deadlift* also elicits a great anterior thigh muscle activity, but with a reduction on erector spinae muscle activity, turning this exercise into an appropriate *Deadlift* variant when athletes have lower back issues.

Hence, coaches, athletes and regular population ought to contemplate these findings when selecting the *Deadlift* exercise and its variants for their training programs, considering the individual training goals.

Supporting information

S1 Checklist. (DOCX)

S1 File. (PDF)

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14 Acute low- compared to high-load resistance training to failure results in greater energy expenditure during exercise in healthy young men

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Abstract

The objective of the present study was to verify the energy expenditure (EE), energy system contributions and autonomic control during and after an acute low-load or high-load resistance training (RT) protocol to momentary failure (MF) in young adults. Eleven young men $(22 \pm 3 \text{ yrs}, 71.8 \pm 7.7 \text{ kg}; 1.75 \pm 0.06 \text{ m})$ underwent a randomized crossover design of three knee extension acute protocols: a low-load RT [30% of their maximal strength (1RM); RT30] or a high-load RT (80% of 1RM; RT80) protocol, with all sets being performed to MF; or a control session (Control) without exercise. Participants were measured for EE, energy system contributions, and cardiac autonomic control before, during, and after each exercise session. Exercise EE was significantly higher for RT30 as compared to RT80. Furthermore, post measurements of blood lactate levels and the anaerobic lactic system contribution were significantly greater for RT30 as compared to RT80. In addition, parasympathetic restoration was lower for RT30 as compared to RT80. In conclusion, a low-load (30% 1RM) RT session produced higher EE during exercise than a high-load (80% 1RM) RT session to MF, and may be a good option for fitness professionals, exercise physiologists, and practitioners when choosing the optimal RT protocol that provides more EE, especially for those who want or need to lose weight.

Introduction

Resistance training (RT) is known to promote several benefits for the practitioners such as increases in energy expenditure (EE), skeletal muscle mass, strength, and power and also reductions in fat mass, visceral and subcutaneous fat, inflammatory markers, lipid profile, and cardiometabolic risk factors [1]. Furthermore, it seems that RT performed with loads equal to or greater than 80% of 1 repetition maximal (1RM) increase the hypertrophic gains and muscle strength in a greater magnitude when compared to lower intensity protocols [2, 3].

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On the other hand, studies have found that similar muscle hypertrophy and strength improvements can result from lifting loads to failure with higher (80% of 1RM) or lower (30% of 1RM) loads [4–7]. However, whether the magnitude of EE generated by low-load (30% 1RM) or high-load (80% 1RM) RT protocols is similar is still undetermined. Thus, it becomes clear that there is a need to investigate whether these RT protocols with different loads but similar muscle mass gains can provide additional EE during and after the training sessions.

In addition, the time to restore parasympathetic modulation after exercise indicates the time under exercise cardiac stress [8]. Autonomic nervous system (ANS) coordinates the cardiovascular adjustments required to supply the exercise metabolic demand and specific metabolic demands are identified by the ANS through different afferent stimuli, among which muscle metaboreflex plays an important role during muscle metabolites accumulation induced by RT protocols, mainly when it is performed to failure [9, 10]. Besides muscle metaboreceptors, many other neural mechanisms such as muscle pressor receptors, cardiopulmonary receptors, carotid and aortic chemo- and baroceptors conduct signals to cardiovascular control nuclei on the brain steam that after interactively processing these signals regulate the sympathetic and parasympathetic efferent neuronal activation or deactivation [11]. Thus, despite we might expect a linear association of EE and autonomic modulation, the different afferent mechanism the brain uses to identify the metabolic need in the body lead to different autonomic adjustments to exercise. In a previous study, Sardeli et al. [10] observed that a low-load RT protocol performed until failure promoted a delayed vagal restoration following an acute session when compared to a high-load RT protocol to failure; However, whether higher EE could be associated to the low- load RT protocol generating a delayed vagal restoration than the high-load RT protocol after the sessions is still unknown.

Since the knowledge of possible differences in EE, energy system contributions and cardiac autonomic control between a high-load or a low-load RT protocol to failure may help fitness professionals and/or exercise physiologists to choose the optimal RT protocol depending on the population considered, the purpose of this study was to compare the energy cost and cardiac autonomic recovery during and after two similar hypertrophic RT protocols [4–7] of low-load (30% 1RM) and high-load (80% 1RM), with all sets being performed until momentary failure (MF) [12]. In addition, since the amount of work performed within the set may contribute to the amount of EE [13] and to a delayed vagal restoration [10] following an RT session, we hypothesized that low-load RT protocol would produce greater EE during exercise and a delayed parasympathetic restoration than high-load RT protocol performed to momentary failure (MF).

Materials and methods

Participants

The disclosure of the project was made by folders and posters in the university campus and internet. Inclusion criteria were as follows: men with a non-active lifestyle (frequency of regular physical activity less than two sessions per week) who had not participated in regular resistance exercise programs for the previous 12 months according to the Baecke Habitual Physical Activity Questionnaire [14]. Exclusion criteria included the following: volunteers who presented in clinical evaluation (physical examination and resting ECG) any pathology or other complications that were risk factors in the practice of the proposed RT exercises.

Thirteen healthy young men (18–30 years old) with no experience in RT were recruited and assigned to a randomized, counterbalanced, crossover design of three acute protocols: a low-load (30% of their 1RM; RT30) or a high-load (80% 1RM; RT80) RT protocol, with all sets being performed to momentary failure (MF); or a control session without exercise (Control);

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

however, two volunteers opted to discontinue their participation in the project for personal reasons, resulting in the final sample of 11 volunteers (Table 1). None of the volunteers were obese, diabetic or using any prescription drugs, supplements or others substances that may affect the present data. All volunteers in the present study were classified as sedentary or irregularly active [14].

The experimental methods and procedures were all approved by the Research Ethics Committee of the State University of Campinas, Brazil.

All participants signed an informed consent document (written) approved by the local University Research Ethics Committee (Protocol nº 890.014).

Experimental design

Prior to baseline testing all participants came to the laboratory and were submitted to two RT familiarization sessions, separated by 72h of rest between them, in order to be acquainted with the range of motion and the proper form for the leg extension machine RT exercise, familiarize themselves with the portable gas analyzer equipment (Oxycon, Carefusion Germany 234 GmbH, Hoechberg, Germany) while testing and all methodologies used in the present study. After 72h of the last familiarization session, volunteers performed the test and re-test of 1RM on the leg extension machine, with a 72h interval between them. One week after the re-testing of 1RM, volunteers underwent the RT30 or RT80 protocol, with all sets being performed to MF; or a control session without exercise (Control), according to the randomization performed.

The acute RT protocols were composed of performing three sets of leg extension machine using the intensity corresponding to the session (30% or 80% of 1RM), with all sets being performed until MF [12] and with one and a half minutes of rest applied between each set. In the Control, volunteers performed all the procedures for determination of EE; however, they remained seated quietly in the leg extension machine during the time of exercise (approximately 8 to 10 min). After the end of the acute sessions, volunteers remained lying on an examination couch in the room for 60 minutes and expired air was collected continuously.

Before the acute sessions, resting EE (REE) was assessed for 30 minutes with the volunteers lying on an examination couch and resting. In addition, volunteers were requested to record all the foods and beverages ingested in the day before the first acute session and instructed to match the same dietary intake patterns before the subsequent acute sessions. During all sessions, breath-by-breath gas exchange was collected with a portable gas analyzer and blood

Age (years)	22 ± 3
Weight (kg)	71.8 ± 7.7
Height (m)	1.75 ± 0.06
Body mass index (kg/m ²)	23.05 ± 2.35
Body fat (%)	16.9 ± 6.1
Fat free mass (kg)	59.5 ± 6.4
One-repetition maximum (kg)	93.1 ± 20.6
Total calories (kcal)	2041 ± 497
Proteins (g)	87.3 ± 31.5
Lipids (g)	81.8 ± 62
Carbohydrates (g)	238.7 ± 74.3

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lactate samples were collected to determine REE, energy system contributions, exercise EE (Exercise EE), excess post-exercise oxygen consumption (EPOC), and total EE of the session (Total EE). Blood lactate samples were collected before (PRE) and after 3 (3min), 5 (5min), 7 (7min), and 60 (60min) minutes of the acute protocols. Heart rate variability (HRV) was recorded before (PRE), post 10 minutes (10min), and post 45 minutes (45min). In addition, subjective perception of effort [15] was applied in the end of the session.

For all EE quantifications, measurements were taken between 7:00–12:00 a.m. in a controlled temperature and humidity environment where the noise was minimal. In order to obtain the closest measurement of their physiological conditions, participants were instructed to sleep well prior to the sessions and to refrain from consuming alcohol and caffeine in the 24 hours preceding the measurements and any physical activity for the 72 hours prior to measurements. In addition, all participants fasted for at least 7 hours before the Control, RT30, or RT80 acute sessions thereby avoiding any variation in EE from feeding; however, water intake was encouraged; thus it is believed that all participants entered the laboratory in a hydrated state. Furthermore, a period of seven days of rest without exercise was used between the experimental protocols to wash out the effects of muscle recuperation.

Anthropometric measures and body composition

Height was measured using a wall-mounted stadiometer with a precision of 0.1 cm, and weight was taken using a calibrated manual scale (Filizola ® S.A., São Paulo, SP, Brazil) with a precision of 0.1 kg. The body composition of the volunteers was estimated by plethysmography in the Bod Pod [™] (COSMED USA, Inc., Concord, CA) body composition system. The same investigator performed all measurement assessments.

Dietary intake

Food records were given to the participants by trained researches who instructed them individually through a presentation of an already completed model food record and photographs of model home measures. Food records for total caloric intake and amount of macronutrients (carbohydrates, lipids, and proteins) were analyzed using the DietPro software program (version 5i).

Blood lactate samples and analyses

For analysis of blood lactate levels, samples (25 μ L) of peripheral blood from the distal phalanges of the hand were collected using lancets (Accu-Chek Safe-T-Pro Uno, Roche Diagnostics GmbH, Indianapolis, IN, USA) and microcapillary tubes. All blood samples were placed in microtubes containing a similar volume (25 μ L) of a 1% NaF solution. Plasma was separated by centrifugation of the samples for 10 minutes at 5,000rpm and stored at -80°C for subsequent analysis. Blood lactate levels were determined using a spectrophotometer (ELx800, Biotek, Winooski, USA) and commercially available kits (Biotecnica, Varginha, Brazil). The peak lactate level was determined by the highest lactate level value found in the three measurements (3min, 5min and 7min) assessed after the acute RT protocols or Control.

Maximal strength assessments

Maximal strength was measured by a one-repetition maximum (1RM) test performed on leg extension machine (Johnson SL153 leg extension machine, Johnson Health Tech. Co., Ltd.), according to descriptions by Brown and Weir [16]. All participants were tested, at baseline, in two separated sessions (test-retest) with 72-h rest between them. To determine the results of

the1RM tests at baseline, we used the value of the highest load obtained after the test-retest. The coefficient of variation and the intraclass correlation coefficient of the 1RM test-retest for leg extension machine were 5.33% and 0.93, respectively.

Acute resistance training protocols

Acute RT protocols comprised performing three sets of knee extension machine (Johnson SL153 leg extension machine, Johnson Health Tech. Co., Ltd.) according to the intensity of the session: low-load (30% of their 1RM; RT30) or high-load (80% of their 1RM; RT80), with all sets being performed to MF and with one and a half minutes of rest applied between each set. The participant started extending the knee from the flexed knee position (~90° knee joint angle; concentric phase) until full extension (~0° knee joint angle), and then flexed the knee (eccentric phase) returning to the ~90° knee joint angle in the knee extension machine. The failure was recognized when the range of motion adopted in the present study to perform the exercise (at least 81 degrees during the concentric and eccentric phase) was not completed, where the range of motion was identified from a hand goniometer to check the angle of extension of the knee, and a metric tape positioned on the side of the equipment to check the position of the weight when the knee was extended [12]. The execution speed of the exercises was one second in concentric action and one second in eccentric action, controlled by a metronome, the exercise was not interrupted by the decrease of the execution speed.

The number of repetitions of each set was recorded and the volume of each set was calculated by multiplying the number of repetitions by the load. Afterwards, total volume was calculated as the sum of each set's volume. All the acute RT protocols were based on the descriptions by Burd et al. [4], Mitchell et al. [5], Morton et al. [6], and Jenkins et al. [7]; thus it is believed that the acute RT protocols performed in the present study can promote similar muscle hypertrophy and strength gains if performed for chronic periods.

Energy expenditure data collection and calculation

REE was calculated by the area under the oxygen uptake (VO₂) curve during the central 20 minutes of the 30 minutes collected from resting, where the initial 5 minutes and 5 final minutes were excluded to avoid fluctuations. The aerobic energy system was calculated by the VO_2 area over time during exercise from which VO₂ from resting was subtracted. To estimate anaerobic alactic energy system we used an exponential model to fit the initial 7 minutes from VO₂ recovery period, considered the post-exercise fast VO₂ kinetics, acc. To calculate anaerobic lactic energy system the lactate accumulation (peak lactate minus resting lactate) was multiplied by the oxygen equivalent (3 ml O_2 .kg-1) and by the participant's body mass. Exercise EE was calculated as the sum of the three energy systems. EPOC was calculated by the area under the 53 minutes remaining of the VO₂ recovery period curve, i.e., 60 minutes of recovering minus the first 7 minutes utilized in the anaerobic alactic calculation. The Total EE was calculated by the sum of the Exercise EE and EPOC. All the variables for energy system contributions were estimated according to Bertuzzi et al. [17]. In addition, the area under the VO₂ curve calculations (trapezoidal method) and energy system contributions estimation were performed using GEDAE-LaB software tools (http://www.gedaelab.org/) and Total EE was calculated using Excel software (Microsoft Corporation, California, USA)

Heart rate variability

Continuous inter-beat (RR) intervals were acquired before and after one hour recovery in supine position using a Polar S810i heart rate monitor (Polar Electro, Kempele, Finland) and Polar ProTrainer 5 software (version 4.0. Kempele, Finland) [18] and analyzed following linear

interpolation of adjacent beats in Kubios HRV software (Version 2.1, Biosignal Analysis and Medical Imaging Group, Kuopio, Finland) [19]. The time and frequency domains from linear and the non-linear indexes of HRV were analyzed. Among time domain indices, mean RR interval (RRi), standard deviation of all normal RR intervals (SDNN), and square root of the mean squared differences of successive RR intervals (RMSSD) were analyzed as representatives of parasympathetic modulation [20, 21]. Frequency domain indices were derived by a fast Fourier transform, which included low frequency (LF: 0.04–0.15 Hz) and high frequency (HF:0.15–0.4 Hz). HF represents parasympathetic modulation, as seen it is almost entirely mediated by the vagus nerve [21]. We opted to use LF in normalized units (LFnu), considering the normalization process tend to minimize the effect of variations in total power on its value; however, LFnu is influenced by parasympathetic and sympathetic modulation [20, 21]. Total power (TP) of the frequencies was used as a global marker of parasympathetic modulation [20, 21].

Statistical analysis

The sample size required was estimated using G*Power software (version 3.1.9.2), with data from the previous study comparing energy expenditure of low- vs. high-load single set resistance exercise [13]. A priori power analysis using an alpha level of 0.05 and an expected power of 0.8 suggested a sample size of 11 participants to achieve a statistical significant difference between low-load vs. high-load in this variable. Data distribution was tested by the Shapiro-Wilk test. The Student paired T-test was used to verify differences between RT30 and RT80 exercise total volume and Borg's subjective perception of effort score. The one way ANOVA for repeated measures, followed by Tukey post hoc test, were performed to verify differences between conditions (RT30 vs. RT80 vs. Control) for energy system contributions, Exercise EE, EPOC, and Total EE. To identify differences between moments and conditions for blood lactate levels and log transformed heart rate variability variables, we used a two-way ANOVA for repeated measures. When significant moments X conditions interactions were detected, the Tukey post hoc test was applied to determine the source of significance. The level of significance was set at p \leq 0.05 for all statistical comparisons. The software used for all analyses was Statistica 6.0 (StatSoft.inc, Tulsa, USA). All data are presented in terms of values of mean \pm SD.

Results

Total repetitions for each set was significantly higher in all sets for RT30 protocol (Set 1: 36 ± 9 ; Set 2: 26 ± 6 ; Set 3: 21 ± 6 repetitions) than the RT80 protocol (Set 1: 9 ± 3 ; Set 2: 8 ± 2 ; Set 3: 7 ± 2 repetitions) (p = 0.0001 to all comparisons). In addition, total volume was significantly higher in the RT30 protocol (2301.4 ± 631.1 kg) than the RT80 protocol (1828.1 ± 690.4 kg) (p = 0.0571). However, no significant difference was found for Borg's subjective perception of effort between RT30 (17 ± 2) and RT80 (16 ± 2) after the end of the acute RT protocols (p > 0.05). In addition, no difference was found for REE before all sessions (Control: 25.9 ± 4.5 Kcal; RT30: 24.7 ± 4.5 Kcal; RT80: 26.5 ± 4.6 Kcal; p > 0.05).

Exercise EE, Total EE and energy system contributions are presented in Fig 1. As expected, Exercise EE for both RT30 (p = 0.0001) and RT80 (p = 0.0001) were greater as compared to Control (Fig 1A). Furthermore, Exercise EE was significantly higher for RT30 as compared to RT80 (p = 0.0243; Fig 1A). Total EE was significantly higher for RT30 (p = 0.0001) and RT80 (p = 0.0001) as compared to Control (Fig 1B), although no significant difference was found for Total EE between RT30 and RT80 (p = 0.9724; Fig 1B). With respect to the energy system contributions, as expected, the aerobic, anaerobic alactic and anaerobic lactic systems contribution were significantly higher for RT30 (p = 0.0001; p = 0.0001, and p = 0.0001, respectively) and

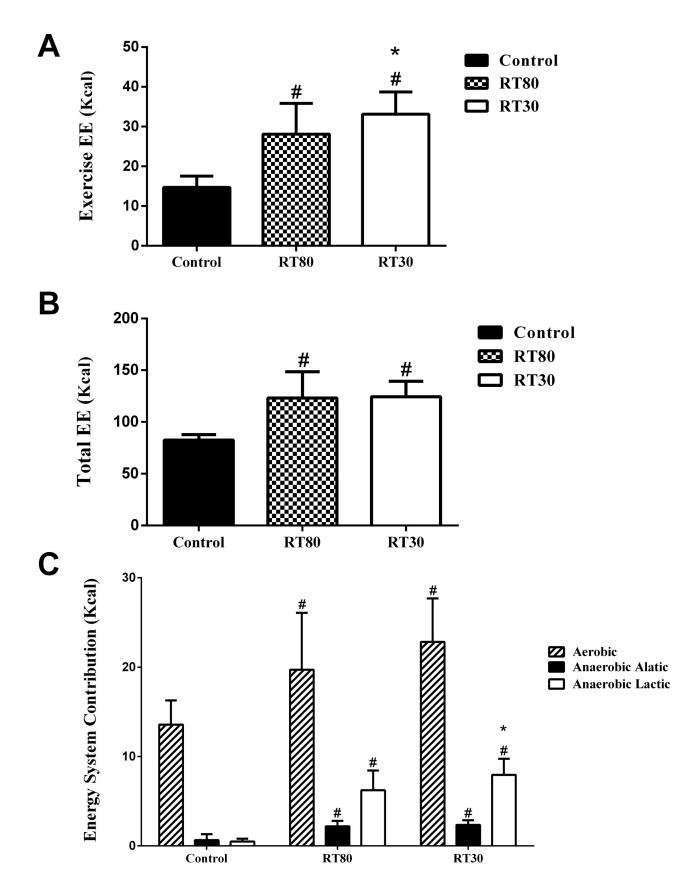


Fig 1. Energy expenditure during exercise (Exercise EE; A), Total energy expenditure (Total EE; B) and Energy system contribution (C) from a low-load (30% of 1RM; RT30) or a high-load (80% of 1RM; RT80) RT protocol performed to momentary failure or a control session without exercise (Control). #significantly different from Control. *significantly different from RT80. Mean \pm SD (n = 11; $p \le 0.05$). Individual data points presented in S1 Dataset.

RT80 (p = 0.0022; p = 0.0001, and p = 0.0001, respectively) when compared to Control (Fig 1C). Also, the anaerobic lactic system contribution was higher in the RT30 protocol than in the RT80 protocol (p = 0.0476; Fig 1C). There were no significant differences for aerobic (p = 0.1349) and anaerobic alactic system (p = 0.7936) between RT30 and RT80 (Fig 1C).

EPOC for RT80 (95.1 ± 18.4 Kcal) was greater as compared to control (75.8 ± 7.6 Kcal p = 0.0260). However, no significant difference was found for EPOC between RT30 (91.4 ± 10.6 Kcal) vs. Control (p = 0.1212) nor for RT30 vs. RT80 (p = 0.7238). Fig.2 represents the schematic evaluation of EPOC after the acute protocols.

Fig.3 represents the blood lactate levels before and after the acute protocols. Significant increases in lactate levels were found in the 3min, 5min and 7min post the exercise period for RT30 and RT80 as compared to Control (p < 0.001 for all comparisons). Furthermore, increased lactate levels were significantly higher for RT30 in the 3min (p = 0.0343), 5min (p = 0.0030) and 7min (p = 0.0002) post exercise than RT80 (Fig.3).

Table 2 shows the HRV before and after the experimental sessions. There was lower parasympathetic modulation (SDNN and RMSSD) in 10min compared to PRE and both RT protocols different of Control at 10min (Table 2). For these parasympathetic indexes, at 45min, RT80 was not different from PRE, while RT30 was still different from PRE and Control for RRi and tended to be different from PRE for RMSSD (p = 0.08). In addition, the reduction of total power in 10min was considerable for RT30 compared to RT80.

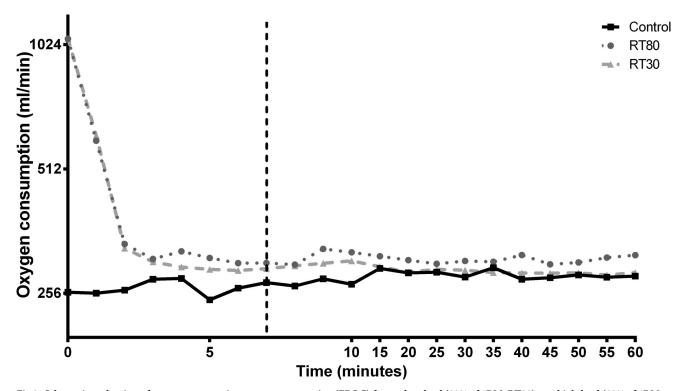


Fig 2. Schematic evaluation of excess post-exercise oxygen consumption (EPOC) from a low-load (30% of 1RM; RT30) or a high-load (80% of 1RM; RT80) RT protocol performed to momentary failure or a control session without exercise (Control). The dashed line represents the initial 7 minutes of recovery used for the calculation of the anaerobic alactic system contribution of the exercise. Individual data points presented in S1 Dataset.

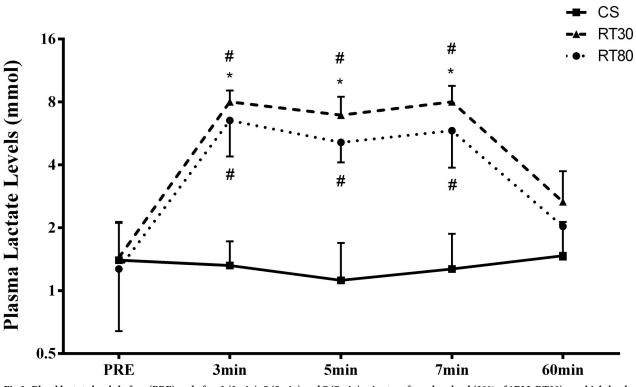


Fig 3. Blood lactate levels before (PRE) and after 3 (3min), 5 (5min) and 7 (7min) minutes after a low-load (30% of 1RM; RT30) or a high-load (80% of 1RM; RT80) RT protocol performed to momentary failure or a control session without exercise (Control). #significantly different from Control. *significantly different from RT80. Mean \pm SD (n = 11; $p \le 0.05$). Individual data points presented in S1 Dataset.

Table 2. Heart rate variability before (PRE) and after 10 (10min) and 45 (45min) minutes after a low-load (30% of 1RM; RT30) or a high-load (80% of 1RM; RT80)
RT protocol performed to momentary failure or a control session without exercise (Control).

	RRi (ms)	SDNN	RMSSD	HF (ms ²)	ТР	LF nu	HF nu
Control							
PRE	1048 ± 165	87 ± 32	94 ± 54	3635 ± 2924	7157 ± 4753	37 ± 20	63 ± 20
10min	1087 ± 165	96 ± 31	113 ± 54	4569 ± 3621	8226 ± 4756	36 ± 20	64 ± 20
45min	1088 ± 166	99 ± 43	107 ± 61	4061 ± 4033	9084 ± 6703	42 ± 17	58 ± 17
RT80							
PRE	1094 ± 174^{a}	93 ± 31^{a}	106 ± 45^{a}	3757 ± 2596^{a}	9500 ± 6619^{a}	42 ± 24	58 ± 24
10min	$907 \pm 117^{\#}$	$56 \pm 15^{\#}$	$46 \pm 26^{\#}$	$1006 \pm 1196^{\#}$	$3314 \pm 1940^{\#}$	56 ± 24	44 ± 24
45min	1015 ± 159^{a}	77 ± 29^{a}	79 ± 42^{a}	2495 ± 2176^{a}	5866 ± 3249^{a}	47 ± 20	53 ± 20
RT30							
PRE	1074 ± 188^{a}	98 ± 44^{a}	115 ± 76^{a}	5746 ± 6747^{a}	9664 ± 7931^{a}	41 ± 25	59 ± 25
10min	$838 \pm 152^{\#}$	$49 \pm 31^{\#}$	$45 \pm 40^{\#}$	$1042 \pm 1774^{\#}$	2670 ± 4393 ^{#,*}	47 ± 12	53 ± 12
45min	948 ± 154 ^{a,b,#}	82 ± 45^{a}	80 ± 66^{a}	3072 ± 4512^{a}	7667 ± 9713^{a}	47 ± 18	53 ± 1

RRi: mean RR interval; SDNN: standard deviation of all normal RR intervals; RMSSD: square root of the mean squared differences of successive RR intervals; HF: high frequency; TP: total power; LFnu: low frequency in normalized units; HFnu: high frequency in normalizes units.

^a Significantly different from 10min

^b Significantly different from PRE

[#] Significantly different from Control

* Significantly different from RT80. Mean ± SD (n = 11; $p \le 0.05$).

Discussion

Studies have demonstrated that RT increases EE both during [23, 24] and immediately post the exercise protocol [25]. To determine whether a low-load (30% of 1RM, RT30) or a high-load (80% of 1RM; RT80) RT protocol, with all sets being performed until MF, can provide different EE during and after an acute session, we tested the energy cost and the energy system contributions in young, healthy and sedentary men. In accordance with our initial hypothesis, the RT30 protocol produced greater EE during exercise as compared to RT80; however, EPOC did not differ between the RT protocols to MF. In addition, we show here that although both protocols produce similar total EE, the RT30 may induce a delayed vagal restoration after the end of the acute sessions.

It has been reported that EE increases as the intensity of RT increases, especially when total volume is matched [26]. However, in a well-controlled study, Mazzetti et al. [23] found no significant differences in total EE after four RT protocols: a light (48% of 1RM), moderate (60% of 1RM), heavy (70% of 1RM) or a heavy with loads equalized to moderate and light RT protocols, concluding that exercise intensity in RT did not affect total EE. In the present study, we observed no difference in total EE between RT30 and RT80, regardless of the fact that RT30 had a total volume of the session significantly higher than the RT80. Taking this into consideration, our results also suggest that RT intensity (load per repetition) may not influence total EE when sets are performed until MF, independent of the equalization in the total volume of the session.

As observed in previous studies, when a single RT set is performed until failure, a lower weight lifted should result in a greater number of repetitions and a heavier weight should result in fewer repetitions [6, 7, 13]. In addition, Scott et al. [13] observed that the energy cost of a single set of bench press performed until the volitional fatigue was higher when loads are performed with lower intensities (37%, 46% or 56% of 1RM) as compared with heavy intensities (70%, 80% or 90% of 1RM), concluding that the amount of work performed within the set may have contributed to the amount of EE during the experimental period; however, this was not sufficient to promote significant alterations in the EPOC data. In the present study, using three sets instead of one and a control session without exercise, both RT30 and RT80 with sets being performed until the MF were able to increase EE during exercise. We also observed that the amount of work performed during RT30 may have promoted a higher contribution from the anaerobic lactic system, generating a greater metabolic perturbation evidenced by greater lactate levels and resulting in a increased exercise EE when compared to RT80 protocol; however, the total amount of EE did not differ between RT30 and RT80, since only RT80 had significantly different EPOC from the Control demonstrating a compensatory effect.

Taking this into account, our results suggest that, when performed until failure, lactate accumulation and clearance is higher during a low-load RT protocol, and this could reflect the increased contribution of the anaerobic lactic system for increasing exercise EE as compared to a high-load RT protocol. Thus, the use of a multiple-sets RT program with low-load and with sets being performed to fatigue seems to be more beneficial to promoting higher rates of EE for those who want or need to lose weight.

Following this higher anaerobic lactic contribution for the higher EE during RT30, this protocol stimulated a lower parasympathetic restoration compared to RT80. We suggest that the higher volume of RT30 contributes to a higher metabolite accumulation such as lactate, which in turn stimulates muscle metaboreceptors and other chemoreceptors leading to its lower parasympathetic modulation [11]. Although higher load exercise could lead to higher sympathetic modulation, when the same RT volume is maintained [27], RT protocols to failure lead to higher sympathetic modulation and slower parasympathetic restoration during recovery [10]. Thus, comparing high-load and low-load RT protocols until failure, the metabolic accumulation (as measured by the blood lactate levels) from higher volume (during RT30) may contribute to parasympathetic recovery.

In contrast with what was expected, the RT protocol that prompted higher EE during exercise and worse parasympathetic restoration (RT30) did not lead to higher increases in EPOC [28]. Considering the delayed parasympathetic recovery for RT30, we speculate that this protocol may have been more efficient regarding exercise increases in sympathetic modulation, blood supply (we did not measured these factors in the present study) and energy production during exercise, which preserved the energetic storage and reduce the demand for EPOC [29]. In fact, higher sympathetic outflow during exercise enables higher oxygen consumption [28, 30], which likely occurs in low-load RT protocols [13], such as the RT30 used in the present study.

In addition, we also suggest that a low-load RT protocol until failure is physiologically more efficient to cardiovascular function because the higher dynamic component may facilitates the local vasodilation (functional sympatholysis), the venous return and mobilizes blood from the splanchnic area to exercised muscles to a higher extent [10]. Furthermore, previous studies that have observed an increased EPOC with higher EE and glycolytic demand probably found these results due to the inclusion of post-exercise fast VO₂ kinetics in the EPOC calculation [26] which is the most O2 costly phase and closely represents the anaerobic contribution of exercise [17, 31-34].

In our study we analyzed both fast and slow components of EPOC, whereas the fast component was analyzed in the first 7 minutes post exercise and considered the anaerobic alactic energy system contribution of the exercise. The remaining 53 minutes were considered as the slow EPOC component. The fast component can be considered as a good measurement of the anaerobic alactic contribution, being responsible for the restoration of muscle adenosine triphosphate (ATP) and creatine phosphate stores [26, 30]. While the slow component is not yet well understood, it can be considered as a replenishment of oxygen stores in blood and muscle, lactate removal, and increased body temperature, circulation and ventilation. An increased triglyceride/fatty acid cycling, and a shift from carbohydrate to fat as substrate source, may explain a substantial part of the prolonged EPOC component after exhaustive exercise [26, 29]. As observed in our results, higher intensity training has a better effect for increasing EPOC versus lower intensity training, even with differences in the total volume of the session. This supports previous studies showing that a more intense exercise has better effects on EPOC when volumes are matched (Thornton & Potteiger, 2002); however, little is known when exercises are not matched [29]. Nevertheless, it seems that EPOC after RT is influenced by the intensity of the training and not by the total volume of training [35].

It is important to acknowledge that the number of sets and RT intensities used in the present study is based on previous studies that demonstrated similar muscle hypertrophy and strength improvements when lifting loads to failure with higher (80% of 1RM) or lower (30% of 1RM) loads [4–7]. In addition, EE rates from our data were from an acute perspective and using only one RT exercise. Future studies comparing the energy cost of a single RT session with low or high loads until failure could be conducted using more exercises or applying the same procedures as used in the present study to experienced RT individuals, overweight/obese or older participants. To this end, we recognize limitations of energy system contributions and energy expenditure estimations on intermittent exercises; however, the calculation approach used in this study can be considered a good option for calculating the EE of the organism as a whole, at least until the emergence of a gold standard [17].

In conclusion, a low-load (30% of 1RM) RT session produced higher EE during exercise as compared to a high-load (80% of 1RM) RT session with exercises being performed to the

point of MF in young, healthy and sedentary men. These results can aid fitness professionals and/or exercise physiologists when choosing the optimal RT protocol that provides more EE without the expectation that strength or muscle mass gains would be compromised, especially for those who want or need to lose weight. However, the greater glycolytic contribution of a low-load RT session resulted in a delayed parasympathetic return; Thus, the magnitude of cardiovascular challenge should also be considered.

Supporting information

S1 Dataset. (XLSX)

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15 Monitoring exercise-induced muscle damage indicators and myoelectric activity during two weeks of knee extensor exercise training in young and old men

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Abstract

This study considered the effects of repeated bouts of short-term resistive exercise in old (age: 64.5 ± 5.5 years; n = 10) and young men (age: 25.1 ± 4.9 years; n = 10) who performed six knee extension exercise bouts over two weeks using various markers of exercise-induced muscle damage and electromyographic activity. We found that time-course changes in quadriceps isometric torque, creatine kinase activity, and muscle soreness in the two groups were similar. However, recovery in the acute torque deficit was mediated by more favourable electromyographic activity changes in the young group than in the older adults group. Muscle elastic energy storage and re-use assessed with dynamometry was selectively improved in the young group by the end of the protocol. Serum myoglobin concentration increased selectively in old group, and remained elevated with further bouts, suggesting higher sarcolemma vulnerability and less effective metabolic adaptation in the older adults, which, however, did not affect muscle contractility.

Introduction

Aging induces-muscle wasting (sarcopenia) and strength loss are well-known processes. By the age of 70 years, the skeletal muscle cross-sectional area and muscle strength are reduced by 25–30% and 30–40%, respectively [1]. The loss of muscle strength continues to decrease by 1–2% per year [2]. Scientists and gerontologists design intervention programs to target general weakness, and it has been recommended that high-intensity resistive exercise be used to delay sarcopenia but may be limited due to muscle injury and repair [3].

While high force (especially eccentric-biased) exercise has strength- and growth-promoting effects in skeletal muscle [4,5], the high tension applied during such contractions may induce

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myofibrillar disruption, delayed onset muscle soreness, elevation of muscle proteins in serum, and temporary strength loss in both young and old humans [6,7,8,9,10]. These exercise-induced muscle damage (EIMD) markers usually peak between 24–48 hours after exercise and recover between days 5 and 7 in antigravitational muscles [11].

The responsiveness to a single bout of eccentric exercise has been extensively investigated in young and old humans with varying outcomes that may be the result of different methodologies of EIMD quantification. For example, evidence exists that old humans are more susceptible to EIMD compared to young humans, and recovery is delayed after a single bout of eccentric exercise when inflammatory markers are measured [12,13]. In contrast, studies using the conventional indirect EIMD markers (serum enzymes and proteins, strength, soreness) have demonstrated greater damage in young versus old subjects [14,15].

The repeated bout effect has also been studied, and it is generally accepted that, regardless of age, EIMD markers show less severe changes after the second exercise bout, compared with the first [16,17]. The phenomenon is often explained by neural adaptation [18,19], allowing the muscle to better distribute the workload among fibres; however, more recent studies have demonstrated that reduced inflammation [20] or remodelling of the extracellular matrix [21] may contribute to protection against a second injury. The effect of age on the repeated bout effect was studied by Lavender & Nosaka [15], using indirect EIMD markers, and it was demonstrated that when the two eccentric exercise bouts were performed with a four-week hiatus, the protective effect conferred by the first bout was less pronounced in the old group. These results were confirmed by Gorianovas et al. [14], who applied two stretch-shortening cycle exercise bouts separated by two weeks. In contrast to these studies, a similar repeated bout effect was found in young and old women when the first eccentric exercise bout was repeated seven days after the first [7]. The inconsistency in the outcomes of these studies may originate from several factors, such as different experimental designs, different criterion measures, and different ages and genders of the subjects studied.

Two important problems emerge from the studies of how long the protective effect lasts in older adults. First, despite the fact that protection may play a role in early neuroadaptation, no myoelectric activity measurements were made [9,22]. Second, the recovery periods between bouts used in the experiments were unrealistic; therefore, results are less informative when a systematic exercise intervention program is to be designed to target muscle weakness. Numerous studies demonstrate that three sessions per week is optimal for ageing individuals for the development of muscle strength and size [4,23,24]. Though evidence exists that muscle strength can increase in as short as two weeks (six sessions) of resistance training in the older adults [25,26], experiments do not provide clear evidence about the time-course of EIMD and early recovery/adaptation in young versus ageing muscle when the bouts are systematically repeated.

Another important issue in the EIMD studies is that a loss of acute performance was observed in muscle mechanical properties such as dynamic and isometric force, rate of force development and stretch–shortening cycle (SSC) dynamics [27,28,29]. However the magnitude of the deficit and the recovery time, after a single exercise bout, varied. Furthermore the time course of loss of performance, recovery and adaptation were not similar during an 11-day-long exercise program using seven bouts [22]. Ageing-induced impairment of the aforementioned mechanical properties raises problems in the older adults by increasing risks of falls and mobility disability, and impairment of these mechanical properties is thought to be related to sarcopenia [30,31,32]. Thus, we propose that, in the early phase of exercise, the magnitude of adaptations in different mechanical properties are age-dependent because old individuals demonstrated a greater loss in rapid force producing capacity after four days of lower

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limb disuse and an incomplete recovery in all strength properties during the seven-day active period compared to young controls [33].

In the present study, a two-week-long quadriceps exercise intervention consisting six bouts was designed to examine the short-term adaptability of young and old human muscles. Specifically, we tested the hypotheses that: (i) the time-course of changes in EIMD markers (serum protein levels, isometric torque, muscle soreness) and myoelectric activity are different in young versus old subjects, and (ii) the two-week changes in rate of torque development (RTD) and SSC function are different in young versus old subjects.

Materials and methods

Subjects

Ten healthy, physically active young men (age: 25.1±4.9 years, height: 176±6.9 cm, weight: 72.4±17.6 kg) and older adults men (age: 64.5±5.5 years, height: 176.2±8.8 cm, weight: 80.3±10 kg) participated in the study. The older adults group was a group of acquaintances of the university, whereas the young subjects were students. Subjects underwent a medical screening and completed training and a health status questionnaire before the beginning of the study. Major exclusion criteria were as follows: current knee injuries, previous hip surgery, and existing muscle pain, endocrine disorders such as diabetes, cardiovascular diseases, and pelvic inflammatory disease. The subjects were advised to avoid any vigorous physical activity or unaccustomed exercises, to maintain their normal dietary and sleep habits, and not to take any anti-inflammatory drugs (e.g. non-steroidal anti-inflammatory agent) or nutritional supplements (e.g. vitamins, protein/amino acids) during the experimental period. One week before the first day of the investigation, the subjects reported to the laboratory for a familiarisation session during which they were acquainted with the testing equipment. Subjects gave written informed consent according to the Declaration of Helsinki after receiving both a verbal and a written explanation of the experimental protocol and its potential risks. The present study was approved by the University of Pecs Ethical Committee. (file number: 4817).

Design and procedures

Subjects performed six eccentric-concentric exercise bouts over two weeks (Table 1). There were five test sessions: before bout 1, and at 24 h, 48 h, one week and two weeks after bout 1 (Table 1). In these sessions, we assessed maximal voluntary isometric (MVC) torque and the myoelectric (EMG) activity of the quadriceps femoris muscle, and we also measured the levels of different serum markers. All exercises and tests were performed in the morning (between 9:00 and 12:00). Blood samples were always taken before exercise or test sessions. In test sessions 1 and 5, we also determined the RTD and SSC function.

Mechanical muscle properties

Multicont II dynamometer (Mediagnost, Budapest and Mechatronic Ltd., Szeged, Hungary) was used for testing the mechanical properties of the quadriceps muscle. Subjects were seated on the padded seat of the dynamometer and performed three maximal voluntary isometric contractions (MVCs) at 70° of knee flexion (0° = full extension). Subjects were instructed to generate the highest possible torque as quickly as possible. Peak torque and RTD were determined offline from the torque–time curves. RTD was quantified as RTD (Nm/ms) = dM (Nm) / dt (ms), where M is the torque and t is the time in milliseconds. RTD was determined for the first 30-, 50-, 100-, and 200-ms intervals from the onset of the contraction. The greatest

	Day 1 (Test 1)	Day 2 (Test 2)	Day 3 (Test 3)	Day 4	Day 5	Day 6	Day 7	
Serum markers	*	*	*					
Isometric MVC	*	*	*					
SSC function	*							
Muscle soreness	*	*	*					
Exercise bout	*		*		*			
	Day 8 (Test 4)	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15 (Test 5)
Serum markers	*							*
Isometric MVC	*							*
SSC function								*
Muscle soreness	*							*
Exercise bout	*		*		*			

Table 1. Exercise bouts, test sessions, and tests/measurements performed during the experiment.

Data obtained from test session 1 were considered as baseline data.

MVC = maximal voluntary contraction

SSC = stretch-shortening cycle

values obtained from these measurement intervals were averaged for every subject and were used for statistical analysis.

MVC torque was also measured at a 30° knee angle to determine the trigger threshold for initiating the SSC test contraction. To determine SSC function, subjects performed a quadriceps SSC contraction during which the dynamometer rapidly applied a preset amount of energy to stretch the quadriceps [22]. The eccentric phase of the contraction started at 30° of knee flexion and the subject had to exert force against the lever arm as fast and forcefully as possible. When the subject reached 60% of isometric MVC torque measured previously at 30° of knee flexion, the lever arm of the dynamometer started to rotate in the direction of knee flexion. Subjects were instructed to resist the rotating lever arm maximally, stop it within the shortest range of motion (eccentric phase), and then extend the knee without a time delay and as quickly as possible to 30°. The initial velocity of the lever arm was 300° · s⁻¹ and the preset amount of stretch-load (expressed in Joules) was half of the baseline isometric MVC torque. The applied stretch-load represents the amount of work the lever arm of the dynamometer performed on the shank to flex the knee joint. As the eccentric knee flexion progresses, the energy stored in the servomotor diminishes to zero (the lever arm stops) and some of the energy is stored in the quadriceps muscle. The instructions given to the subjects ensured that the transfer of energy that stretched the quadriceps muscle occurred in a short time and over a small range of motion so that the concentric contraction (i.e., knee extension) would start without delay. During the concentric phase, the dynamometer motor was automatically turned off and provided resistance through friction and the inertia of the lever arm and lower leg (S1 Video). Torque and knee angle as a function of time were recorded for each contraction, and similar to Kyröläinen et al. [34], we calculated the negative (Wssc⁻) and positive mechanical work (Wssc⁺) during the SSC by integrating the torque–position curve between the boundaries of the range of motion:

$$W(J) = \int\limits_{ heta_1}^{ heta_n} M_{(heta)} \cdot d heta$$

where M = torque, $d\theta = \text{angular displacement}$, and $\theta 1$ and θn represent the first and the last knee angle data points, respectively. Total time to complete the eccentric (Tssc⁻) phase, the concentric phase (Tssc⁺), and the entire SSC contraction (Tssc), were also determined.

To examine the ability to store and re-use elastic energy, a pure concentric contraction was performed after the SSC test. For each subject, this contraction started exactly at the knee angle where the dynamometer lever was stopped during the SSC test (transition phase) and ended in the 30° position. Subjects fully relaxed their quadriceps and then performed maximal effort knee extension during which, similar to the concentric phase of the SSC described above, the dynamometer motor was turned off, and provided resistance through friction. For this contraction, mechanical work (Wcon) was calculated as in the equation above. To investigate the ability to store and re-use elastic energy, positive SSC work and the pure concentric work ratio (Wssc⁺/Wcon) were determined.

Serum markers

Ten-milliliter blood samples were collected from an antecubital vein by a standard vein puncture technique using disposable needles, and were placed in Vacutainer plain tubes (Becton-Dickinson). After clotting, the blood sample was centrifuged at 1500 *g* for 10 min to obtain serum. Serum were stored at -80°C until being analysed for creatine kinase (CK) activity and myoglobin (Mb) concentration. CK activities were determined using a standard routine laboratory test (kinetic optimized UV method, COBAS INTEGRA[®] 400 plus, Roche Diagnostics GmbH, Mannheim, Germany). Using this methodology, the reference range of CK was 0–200 IU·l⁻¹. For Mb determination, an automated chemiluminescence immunoassay was applied (Immulite 1000, Siemens Healthcare Diagnostics GmbH, Marburg, Germany). The upper reference limit at 97.5 percentile for Mb was 70 µg L⁻¹; however, based on the manufacturer's suggestions, the median value was 25 µg L⁻¹.

Muscle soreness

Subjects reported the soreness level sensed during the MVC test contractions. Subjects were asked to mark a point on a visual analogue scale of 50 mm in length, where 0 mm signified "no pain" and 50 mm signified "extremely painful". The length of the line from 0 to the marked point provided a numeric measure of soreness [18].

EMG activity

Surface electromyography (EMG) signals were recorded (1000 Hz sampling rate) from the vastus lateralis (VL) and vastus medialis (VM) muscles (Noraxon, Scottsdale, USA). The skin was carefully prepared by shaving, rubbing, and cleaning with alcohol. Dual Ag/AgCl electrodes (Noraxon, Scottsdale, USA) with a 20-mm inter-electrode distance were placed over the muscle belly, in accordance with SENIAM recommendations (www.seniam.org). The raw EMG signals were rectified, filtered and smoothed using the root mean square (RMS) method with a 200 ms smoothing window. The peak EMG activity of VL and VM were averaged and used for data analysis.

Dynamometric exercise

The exercise bouts consisted of maximal effort knee extensions performed on the dynamometer as described previously. Subjects trained using the right limb only. Each bout started with a warm-up of five minutes of cycling on a cycle ergometer, followed by stretching the knee extensor muscles. After warming up, subjects performed 4 sets of 15 repetitions eccentricconcentric contractions at $60^{\circ} \cdot s^{-1}$ constant angular velocity over 60° of range of motion, between 20° and 80° of knee joint position. Subjects were encouraged to maximally resist the dynamometer's rotating lever arm during the eccentric phase and then to extend the knee forcefully during the concentric phase. A one-second rest was provided between repetitions and a two-minute rest was provided between sets. Peak torques achieved during the exercise contractions were averaged for every subject in every bout. Verbal encouragement was given to subjects during the exercise.

Statistical analysis

Descriptive statistics (mean values and standard deviations) were computed for the measured and calculated variables. All variables were checked for normality. Between-group differences in the baseline values were determined using independent t-tests (all mechanical variables, EMG activity) and Mann-Whitney U tests (CK, Mb). Exercise-induced changes in all mechanical variables and EMG activity were analysed using a two-way (group by time) mixed model analysis of variance (ANOVA). In case of significant interaction or time main effects, the Bonferroni correction was used for post-hoc analysis to perform pairwise comparisons. We determined the training effects across time in dependent variables such as Mb and CK using a nonparametric Friedman ANOVA. To test differences in these variables, the Wilcoxon matched pairs test was used for post hoc analysis. Because muscle soreness was measured on the ordinal scale, differences were determined using the nonparametric Mann-Whitney U test. The significance level was set at p<0.05.

Results

Barring the Wssc⁺/Wcon ratio, the baseline values for the mechanical variables and EMG activity were significantly greater in the young group (p<0.05). There was no between-group difference in baseline CK activity and Mb level.

A significant between-group difference was found in the exercise torques achieved during the six bouts (young = 224 ± 37 Nm; old = 177 ± 37 Nm; p = 0.001).

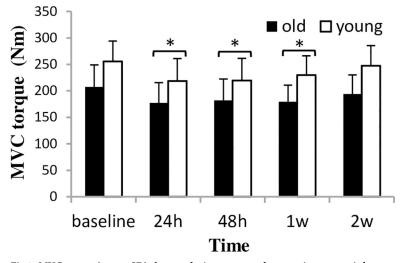
A significant time main effect was found for MVC torque ($F_{4.15} = 20.03 \text{ p} = 0.000$), without group by time interaction. MVC torque decreased uniformly in the two groups 24 h after the first bout and returned to baseline after two weeks (Fig 1).

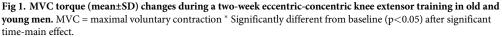
A significant time main effect ($F_{4.15} = 13.33$, p = 0.000) and group by time interaction ($F_{4.15} = 3.60$, p = 0.030) was found for RTD (Fig 2). RTD was reduced only in the young group 24 h after bout 1 (p = 0.03), and tended to increase at the last test session (p = 0.090). Despite the significant time main effect, no change in RTD was observed for the old group during the experiment.

There was no significant time effect in EMG activity; however, the significant group by time interaction ($F_{4.15} = 2.58$, p = 0.047) suggests that the two groups responded differently (Fig 3). The post-hoc analyses revealed that EMG activity in the old group decreased at 24 h (p = 0.021) and only recovered by the end of the experiment. In contrast, EMG activity in the young group tended to increase from baseline to 24 h after bout 1 (p = 0.089); however, this elevation remained non-significant also at later measurement times.

Muscle soreness peaked at 24 h after the first bout in both groups (significantly different from baseline, p = 0.000), and with further bouts, it gradually decreased and returned to the baseline level at the last test session (Fig 4). We found no between-group difference in muscle soreness at any of the measurement times.

CK activity peaked at 24 h after bout 1 (p = 0.001) and then gradually returned to the baseline level by the last test session in both groups (Fig 5). Between-group difference in CK

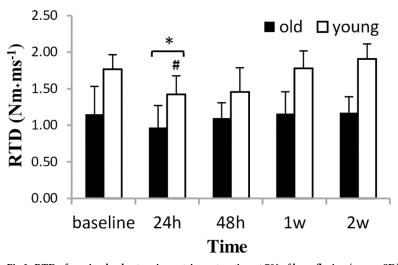


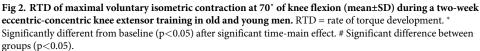


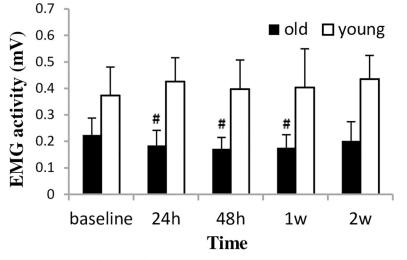
activity was found only at 1 week after bout 1. Mb level was unchanged in the young group throughout the experiment (Fig 6). However, in the old group, Mb level increased significantly and peaked at 24 h after bout 1 (p = 0.001), and in the last test session it was still elevated (p = 0.039).

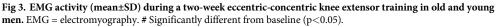
Table 2 shows the within-group percent changes in MVC torque, RTD, muscle soreness, CK, and Mb from baseline to 24 h, 48 h, week 1, and week 2. Between-group comparisons (using old men's values expressed as percent of young) are also shown for 24h, 48h, week 1 and week 2 test periods.

<u>Table 3</u> shows the two-week changes in SSC function. The only significant group by time interaction was found for the Wssc⁺/Wcon ratio ($F_{1.18} = 3.20$, p = 0.041), and the post-hoc test









revealed that by the last test session it increased significantly in the young group (p = 0.026) and remained unchanged in the old group. A significant time main effect was found for Tssc⁺ ($F_{1.18} = 0.73$, p = 0.039), which reduced uniformly in the two groups by the last test session. All other SSC properties were unchanged.

Discussion

Though the time course of change and isometric MVC torque shows a similar pattern, elastic energy storage and re-use improved selectively in the young group at the end of the two-week-long exercise intervention. Furthermore, we demonstrated that old men respond with lowered EMG activity, elevated Mb level, and without RTD deficit in the early phase (24 h after bout 1) of the exercise, compared with the young.

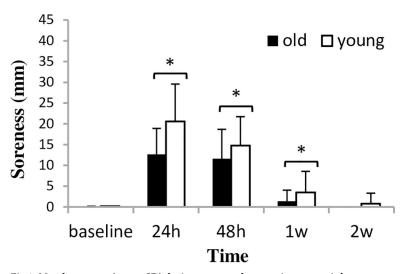
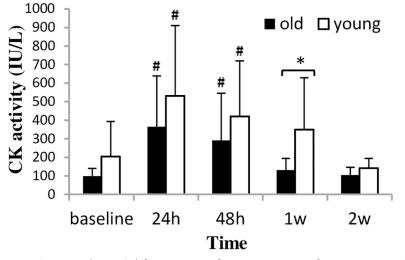
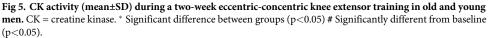


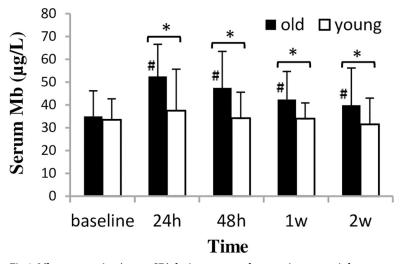
Fig 4. Muscle soreness (mean \pm SD) during a two-week eccentric-concentric knee extensor training in old and young men. * Significantly different from baseline (p<0.05) after significant time-main effect.

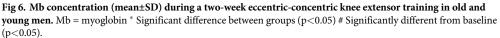




At baseline, the young men demonstrated 40% greater EMG activity, isometric torque, rate of torque development, and SSC function, while the Wssc⁺/Wcon ratio was similar in the young and old subjects. Age-associated losses in strength, not caused by neurological deficits or muscle disease, were seen as a change in isometric torque and EMG in the old group as reported by others [35,36,37]. The Wssc⁺/Wcon ratio represents the ability to store and re-use elastic energy during SSC and it is agreed that this property is less affected by ageing [38].

A single bout of eccentric-biased exercise induces myofibrillar ruptures, elevations of muscle proteins in serum, reduced voluntary force, and delayed onset muscle soreness on the subsequent day [18,39,40,41,42,43]. At 24 h after bout 1, our subjects demonstrated 15% decline in isometric MVC torque, muscle soreness developed, and CK activity was three-fold higher than at baseline and was consistent with previously reported data for young and old humans





		24h			48h	1w		2w		
		old	young	old	young	old	young	old	young	
MVC torque	within	-15	-14	-12	-14	-10	-11	-3	-3	
	between	77		80		73			73	
RTD	within	-16	-20	-4	-18	1	1	2	8	
	between	54			68	49		38		
SOR	within	632	970	480	735	71	135	0	40	
	between	57		78		75		60		
СК	within	265	161	191	106	32	72	1	-31	
	between	54			56	140		66		
Mb	within	51	11	36	2	21	2	14	-6	
	between	138			137		123	124		

Table 2. Percent change from baseline to 24h, 48h, 1w and 2w within each group, and between group comparisons (using old men's values expressed as percent of young) at 24h, 48h, 1w and 2w.

MVC = maximal isometric voluntary torque

RTD = rate of torque development

SOR = muscle soreness level sensed during the MVC test contractions

CK = creatine kinase activity

Mb = myoglobin concentration

in similar protocols in which antigravitational muscles were exercised [7,11]. The impaired contractility is associated with failures in excitation-contraction coupling, eventually leading to loss of voluntary force. CK enzyme efflux from muscle tissue to serum is a normal response as the membrane ruptures or its permeability changes after vigorous exercise [44]. The aforementioned EIMD markers in our studies did not differentiate the acute responsiveness of young and old subjects, which is in agreement with the results of previous studies using the same markers [7]. However, the group by time interaction in EMG activity, Mb, and RTD suggests that the two groups responded differently to the first exercise bout. EMG activity reduced by 18% in the old group, while a non-significant trend to increase was seen in the young group

Variables	Old					Yo	ung	2-week % change and time main effect		Interaction	
	Baseline		2 weeks		Baseline		2 weeks				
W _{ssc+} (J)	49.26	±12.62	52.34	±11.75	74.01	±14.69	80.75	±18.6	8%	p = 0.033	p = 0.402
$W_{ssc-}(J)$	85.24	±17.17	81.31	±14.71	109.89	±16.78	107.32	±17.17	-4.3%	p = 0.130	p = 0.745
W _{ssc+} /W _{con}	1.12	±0.32	1.02	±0.11	1.11	±0.19	1.19	±0.21	-0.9%	p = 0.850	p = 0.041
T _{ssc-} (ms)	231	±39	234	±64	234	±32	237	±32	1.2%	p = 0.758	p = 0.990
T _{ssc+} (ms)	290	±134	258	±107	207	±46	198	±60	-8.2%	p = 0.039	p = 0.644
T _{ssc} (ms)	521	±141	492	±156	441	±60	435	±85	-3.7%	p = 0.548	p = 0.684

Table 3. Effects of two-week eccentric—concentric quadriceps exercise training on SSC function.

Mean values (\pm SD) for the individual groups and percent change from baseline to two weeks for the two groups combined (time main effect) are presented. SSC = stretch-shortening cycle

W_{ssc+} = mechanical work in the positive (concentric) phase of the SSC test contraction

W_{ssc-} = mechanical work in the negative (eccentric) phase of the SSC test contraction

 W_{ssc+}/W_{con} = ratio of the positive work in the SSC test contraction and the work in the pure concentric test contraction

T_{ssc-} = time to complete the negative (eccentric) phase in the SSC test contraction

 $\rm T_{\rm ssc+}$ = time to complete the positive (concentric) phase in the SSC test contraction

 $T_{\rm ssc}$ = time to complete the entire SSC test contraction

24 hours after bout 1. The acute gain in EMG activity is often explained with the compensation mechanism: when some fibres are irresponsive, increased motor unit synchronicity could be a compensation strategy to maintain force in the damaged muscle [45]. Muscle soreness is often suggested to inhibit central drive; however, this was not the case in our study because there was no difference in soreness between groups [46]. Perhaps the increase in EMG activity in the young group is due to a lack of full motor unit pool recruitment that improves following exercise (repeated test effect). Furthermore, we assume that in the old people the sarcolemma damage was more. Damage to the sarcolemma reduces the efficiency of the electrical conductivity and the strength of the contraction [47].

Twenty-four hours after bout 1, RTD was reduced only in the young group. RTD, which represents contractile speed, at least in part, relies on the activation of fast motor units. Because both fast motor unit content and their recruitment is reduced in the ageing muscle [48,49], we suggest that in the young subjects, the exercises used in our experiment probably damaged more fast motor units than in the old subjects. Furthermore, the power reduction was more than two fold lower in the older adults immediately after a bout of eccentric contractions, compared to young subjects [50], maybe due to the selective fatigue of fast motor units. Therefore, some fast motor units most likely became overloaded in our old subjects during the exercise because of fatigue or because they were not recruited at all preventing significant damage to the contractile units.

It is interesting in the present study that although CK responses were similar in the two experimental groups, Mb levels were elevated in the old group 24 hours after bout one. This result would occur if an aging-induced shift in muscle fiber type was present. Old humans demonstrate larger ratio of type I fibers [49], compared with young. Type I fibers contain more myoglobin than type II, however, type II fibers are more susceptible to injury. As a result, during microdamage, more myoglobin is released in the old people, however, CK activity and soreness tend to be greater in the young. On the other hand, Mb level is often considered a marker of sarcolemma vulnerability [49], therefore more sarcolemma can be ruptured after eccentric exercise in old people.

Finally, a potential factor for Mb release might be the transient decrease of energy (ATP) of the skeletal muscle cells during strenuous physical exercise. During muscle contractions, the initially low ATP turnover rate might increase 100-fold to maintain cellular ATP at constant level [51]. However, in the old group, the energy status of the muscle cells might not recover within 48 h, which shows the weaker metabolic adaptation capacity of the old subjects.

From 48 h after bout 1, the most-often-measured markers, isometric torque, CK, and soreness recovered uniformly in the two groups, which is in agreement with previous data found in young subjects showing weaker symptoms when exercise is continued [52]. A parallel change in isometric torque and EMG was observed in the old group, suggesting that, after the initial reduction, recovery of maximal force was mediated by gradually increasing neural drive. In contrast, the isometric torque recovery was accompanied with unchanged EMG activity in the young group, but it is important to note that EMG tended to be greater in test sessions 2 to 5 than the baseline. Therefore, in the early phase of a high force resistance exercise, young men either maintain normal neural drive or tend to show early favorable adaptation. In contrast, this mechanism is delayed in the old subjects, and adaptation may be expected beyond two weeks. Also, the slower EMG recovery in the old men can be explained by higher sarcolemmal damage, supported by the marked Mb elevation in the old subjects.

Despite the initial deficit, the young subjects recovered quickly in RTD and tended to improve by the end of the two-week protocol. In contrast, old subjects demonstrated no change in RTD during the entire period. Similarly, no adaptation in RTD was observed for the old subjects after 10 weeks of slow velocity eccentric exercise showing no adaptation in rapid contractility [4]. This lack of responsiveness in aging men can again be explained by the low number of fast motor units and/or their delayed recruitment with additional exercise bouts.

A novel observation in the present study is that after bout 1, Mb level remained elevated in the old group, despite the return of CK to normal levels. In contrast, Mb level was unchanged in the young group. In our opinion, persistent elevated Mb concentration might be an indicator of the extent of adaptability of skeletal muscle to physical exercise.

The data discussed above should be interpreted with caution. Studies of the repeated bout effect usually measured effects at 24 hours after bout 1 and bout 2 with the recovery of a few days or weeks between exercise bouts [18,40,52,53]. Instead of demonstrating the repeated bout effect, our aim was to measure the damage markers and EMG activity on days when an actual bout was to be performed (72 h after bout 3 and bout 6). Therefore, it is difficult to compare our results with those obtained from previous repeated bout effect studies because of the different measurement times. Instead, we evaluated the pre-bout physiologic status of our subjects rather than the acute post-bout responses during a realistic multi-bout exercise program.

Maintaining SSC function is important in the older adults because it is associated with muscle mechanical efficiency, movement economy, and fatigability [54]. In our study, the twoweek adaptation to eccentric concentric exercise in SSC function was age-dependent and was specific to the SSC property measured.

The significant time main effect in positive work and the lack of significance in negative work suggest that concentric contractility improved selectively in both old and young subjects. Furthermore, the decrease in time to complete the concentric phase of the SSC would result in an increase in positive work observed.

Not surprisingly, the gain in positive work resulted from using maximal effort concentric muscle actions and, therefore, was not due to task specific effects. In addition, the concentric action in the exercise started immediately after the eccentric action, when the highest tension usually develops. Therefore, subjects initiated the concentric phase with maximally activated quadriceps, enhancing quick adaptation in concentric work. However, negative work was unchanged in both groups. Since our exercise protocols contain both type of contractions, the lack of improvement in an eccentric contractility was surprising. Generally, strength adaptation to eccentric exercise is more pronounced than strength gains due to concentric exercise as reported for long-term interventions [55,56,57]. It is possible that pain, which is more pronounced during eccentric action, prevented subjects from fully activating the quadriceps during the eccentric phase, resulting in insufficient stimulus for adaptation in eccentric work. Despite the fact that concentric contractility improved regardless of age, enhanced elastic energy storage and re-use (indicated by increased Wssc⁺/Wcon ratio) was shown only in young men at the end of the two-week protocol. The group by time interaction in the Wssc⁺/ Wcon ratio can be explained by the fact that the older adults demonstrated high variability in this muscle property, which prevented the detection of significant improvements. Still, the adaptation in some of our old subjects was notable.

It is important to note that SSC function improved along with no change in isometric torque during the two-week experiment. It seems that the transfer effect of the present exercise was more favourable to SSC function versus isometric contractility, probably because of its dynamic nature. Adaptation in isometric versus dynamic contractility showed small sensitivity (6% vs. 23% change) in old humans even after 10 weeks of dynamic exercise [4], supporting our finding. In addition, short-term isometric torque adaptation in young humans was seen only after 3 days tapering from a vigorous exercise program (7 quadriceps exercise bouts within 8 days). Finally, elastic energy storage and re-use relies (in part) on the series elastic muscle components, which remain intact in the presence of myofibrillar damage [28], allowing faster adaptation in this mechanical property. An important limitation in the present protocol was that SSC properties were measured only at test 1 (baseline) and test 5 (two weeks after bout 1); therefore, we have no information on how SSC function declines acutely and recovers in old and young subjects. However, in other studies, subjects have adapted quickly to SSC tests even when no additional exercise was performed [22]. Therefore, we omitted the SSC tests from test 2, 3, and 4.

Another limitation was that functional tests were not used to measure subjects' mobility and functionality. However, the EIMD symptoms were small and the low sensitivity of functional tests would have probably prevented us from detecting changes in two weeks. Finally, delayed responses in common central and peripheral fatigue markers (voluntary activation, muscle contractility and membrane excitability, cortico-spinal excitability, and reflex response) using twitch interpolation and transcranial magnetic stimulation techniques should also be measured in order to reveal neuro-mechanical mechanisms responsible for the 'secondary strength deficit' and its recovery. Also, we were unable to measure motor unit firing frequency, which may change with ageing.

In summary, a two-week long high-intensity quadriceps exercise induced a similar time course of changes in isometric torque, CK activity and muscle soreness in young and old men. In contrast, the recovery was mediated by different myoelectric changes. In contrast with the young, RTD remained unchanged in the old subjects, probably because of the smaller amount of larger motor units and/or their de-recruitment. Finally, persistent elevations in Mb levels suggest higher sarcolemma vulnerability and less effective metabolic adaptation in the older adults, which, however, did not affect muscle contractility. Therefore, the design of exercise interventions to avoid overuse and loss of motivation and interest in older adults should be considered by gerontologists and strength specialists.

Supporting information

S1 Video. The stretch-shortening cycle (SSC) test using Multicont II dynamometer. (AVI)

Author Contributions

Conceptualization: Zoltán Heckel, Márk Váczi. Data curation: Zoltán Heckel, József Laczkó, Márk Váczi. Formal analysis: Zoltán Heckel, Márk Váczi. Funding acquisition: Márk Váczi. Investigation: Zoltán Heckel, Tamás Atlasz, Éva Tékus, Tamás Kőszegi, Márk Váczi. Methodology: Márk Váczi. Project administration: Zoltán Heckel, Tamás Atlasz, Éva Tékus, Tamás Kőszegi. Resources: Márk Váczi. Software: Zoltán Heckel, József Laczkó. Supervision: Márk Váczi. Validation: Tamás Kőszegi, József Laczkó. Visualization: Zoltán Heckel. Writing - review & editing: Tamás Atlasz, Éva Tékus, Tamás Kőszegi.

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