PHYSICAL EDUCATION AND NUTRITION FOR LIFELONG FITNESS AND HEALTH

HAARIKRISHNNA KUMAR

Physical Education and Nutrition for Lifelong Fitness and Health

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Haarikrishnna Kumar

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1

Cardiometabolic Health in Relation to Lifestyle and Body Weight Changes 3–8 Years Earlier

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Abstract: The degree to which individuals change their lifestyle in response to interventions differs and this variation could affect cardiometabolic health. We examined if changes in dietary intake, physical activity and weight of obese infertile women during the first six months of the LIFEstyle trial were associated with cardiometabolic health 3–8 years later (N = 50–78). Lifestyle was assessed using questionnaires and weight was measured at baseline, 3 and 6 months after randomization. BMI, blood pressure, body composition, pulse wave velocity, glycemic parameters and lipid profile were assessed 3–8 years after randomization. Decreases in savory and sweet snack intake were associated with lower HOMA-IR 3–8 years later, but these associations disappeared after adjustment for current lifestyle. No other associations between changes in lifestyle or body weight during the first six months after randomization with cardiovascular health 3–8 years later were observed. In conclusion, reductions in snack intake were associated with reduced insulin resistance 3–8 years later, but adjustment for current lifestyle reduced these associations. This indicates that changing lifestyle is an important first step, but maintaining this change is needed for improving cardiometabolic health in the long-term.

Keywords: dietary intake; physical activity; body weight; lifestyle change; cardiometabolic health; long-term follow-up

1. Introduction

Unhealthy diet, physical inactivity and a BMI over 25 kg/m^2 are well known risk factors for cardiovascular diseases (CVDs). It is therefore that primary prevention of CVDs focuses, among others,

on improving these lifestyle factors and reducing body weight [1]. Observational studies showed graded relationships between healthy changes in dietary intake, physical activity and body weight over time and future cardiometabolic health. For example, improvements in diet [2], or transition to a more active lifestyle [3] lowered risk of coronary heart disease among women in the Nurses' Health Study.

Randomized controlled trials (RCTs) showed that improving lifestyle and reducing body weight improved cardiometabolic health [4,5]. However, although lifestyle interventions can improve cardiometabolic health, the effectiveness of lifestyle interventions varies [6,7]. For example, a meta-analysis including 54 RCTs to determine the effect of aerobic exercise on blood pressure showed that net change in blood pressure after following a physical activity intervention varied from −16.7 to 3.9 mmHg for systolic blood pressure and from −11.0 to 11.3 mmHg for diastolic blood pressure [8]. Despite the large variation in lifestyle and body weight change, the effects of a lifestyle intervention are often described as a randomized group comparison. By studying the effect of absolute change in lifestyle and body weight, more knowledge can be gathered on dose-response relationships and potential thresholds between lifestyle and body weight change with later life cardiometabolic health. This information will aid in improving primary prevention.

The LIFEstyle RCT enrolled obese infertile women, and allocated them to a six-month diet and physical activity intervention or to prompt infertility treatment [9–11]. We previously showed that the intervention lowered the intake of high caloric snacks and beverages and increased physical activity in the short term [12], reduced body weight [11] and improved cardiometabolic health at the end of the six-month intervention period [13]. Furthermore, women allocated to the intervention group reported a lower energy intake at 5.5 years after randomization [14]. Individual responses to the lifestyle intervention varied largely among study participants. The mean weight change in the intervention group was −4.4 kg and the standard deviation of 5.8 kg underlines this large variation [11]. Also women allocated to the control group changed their lifestyle: 10.5% of the women in the control group lost 5% or more of their original body weight [11]. Hence we here investigate individual changes in lifestyle and body weight and relate these changes in dietary intake, physical activity and weight during the first six months after randomization to cardiometabolic health 3–8 years later.

We hypothesized that women who increased their intake of vegetables and fruit, decreased their intake of sugary drinks and snacks, became more physically active and lost more weight during the first six months after randomization had a better cardiometabolic health 3–8 years after randomization compared to women who did not show these improvements in lifestyle and weight. We therefore examined if the change in dietary intake, physical activity and body weight of obese infertile women, combining the intervention and control group, over the first six months of a preconception lifestyle intervention study was associated with their cardiometabolic health 3–8 years after the start of the study.

2. Materials and Methods

2.1. Study Population

The study population comprises all women who participated in the follow-up of the LIFEstyle study. The LIFEstyle study was a multicenter randomized controlled trial (RCT), conducted between 2009 and 2014 in the Netherlands [9–11]. In total, 577 women between 18 and 39 years old, with a BMI of $\geq$29 kg/m^2 were randomized into a six-month structured lifestyle intervention (intervention group) or infertility care as usual (control group). The lifestyle intervention focused on eating a healthy diet according to the Dutch Dietary Guidelines 2006 [15], including a caloric reduction of 600 kcal/day but not below 1200 kcal/day, and being physically active 2–3 times a week for at least 30 min at moderate intensity (60–85% of maximum heart rate frequency). Women were additionally advised to increase physical activity in daily life by taking at least 10,000 steps per day.

At 3–8 years after randomization, 574 women were eligible to participate in the follow-up study of the LIFEstyle RCT [16]. During the follow-up study, data were collected using questionnaires and physical examinations. In the current study, we included all women that participated in the physical examinations (N = 111; Figure 1).

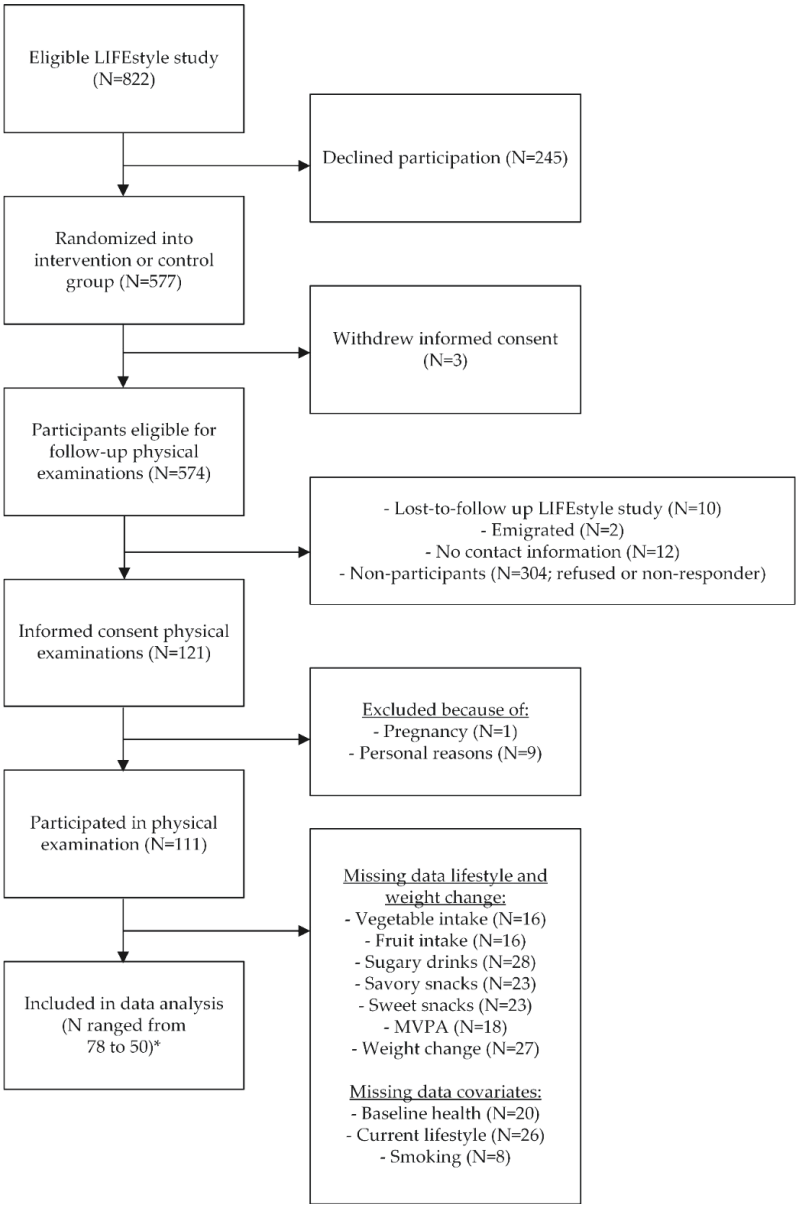

Figure 1. Flow-chart of the study participants. MVPA = Moderate to Vigorous Physical Activity * N depends on missing data in the combined models including independent variables and covariates.

The study was conducted in accordance with the Declaration of Helsinki and all procedures were approved by the Medical Ethics Committee of the University Medical Centre Groningen, the Netherlands (METc 2008/284). Written informed consent was obtained from all participants at the start of the LIFEstyle study as well as the start of the follow-up.

2.2. Assessment of Dietary Intake, Physical Activity and Body Weight to Calculate Change

In order to calculate change in lifestyle and body weight over the first six months of the study, we used dietary intake, physical activity and body weight measures collected at baseline, 3 and 6 months after randomization in the lifestyle intervention study. Dietary intake was examined using a 33-item food frequency questionnaire (FFQ) consisting of two parts. The first part was based on the standardized questionnaire on food consumption used for the Public Health Monitor in the Netherlands [17], asking about the type of cooking fats used, the consumed type of bread, frequency of breakfast use, frequency of consumption and portion size of vegetables, fruits and fruit juice. The questions on the intake of fruit, fruit juice and cooked vegetables were validated against two 24-h recalls. The estimated intake of fruit and fruit juice consumption based on the questionnaire showed fairly strong comparability with the intake based on the two 24-h recalls, however the comparability for cooked vegetables was weak [17]. The second part consisted of additional questions about savory and sweet snack intake and the intake of soda. For all foods, frequency of consumption per week or per months was asked and portion size was asked per standard household measure. The presented portion sizes and food groups in the current study were pre-specified in the questions of the FFQ. Dietary intake was studied as the intake of vegetables (raw as well as cooked; grams/day), fruits (grams/day), sugar containing beverages (fruit juice and soda; glasses/day), savory snacks (crisps, pretzels, nuts and peanuts; handful/week) and sweet snacks (biscuits, pieces of chocolate, candies or liquorices; portion/week). One portion of sweet snacks included 2 biscuits, or 2 pieces of chocolate, or 5 candies, or 5 pieces of liquorice.

Physical activity was examined using the validated Short QUestionnaire to ASsess Health-enhancing physical activity (SQUASH) questionnaire [18]. This questionnaire asked about the number of days per week, the average time per day or week (hours and/or minutes), and the intensity (low, moderate, high) of physical activity in four domains: commuting activities, leisure time activities, household activities, and activities at work and school. Physical activity was studied as total moderate to vigorous physical activity (MVPA; hours/week).

Body weight (kg) was measured during hospital visits at baseline, 3 months and 6 months after randomization by trained research nurses that were not involved in the lifestyle intervention coaching.

2.3. Cardiometabolic Health at Follow-Up

Cardiometabolic health 3–8 years after randomization was examined in a mobile research vehicle by two researchers, using a standardized research protocol. Women were asked not to eat or drink from 90 min onwards before the mobile research vehicle arrived at their home. They were additionally asked not to drink caffeine containing beverages or to smoke from 12 h onwards before the physical examination. Height and current weight were measured to calculate current BMI. Height was measured to the nearest 0.1 cm using a wall stadiometer (SECA 206; SECA, Germany) on bare feet, with heels flat on the ground at an angle of 90 degrees, head in Frankfort horizontal position and heels, back and shoulders straight against the wall. Current weight was measured in underwear to the nearest 0.1 kg using a digital weighting scale (SECA 877; SECA, Germany), while the participant was standing still and looking straight ahead. All measurements were done twice, and in case of >0.5 cm difference in height and >0.5 kg difference in weight, a third measurement was performed. After sitting quietly for 5 min, blood pressure was measured three times at heart level, at the non-dominant arm, using an automatic measurement device (Omron HBP-1300; OMRON Healthcare, The Netherlands) with appropriate cuff size. The three measurements were done using a 30 s time interval and women were not allowed to talk in between, move, cross their legs or tense their arm muscles. Body composition

was measured twice by bio-electrical impedance (BIA; Bodystat 1500; Bodystat Ltd., Isle of Man, UK) after lying quietly for 5 min. On beforehand participants were asked to take of any jewelry, belts, piercings, etc. which could affect the BIA measurements. After cleaning the skin with alcohol, electrode strips were attached at the dorsal side of the left hand and foot with at least 3–5 cm in between the two electrodes. Women were instructed not to talk in between the measurements and attention was paid that arms and legs did not touch other parts of the body. A third measurement was performed in case the impedance or resistance differed >5Ω. Fat mass and fat free mass were calculated using equation of Kyle and colleagues [19]. Immediately after the BIA measurement, still in supine position, carotid-femoral pulse wave velocity (PWV) was measured twice using the Complior Analyse (Complior; Alam Medical, Saint-Quentin-Fallavier, France). Mechanotransducer censors were placed at the carotid artery on the right side and on the femoral artery on the left side. Blood pressure in lying position was measured once before the actual measurement started and entered in the Complior software. Directly after the measurement, distance between both censors was measured and also entered in the Complior software. In case of >10% difference in PWV between both measurements, a third measurement was done. The following equation was used to calculate PWV: PWV = 0.8 × (distance between the carotis and fermoralis measuring site/Δ time between upstrokes of pressure waves) [20].

Apart from the physical examinations, a trained nurse visited the participants at home to draw a venous blood sample after an overnight fast. All venous blood samples were analyzed at the biochemical laboratory of the Amsterdam UMC. We examined metabolic health by fasting serum concentrations of glucose (Roche cobas 8000, c702; Roche Diagnostics, Rotkreuz, Switzerland) and insulin (Centaur XP; Siemens, Munich, Germany), triglycerides (Roche cobas 8000, c702; Roche Diagnostics, Switzerland), total cholesterol (Roche cobas 8000, c502; Roche Diagnostics, Switzerland) and high density (HDL-C) lipoprotein cholesterol (Roche cobas 8000, c702; Roche Diagnostics, Switzerland). Low density (LDL-C) lipoprotein cholesterol was calculated using the following formula; (total cholesterol) − (high density lipoprotein cholesterol) − 0.45 × (triglycerides). Furthermore, insulin resistance (HOMA-IR) was calculated as fasting insulin concentration in μU/mL multiplied by fasting glucose concentration in mmol/L divided by 22.5. We additionally examined if metabolic syndrome was present or not. If present, participant met at least three of the following criteria determined by the American Heart Association: glucose ≥ 5.6 mmol/L, HDL-C < 1.3 mmol/L, triglycerides ≥ 1.7 mmol/L, waist circumference ≥ 88 cm or blood pressure ≥ 130/85 mmHg [21].

2.4. Statistical Analysis

We used linear regression models to study the association between the change in dietary intake, physical activity, and weight during the first six months after randomization and cardiometabolic health 3–8 years later. Results are displayed as betas (β) and 95% confidence intervals (C.I.). For metabolic syndrome, logistic regression was used and results are displayed as odds ratios (OR) and 95% C.I. We recalculated the intake of vegetables and fruits into 10 g/day dividing the change by ten. Our regression models therefore display the effect on cardiometabolic health per 10 g change of vegetable intake and fruit intake per day.

For the descriptive statistics the change in lifestyle and body weight over time was calculated by subtracting the baseline measurement from the last know measurement at preferably 6 months or otherwise 3 months after randomization (Table S1). In most women, the last known measurement was at six months after randomization, but when missing, the measurement at three months after randomization was used ($N = 5$). This means that a higher change score for vegetable intake, fruit intake, and MVPA is healthier, while a higher change score for sugary drink intake, savory and sweet snack intake, and weight change (higher means weight gain instead of weight loss) is unhealthier. In our regression models, the change in vegetable intake, fruit intake and MVPA was calculated by subtracting the baseline measurement from the last known measurement. The change in sugary

drinks, savory and sweet snack intake and body weight was calculated by subtracting the last known measurement from the baseline measurement. An increase in change score in our regression models is favorable, reflecting an increase in vegetable intake, fruit intake and MVPA, and a decrease in sugary drink intake, savory and sweet snack intake and body weight.

All crude regression models were corrected for baseline cardiometabolic health, depending on the outcome variable (e.g., BMI at follow-up was corrected for BMI at baseline), with exception of fat mass, fat free mass, and PWV as these outcomes were not measured at baseline. We therefore corrected fat mass and fat free mass models for baseline BMI [22], and PWV models for baseline systolic blood pressure [23]. Based on literature [24] and the associations with both the change variables and the outcome variables, we corrected the adjusted regression models additionally for (1) smoking at follow-up (yes/no; self-reported by means of a questionnaire); (2) current dietary intake, depending on which food group is added into the model; e.g., if we examined the association between the change in vegetable intake during the first six months after randomization and current BMI, we corrected for current vegetable intake, and when examining the effect of MVPA we corrected for total current energy intake (kcal/day). Current dietary intake, i.e., dietary intake during follow-up, was assessed with the same 33-item FFQ as described previously [17]; (3) current MVPA (min/day), i.e., MVPA during follow-up, which was measured for seven consecutive days using the triaxial Actigraph wGT3X-BT or GT3X+ accelerometer [25]. Freedson cut-off points were used to determine the number of minutes per day in MVPA ($\geq$1952 counts/min) [26]. We additionally added randomization group (intervention/control group) and time between randomization and follow-up (years) into the adjusted model to see if this affected the effect estimates. As women got pregnant during the LIFEstyle study, some diet, physical activity and weight measurements were collected during early pregnancy. We therefore once excluded measurements collected during pregnancy to see if effect estimates changed.

Statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS) version 24 for Windows (SPSS, Chicago, IL, USA). p-values <0.05 were considered statistically significant.

3. Results

Of the 577 women randomly allocated to the intervention and control group during the trial, 574 women were eligible to participate in the physical examinations at 3–8 years after the intervention (Figure 1). Of these eligible women, 121 were willing to participate in the follow up study and signed informed consent (21.1%) and we collected data of 111 women. Because of missing data regarding the change in dietary intake, physical activity, weight and the covariates, we were able to include 50 up to 78 women in our regression analyses.

Mean age of the women during physical examination was 36.4 years (SD = 4.3), most of them were Caucasian (94.6%), had an intermediate vocational education (49.1%) and were obese at 3–8 years after the intervention (mean BMI = 35.5 kg/m2 (SD = 5.3); Table 1). Baseline characteristics, collected during the LIFEstyle RCT, did not differ between participants (N = 111) and non-participants (N = 463) of the follow-up study (N = 111), with exception of ethnicity and the change in sweet snack intake (Table S1). Participants were more often of Caucasian origin (94.6%) compared to non-participants (85.7%). Additionally, the change in sweet snacks during the first six months after randomization was lower in the participants ($-$0.1 portions/week (SD = 5.6)) compared to the non-participants ($-$3.3 portions/week (SD = 10.2)).

Table 1. Characteristics and cardiometabolic health of the study population (N = 111).

Age at follow-up (years; mean; SD)	36.4 (4.3)
Caucasian (N; %)	105 (94.6)
Education level (N; %)	
No education or primary school (4–12 years)	1 (0.9)
Secondary education	25 (23.6)
Intermediate Vocational Education	52 (49.1)
Higher Vocational Education or University	28 (26.4)
Body Mass Index at randomization (kg/m^2; mean; SD)	35.7 (3.0)
Current smoker at follow-up (yes; N; %)	16 (15.5)
PCOS (yes; N; %)	43 (38.7)
Nulliparous at follow-up (yes; N; %)	21 (20.8)
Familial predisposition cardiovascular diseases (yes; N; %)	92 (89.3)
Gestational diabetes (yes; N; %) *	18 (17.5)
(Pre-)eclampsia (yes; N; %) *	16 (15.5)
HELLP syndrome (yes; %; N) *	7 (6.8)
Randomization group (intervention group; N; %)	50 (45.0)
Cardiovascular outcomes at follow-up	
Body Mass Index 3–8 years after randomization (kg/m^2; mean; SD)	35.5 (5.3)
Systolic blood pressure (mmHg; mean; SD)	120.4 (14.4)
Diastolic blood pressure (mmHg; mean; SD)	81.7 (9.5)
Fat mass (% of total body weight; mean; SD)	43.1 (4.3)
Fat free mass (kg; mean; SD)	56.9 (6.5)
Pulse Wave Velocity (m/s; mean; SD)	7.2 (2.0)
Metabolic outcomes at follow-up	
Glucose (mmol/L; mean; SD)	5.3 (0.7)
Insulin (pmol/L; mean; SD)	81.7 (53.1)
HOMA-IR (mean; SD)	3.4 (2.8)
Triglycerides (mmol/L; mean; SD)	1.2 (0.8)
Total cholesterol (mmol/L; mean; SD)	4.6 (0.9)
LDL-C (mmol/L; mean; SD)	2.8 (0.8)
HDL-C (mmol/L; mean; SD)	1.3 (0.3)
Metabolic syndrome at follow-up (yes; N; %)	40 (40.0)

PCOS = Polycystic Ovary Syndrome; HELLP = syndrome characterized by hemolysis (H), elevated liver enzymes (EL) and low platelet count (LP); HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; LDL-C = low-density lipoproteins cholesterol; HDL-C = high-density lipoproteins cholesterol. * Diagnosed with these pregnancy complications during any pregnancy in the past.

Table 2 shows the change in dietary intake, physical activity and body weight during the first six months of the LIFEstyle study in our study population.

We did not observe the hypothesized associations between increases in vegetable intake, fruit intake and MVPA during the first six months of the LIFEstyle study with a more favorable BMI, blood pressure, body composition, PWV and metabolic health 3–8 years later (Table 3). Furthermore, decreased sugary drink intake and body weight were not associated with a more favorable cardiovascular health 3–8 years later. A decrease in savory snacks intake during the first six months after randomization was associated with a lower HOMA-IR at follow-up (crude model: −0.16 (−0.32; −0.001); p = 0.049). This association disappeared after adjustment for smoking, current savory snack intake and current MVPA (adjusted model: −0.09 (−0.28; 0.09); p = 0.33). Changes in savory snack intake were not associated with women's BMI, blood pressure, body composition, PWV or other metabolic outcomes at follow-up. Furthermore, a decrease in sweet snacks intake during the first six months after randomization was associated with a lower HOMA-IR at follow-up (crude model: −0.16 (−0.06; −0.06); p = 0.003). Also this association disappeared after adjustment for smoking, current sweet snack intake and current MVPA (adjusted model: 0.01 (−0.09; 0.12); p = 0.84).

Table 2. Change in dietary intake, physical activity and weight during the first six months of the LIFEstyle study in the study population included for follow-up *.

Change in	*N*	Mean (SD)	Median (IQR)
Vegetable intake (grams/day)	95	1.92 (58.70)	3.57 (−28.57; 35.71)
Fruit intake (grams/day)	95	25.56 (75.61)	14.29 (0.00; 85.71)
Sugary drinks (glasses/day)	83	−0.43 (1.81)	0.00 (−0.84; 0.21)
Savory snacks (handful/week)	88	−1.67 (5.57)	0.00 (−4.18; 0.57)
Sweet snacks (portion/week)	88	−0.15 (5.63)	0.00 (−2.61; 1.62)
Total MVPA (hour/week)	93	1.05 (12.1)	0.83 (−3.25; 6.13)
Body weight (kilograms)	84	−2.62 (5.10)	−2.10 (−4.68; 0.68)

MVPA = Moderate to Vigorous Physical Activity. * For all variables the change was calculated as preferably 6 months or otherwise 3 months minus baseline. If a higher score on the change variable is favorable or not depends on the independent variable of interest: A higher change score for vegetable intake, fruit intake, and MVPA is healthier, while a higher change score for sugary drink intake, savory and sweet snack intake, and weight change (higher means weight gain instead of weight loss) is unhealthier.

Table 3. Association between the change in dietary intake and physical activity during the preconception lifestyle intervention and cardiometabolic health 3–8 years after randomization †.

	N	Crude Model *		Adjusted Model **	
		β (95% C.I.)	*p*	β (95% C.I.)	*p*
Change in Vegetable Intake (10 g/day)					
Body Mass Index (kg/m^2)	76	−0.02 (−0.23; 0.19)	0.85	−0.04 (−0.25; 0.17)	0.71
Systolic blood pressure (mmHg)	74	0.50 (−0.07; 1.07)	0.08	0.49 (−0.11; 1.09)	0.11
Diastolic blood pressure (mmHg)	74	0.31 (−0.05; 0.68)	0.09	0.32 (−0.06; 0.70)	0.10
Fat mass (% of total body weight)	76	−0.04 (−0.21; 0.13)	0.64	−0.04 (−0.21; 0.14)	0.68
Fat free mass (kg)	76	0.14 (−0.14; 0.41)	0.33	0.10 (−0.18; 0.38)	0.47
Pulse Wave Velocity (m/s)	60	0.02 (−0.09; 0.14)	0.66	−0.004 (−0.13; 0.12)	0.95
Glucose (mmol/L)	64	−0.01 (−0.05; 0.02)	0.46	−0.01 (−0.05; 0.02)	0.52
Insulin (pmol/L)	63	0.56 (−1.35; 2.47)	0.56	1.03 (−0.94; 3.01)	0.30
HOMA-IR	61	0.001 (−0.11; 0.11)	0.99	0.02 (−0.10; 0.14)	0.72
Triglycerides (mmol/L)	64	−0.02 (−0.04; 0.01)	0.16	−0.01 (−0.03; 0.01)	0.33
Total cholesterol (mmol/L)	64	0.01 (−0.02; 0.05)	0.42	0.02 (−0.01; 0.06)	0.24
LDL-C (mmol/L)	64	0.02 (−0.01; 0.05)	0.15	0.02 (−0.01; 0.05)	0.14
HDL-C (mmol/L)	64	−0.002 (−0.01; 0.01)	0.70	−0.001 (−0.01; 0.01)	0.92
Change in fruit intake (10 g/day)					
Body Mass Index (kg/m^2)	78	0.08 (−0.10; 0.26)	0.37	0.13 (−0.07; 0.32)	0.20
Systolic blood pressure (mmHg)	76	−0.09 (−0.59; 0.41)	0.72	−0.31 (−0.87; 0.25)	0.28
Diastolic blood pressure (mmHg)	76	0.00 (−0.32; 0.33)	>0.99	−0.04 (−0.41; 0.32)	0.81
Fat mass (% of total body weight)	78	0.10 (−0.05; 0.24)	0.19	0.12 (−0.04; 0.28)	0.13
Fat free mass (kg)	78	0.20 (−0.04; 0.44)	0.09	0.25 (−0.01; 0.52)	0.06
Pulse Wave Velocity (m/s)	63	−0.05 (−0.16; 0.05)	0.30	−0.05 (−0.16; 0.07)	0.39
Glucose (mmol/L)	66	0.03 (−0.01; 0.06)	0.12	0.01 (−0.02; 0.05)	0.41
Insulin (pmol/L)	65	0.52 (−1.26; 2.30)	0.56	0.52 (−1.40; 2.44)	0.59
HOMA-IR	63	−0.01 (−0.11; 0.10)	0.89	−0.02 (−0.14; 0.09)	0.72
Triglycerides (mmol/L)	66	−0.004 (−0.03; 0.02)	0.67	0.00 (−0.02; 0.02)	0.97
Total cholesterol (mmol/L)	66	0.03 (−0.002; 0.06)	0.07	0.02 (−0.01; 0.05)	0.27
LDL-C (mmol/L)	66	0.02 (−0.003; 0.05)	0.08	0.02 (−0.01; 0.04)	0.26
HDL-C (mmol/L)	66	0.003 (−0.01; 0.01)	0.57	−0.002 (−0.01; 0.01)	0.73

Table 3. *Cont.*

	N	Crude Model *		Adjusted Model **	
		β (95% C.I.)	*p*	β (95% C.I.)	*p*
Change in sugary drink intake (glass/day)					
Body Mass Index (kg/m^2)	62	0.46 (−0.71; 1.62)	0.44	0.42 (−0.79; 1.62)	0.49
Systolic blood pressure (mmHg)	60	2.04 (−1.55; 5.63)	0.26	2.34 (−1.34; 6.02)	0.21
Diastolic blood pressure (mmHg)	60	1.39 (−0.96; 3.74)	0.24	1.55 (−0.91; 4.00)	0.21
Fat mass (% of total body weight)	62	0.56 (−0.38; 1.50)	0.24	0.54 (−0.46; 1.54)	0.28
Fat free mass (kg)	62	0.58 (−1.15; 2.31)	0.50	0.48 (−1.32; 2.28)	0.60
Pulse Wave Velocity (m/s)	50	0.19 (−0.37; 0.76)	0.50	0.16 (−0.44; 0.75)	0.60
Glucose (mmol/L)	54	−0.03 (−0.27; 0.22)	0.84	−0.04 (−0.29; 0.21)	0.76
Insulin (pmol/L)	53	2.01 (−8.17; 12.19)	0.69	3.70 (−7.04; 14.44)	0.49
HOMA-IR	51	−0.20 (−0.90; 0.51)	0.57	−0.02 (−0.77; 0.72)	0.95
Triglycerides (mmol/L)	54	0.02 (−0.13; 0.16)	0.84	0.03 (−0.13; 0.18)	0.74
Total cholesterol (mmol/L)	54	0.07 (−0.11; 0.26)	0.44	0.06 (−0.13; 0.25)	0.56
LDL-C (mmol/L)	54	0.12 (−0.04; 0.29)	0.13	0.10 (−0.06; 0.27)	0.21
HDL-C (mmol/L)	54	−0.06 (−0.13; 0.01)	0.07	−0.07 (−0.13; 0.003)	0.06
Change in savory snack intake (handful/week)					
Body Mass Index (kg/m^2)	72	0.05 (−0.20; 0.29)	0.72	0.21 (−0.06; 0.47)	0.12
Systolic blood pressure (mmHg)	70	0.25 (−0.47; 0.97)	0.49	0.45 (−0.35; 1.26)	0.26
Diastolic blood pressure (mmHg)	70	0.18 (−0.29; 0.65)	0.44	0.38 (−0.14; 0.90)	0.15
Fat mass (% of total body weight)	72	0.003 (−0.20; 0.20)	0.98	0.09 (−0.13; 0.31)	0.42
Fat free mass (kg)	72	−0.02 (−0.39; 0.36)	0.94	0.21 (−0.19; 0.60)	0.30
Pulse Wave Velocity (m/s)	58	0.04 (−0.06; 0.15)	0.44	0.04 (−0.08; 0.16)	0.49
Glucose (mmol/L)	62	−0.03 (−0.08; 0.02)	0.25	−0.02 (−0.08; 0.04)	0.57
Insulin (pmol/L)	61	−1.57 (−4.08; 0.95)	0.22	0.04 (−2.81; 2.88)	0.98
HOMA-IR	59	−0.16 (−0.32; −0.001)	0.049	−0.09 (−0.28; 0.09)	0.33
Triglycerides (mmol/L)	62	−0.02 (−0.06; 0.01)	0.17	−0.02 (−0.06; 0.02)	0.29
Total cholesterol (mmol/L)	62	−0.01 (−0.06; 0.04)	0.65	−0.02 (−0.08; 0.03)	0.42
LDL-C (mmol/L)	62	−0.001 (−0.04; 0.04)	0.95	−0.01 (−0.06; 0.04)	0.74
HDL-C (mmol/L)	62	−0.002 (−0.02; 0.02)	0.81	−0.01 (−0.03; 0.01)	0.42
Change in sweet snack intake (portion/week)					
Body Mass Index (kg/m^2)	72	−0.10 (−0.29; 0.09)	0.30	−0.04 (−0.26; 0.17)	0.69
Systolic blood pressure (mmHg)	70	0.34 (−0.23; 0.92)	0.23	0.30 (−0.38; 0.98)	0.38
Diastolic blood pressure (mmHg)	70	0.25 (−0.11; 0.62)	0.17	0.30 (−0.13; 0.74)	0.17
Fat mass (% of total body weight)	72	−0.09 (−0.24; 0.06)	0.24	−0.04 (−0.21; 0.13)	0.65
Fat free mass (kg)	72	−0.04 (−0.32; 0.24)	0.76	0.04 (−0.28; 0.36)	0.79
Pulse Wave Velocity (m/s)	58	0.05 (−0.05; 0.16)	0.29	0.04 (−0.07; 0.14)	0.47
Glucose (mmol/L)	62	−0.03 (−0.07; 0.001)	0.06	−0.002 (−0.04; 0.04)	0.90
Insulin (pmol/L)	61	−1.16 (−2.84; 0.53)	0.18	0.57 (−1.37; 2.52)	0.56
HOMA-IR	59	−0.16 (−0.26; −0.06)	0.003	0.01 (−0.09; 0.12)	0.84
Triglycerides (mmol/L)	62	−0.02 (−0.04; 0.01)	0.17	−0.02 (−0.05; 0.01)	0.12
Total cholesterol (mmol/L)	62	0.02 (−0.01; 0.06)	0.18	0.01 (−0.04; 0.05)	0.71
LDL-C (mmol/L)	62	0.02 (−0.01; 0.05)	0.12	0.02 (−0.02; 0.05)	0.41
HDL-C (mmol/L)	62	0.004 (−0.01; 0.02)	0.52	−0.002 (−0.02; 0.01)	0.82
Change in total MVPA (hour/week)					
Body Mass Index (kg/m^2)	76	−0.03 (−0.14; 0.09)	0.64	0.05 (−0.07; 0.16)	0.43
Systolic blood pressure (mmHg)	74	0.14 (−0.19; 0.46)	0.41	0.11 (−0.24; 0.47)	0.53
Diastolic blood pressure (mmHg)	74	−0.10 (−0.31; 0.10)	0.32	−0.09 (−0.31; 0.14)	0.44
Fat mass (% of total body weight)	76	−0.01 (−0.11; 0.08)	0.76	0.03 (−0.07; 0.13)	0.51
Fat free mass (kg)	76	0.09 (−0.06; 0.24)	0.25	0.14 (−0.02; 0.30)	0.09
Pulse Wave Velocity (m/s)	61	0.02 (−0.05; 0.09)	0.56	0.003 (−0.07; 0.07)	0.94
Glucose (mmol/L)	64	−0.01 (−0.03; 0.01)	0.26	−0.01 (−0.03; 0.02)	0.61
Insulin (pmol/L)	63	−0.59 (−1.74; 0.56)	0.31	−0.25 (−1.48; 0.99)	0.69
HOMA-IR	61	−0.03 (−0.10; 0.04)	0.39	−0.003 (−0.08; 0.07)	0.94
Triglycerides (mmol/L)	64	−0.001 (−0.02; 0.01)	0.86	0.002 (−0.01; 0.02)	0.76
Total cholesterol (mmol/L)	64	−0.01 (−0.03; 0.01)	0.38	−0.01 (−0.03; 0.02)	0.54
LDL-C (mmol/L)	64	−0.01 (−0.03; 0.01)	0.22	−0.01 (−0.03; 0.01)	0.42
HDL-C (mmol/L)	64	0.004 (−0.003; 0.01)	0.28	0.002 (−0.01; 0.01)	0.60

Table 3. *Cont.*

	N	Crude Model *		Adjusted Model **	
		β (95% C.I.)	*p*	β (95% C.I.)	*p*
Change in body weight during the intervention (kilograms)					
Body Mass Index (kg/m^2)	66	−0.12 (−0.35; 0.11)	0.31	−0.15 (−0.36; 0.06)	0.17
Systolic blood pressure (mmHg)	65	−0.27 (−0.89; 0.35)	0.38	−0.32 (−0.95; 0.32)	0.32
Diastolic blood pressure (mmHg)	65	−0.19 (−0.61; 0.23)	0.38	−0.22 (−0.65; 0.21)	0.31
Fat mass (% of total body weight)	66	−0.02 (−0.20; 0.16)	0.79	−0.04 (−0.22; 0.14)	0.65
Fat free mass (kg)	66	−0.14 (−0.42; 0.14)	0.32	−0.18 (−0.44; 0.08)	0.17
Pulse Wave Velocity (m/s)	53	0.02 (−0.07; 0.11)	0.63	0.02 (−0.07; 0.10)	0.69
Glucose (mmol/L)	55	0.001 (−0.05; 0.05)	0.98	−0.004 (−0.05; 0.04)	0.88
Insulin (pmol/L)	54	1.97 (−0.67; 4.61)	0.14	1.53 (−0.94; 3.99)	0.22
HOMA-IR	53	0.12 (−0.04; 0.28)	0.13	0.09 (−0.05; 0.24)	0.21
Triglycerides (mmol/L)	55	−0.01 (−0.03; 0.02)	0.65	−0.01 (−0.03; 0.02)	0.58
Total cholesterol (mmol/L)	55	−0.01 (−0.05; 0.04)	0.74	−0.004 (−0.05; 0.04)	0.87
LDL-C (mmol/L)	55	−0.01 (−0.05; 0.03)	0.61	−0.01 (−0.05; 0.03)	0.61
HDL-C (mmol/L)	55	0.01 (−0.01; 0.03)	0.24	0.01 (−0.001; 0.03)	0.06

MVPA = Moderate to Vigorous Physical Activity; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; LDL-C = low-density lipoproteins cholesterol; HDL-C = high-density lipoproteins cholesterol. † Change was calculated as preferably 6 months or otherwise 3 months minus baseline for vegetable intake, fruit intake and MVPA, and for sugary drink intake, snack intake and body weight, change was calculated as baseline minus preferably 6 months or otherwise 3 months. * The crude model is adjusted for baseline health outcomes and baseline lifestyle behavior. ** Model further adjusted for smoking (yes/no), current diet behavior depending on the variable of interest (e.g., in case of change in vegetable intake the model is adjusted for current vegetable intake, in case of MVPA the current dietary behavior is defined as current kcal intake) and current MVPA (min/day).

Dietary intake, physical activity and weight change during the first six months after randomization were not associated with having metabolic syndrome at follow-up (Table 4).

Table 4. Association between the change in dietary intake and physical activity during the preconception lifestyle intervention and metabolic syndrome 3–8 years after randomization †.

Change in	*N*	Crude Model *		Adjusted Model **	
		OR (95% C.I.)	*p*-Value	OR (95% C.I.)	*p*-Value
Vegetable intake (10 g/day)	59	1.01 (0.90; 1.14)	0.87	1.01 (0.89; 1.14)	0.93
Fruit intake (10 g/day)	61	1.00 (0.90; 1.11)	0.99	1.01 (0.91; 1.12)	0.87
Sugary drink intake (glasses/day)	49	0.97 (0.38; 2.47)	0.94	0.75 (0.20; 2.87)	0.68
Savory snack intake (handful/week)	57	1.03 (0.89; 1.20)	0.67	1.17 (0.94; 1.44)	0.16
Sweet snack intake (portion/week)	57	1.01 (0.90; 1.12)	0.91	1.02 (0.88; 1.18)	0.76
Total MVPA (30 min/week)	59	1.00 (0.93; 1.07)	0.91	1.04 (0.95; 1.13)	0.42
Weight change (kg)	52	1.06 (0.90; 1.25)	0.49	0.98 (0.76; 1.27)	0.90

MVPA = Moderate to Vigorous Physical Activity. † Change was calculated as preferably 6 months or otherwise 3 months minus baseline for vegetable intake, fruit intake and MVPA, and for sugary drink intake, snack intake and body weight, change was calculated as baseline minus preferably 6 months or otherwise 3 months. * The crude model is adjusted for baseline metabolic syndrome (yes/no) and baseline lifestyle behavior. ** Model further adjusted for smoking (yes/no), current diet behavior depending on the variable of interest (e.g., in case of change in vegetable intake the model is adjusted for current vegetable intake, in case of MVPA the current dietary behavior is defined as current kcal intake) and current MVPA (min/day).

Adding randomization arm into the model (intervention or control group) and time between randomization and follow-up (years) did not change effect estimates. Additionally, effect estimates hardly changed when we excluded women in early pregnancy from our study sample (results not shown).

4. Discussion

A decrease in savory and sweet snacks during the first six months after randomization was associated with lower insulin resistance 3–8 years later. However, these associations became

non-significant after adjustment for current lifestyle. No other associations between changes in lifestyle or body weight during the first six months after randomization with cardiovascular health 3–8 years later were observed.

A reason why we did not observe statistically significant associations between changes in lifestyle and body weight during the first six months after randomization with cardiovascular health 3–8 years later might be the low number of participants included in the follow-up study, and therefore we had low power to observe the hypothesized associations. Furthermore, it might be that there was not enough individual variation in the changes in lifestyle and body weight to find any associations with cardiovascular health at follow-up, which could be explained by the fact that women allocated to the control group participated to a larger extent in our follow-up study than women allocated to the intervention group.

There were multiple associations pointing towards our hypothesis that healthy changes in lifestyle are associated with more favorable cardiovascular health. Our findings that women with a higher intake of fruit ($p = 0.06$) and more MVPA ($p = 0.09$) had a higher fat free mass are in accordance with physiological mechanisms. Muscular activity stimulates the development and maintenance of lean muscle mass [27] and the high fiber content of the diet, associated with fruit intake, is related to lower body fat [28]. Furthermore, women who lost weight during the first six months after randomization tended to have higher HDL-cholesterol ($p = 0.06$). This is in line with findings in other studies and may relate to links between lower visceral fat mass and higher HDL-cholesterol [29].

However, we also observed that women who decreased their sugary drink intake tended to have a lower HDL-cholesterol ($p = 0.06$), which is not in line with our hypothesis and unexpected, assuming that a lower intake of sugary drinks causes a lower BMI, which is associated with improved HDL-cholesterol [29]. We do not know why we observed this: additional corrections for current BMI did not weaken this association and high sugary drink intake was not correlated with high MVPA levels, which is associated with higher HDL-cholesterol [30]. We were not able to analyze the effect of fresh fruit juice on HDL-cholesterol, because the 33-item FFQ do not ask specifically about fresh fruit juice. Evidence showed that fresh fruit juice might be associated with lower HDL-cholesterol [31]. It could therefore be that women specifically reduced their intake of sugar sweetened fruit juice, but not fresh fruit juice, which might have led to the unexpected association between decreases in sugary drinks and lower HDL-cholesterol. However, given the number of associations studied, it might also be that this is a chance finding.

Our results of reduced snack intake and improved HOMA-IR indicate that changing lifestyle is an important first step, but that maintaining a healthy lifestyle is needed for improving cardiometabolic health in the long-term. However, lifestyle change and maintaining those healthy changes on the long-term is notoriously difficult. To sustain long-term intervention adherence and thereby improve cardiometabolic health, it might be helpful to provide extended care by offering long-term individual or group contact to stimulate healthy behavior [32].

An important strength of the current study is the detailed information about the intake of specific foods and beverages, physical activity and body weight during the first six months after randomization into a preconception lifestyle program. This enabled us to gain more knowledge about lifestyle and body weight changes during the first six months after randomization in association with cardiometabolic health 3–8 years after the intervention instead of a randomized comparison between groups. We additionally had good quality data (measured by trained researchers) about cardiometabolic health at the start of the intervention and at 3–8 years after randomization, and were able to take into account women's baseline cardiometabolic health. There are also limitations that should be mentioned. Dietary intake as well as physical activity was measured using self-reported questionnaires. Obese women tend to under-report unhealthy behavior and over-report healthy behavior [33], and women allocated to the intervention group might do this to a larger extent because of social desirability bias [34,35]. However, adding randomization group into our regression models hardly changed the effect estimates, which indicates that the effect of social desirability bias induced

by randomization group is minimal. Furthermore, there was a wide range (3–8 years) in the time between inclusion in the preconception lifestyle intervention study and our follow-up assessment of cardiometabolic health. This wide range might have affected the associations between lifestyle and body weight change with cardiometabolic health at follow-up. However, adding time in between randomization and follow-up into our models hardly changed the effect estimates. Finally, the 33-item FFQ pre-specified two food groups, fruit juice and savory snack intake, including foods known to have favorable as well as unfavorable effects on cardiometabolic health. The question on consumption of fruit juice does not distinguish between fresh fruit juice and sugar sweetened juice, while studies show that the consumption of fresh juice reduces cardiovascular risk factors due to, amongst others, the antioxidant effects and anti-inflammatory effects [31]. Furthermore, the question on savory snack consumption combines the intake of crisps, pretzels, nuts and peanuts into one question, while studies show that nuts and peanuts might be beneficial for cardiovascular health [36]. It might therefore be that an increase in these food groups represents a healthy change instead of an unhealthy change. We recommend future research to use a more extensive FFQ, not pre-specifying these foods into one food group. Future research should replicate our results in a larger study population, preferably with larger variations in lifestyle and weight changes.

5. Conclusions

To conclude, decreases in savory and sweet snack intake were associated with reduced insulin resistance 3–8 years later, but after adjustment for current lifestyle these associations disappeared. No other associations between changes in lifestyle or body weight during the first six months after randomization with cardiovascular health 3–8 years later were observed. Changing lifestyle is an important first step, but maintaining this change is needed to improve cardiometabolic health in the long-term.

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2

Exercise and the Timing of Snack Choice: Healthy Snack Choice is Reduced in the Post-Exercise State

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Abstract: Acute exercise can induce either a compensatory increase in food intake or a reduction in food intake, which results from appetite suppression in the post-exercise state. The timing of food choice—choosing for immediate or later consumption—has been found to influence the healthfulness of foods consumed. To examine both of these effects, we tested in our study whether the timing of food choice interacts with exposure to exercise to impact food choices such that choices would differ when made prior to or following an exercise bout. Visitors to a university recreational center were equipped with an accelerometer prior to their habitual workout regime, masking the true study purpose. As a reward, participants were presented with a snack for consumption after workout completion. Participants made their snack choice from either an apple or chocolate brownie after being pseudo-randomly assigned to choose prior to ("before") or following workout completion ("after"). Complete data were available for 256 participants (54.7% male, 22.1 $\pm$ 3.1 years, 24.7 $\pm$ 3.7 kg/m^2) who exercised 65.3 $\pm$ 22.5 min/session. When compared with "before," the choice of an apple decreased (73.7% vs. 54.6%) and the choices of brownie (13.9% vs. 20.2%) or no snack (12.4% vs. 25.2%) increased in the "after" condition (χ^2 = 26.578, $p < 0.001$). Our results provide support for both compensatory eating and exercise-induced anorexia. More importantly, our findings suggest that the choice of food for post-exercise consumption can be altered through a simple behavioral intervention.

Keywords: compensatory eating; exercise-induced anorexia; food choice; acute exercise; behavioral economics; nudges

1. Introduction

Regular exercise and a healthy diet are important staples of a healthy lifestyle. The beneficial effects of exercise for the treatment and prevention of many physiological and psychological conditions, including diabetes, cardiovascular disease, certain cancers, recovery from stroke, emotional well-being, depression, anxiety, and suicidal behaviors [1–8], are well documented. However, the impact of exercise on overweight and obesity, and particularly its ability to produce meaningful weight loss remains under debate [9]. It is undisputed that exercise increases energy expenditure and thereby has the potential to induce weight loss. However, individual weight loss responses are mixed [10], suggesting that the success of exercise as a weight loss strategy is largely dependent on its effects on the other component of energy balance, i.e., dietary energy intake. A primary barrier to exercise-induced weight loss is compensatory eating, which is defined as an increase in food intake following exercise or physical activity [11].

It is a common belief that exercise stimulates appetite and food intake [12], and as much as 77% of college students report engaging in compensatory eating [11,13]. The mechanisms for this

compensatory increase in food intake following exercise are manifold, but most likely include endocrine pathways that favor food intake to ensure the maintenance of body weight, and more specifically lean body mass [14,15]. On the other hand, there is also evidence in the literature contrary to compensation. Evidence shows that only 19% of intervention studies reported an increase in energy intake after exercise, whereas 65% show no change [16]. Furthermore, it has repeatedly been demonstrated that appetite and hunger are suppressed following exercise, particularly in the immediate post-exercise state [12]. This reduction in perceived hunger has been termed "exercise-induced anorexia" and has been linked to the suppression of orexigenic hormones, such as ghrelin, and concomitant increases in satiety hormones, including peptide YY and glucagon-like peptide 1 [17–19].

While the impact of exercise on energy intake is important from an energy balance perspective, it is critical to understand that energy intake is ultimately the product of food choices, as individuals select foods rather than nutrients under free-living conditions [12]. It has been proposed that exercise participation can impact food selection, modify the sensitivity to sensory cues [12], and alter the reward value of foods with particular sensory and/or macronutrient profiles [20,21]. Hedonic mechanisms controlling food intake are stimulated by the sensory pleasure of eating palatable food and may result in increased food intake [22]. This increase in food intake has been linked to the neuroendocrine factor dopamine, which has been shown to reinforce the pleasure derived from eating highly palatable foods [23,24]. Exercise has been found to reduce addictive behaviors such as drug and alcohol consumption [23], and as such it is possible that exercise may also elicit a reduction in food intake as a result of the rewarding value of food. Previous experiments have shown that the responsiveness of brain regions related to food reward is altered in the post-exercise state [25], such that the brain's neural reward system's response to low energy density foods is increased and the reaction to high energy density is reduced [26–28].

In support of the contradictory findings in the literature, it has been proposed that exercise could either increase or decrease the reinforcing value of energy-dense, palatable foods. For example, the deliberate choice of highly palatable, energy-dense foods (e.g., fatty and/or sweet "treats") in the post-exercise state has been linked to compensatory eating and reduced weight loss success [21]. Others have reported that carbohydrate-rich foods are rated more palatable in the post-exercise state [25,26], possibly reflecting increased carbohydrate utilization during aerobic exercise [29]. Alternatively, exercise could also reduce the consumption of these foods as a result of improved appetite control coupled with a higher motivation to engage in healthy behaviors [28]. These diametrically opposed effects of exercise on food choices could ultimately explain the inter-individual variation in the degree of compensatory eating following exercise interventions [21].

Another important influence on dietary quality is the timing of food choice relative to consumption. Prior research from behavioral economics and psychology suggests that changing the timing of food choice relative to consumption—whether food choices will be consumed immediately or at some later point—influences the healthfulness of the foods chosen [30,31]. Behavioral economic models of choices over time (the most prominent of which is hyperbolic discounting [32]) have been formulated to represent individuals who have inconsistent preferences. These models predict that choices will differ depending on whether the chosen item is to be received immediately or after a delay. When the decision-maker will receive their choice immediately, the models predict that the individual will make less healthy, more impatient decisions than if the receipt is delayed. Individuals whose choices fit this pattern are said to have "present-biased preferences." An upshot of present-biased preferences is that people will often be willing to pre-commit to a healthier behavior if given the opportunity to do so [33,34].

To integrate the impact of biophysical and behavioral effects on post-exercise food intake in a real-life scenario, the overall goal of the present study was to test whether manipulating the timing of the choice of a snack to be received after exposure to exercise would alter food choices such that in the post-exercise state the choice of snacks with varying energy density and health attributes would differ from choices made prior to exercise. Our intervention addresses a simple but important question: *Can*

a simple nudge—changing the time when the choice of a post-exercise snack is made—help individuals select healthier food options? Given the importance of understanding why people make unhealthy dietary choices and how these choices can be discouraged [35], examining how factors such as the timing of food choice that may promote healthy eating in the context of exercising would be beneficial [28]. This is particularly true since behavioral weight loss strategies typically employ a combination of diet and/or exercise. However, as mentioned previously, evidence suggests that increases in food consumption in response to exercise can derail weight loss efforts [16]. Knowledge about the degree to which food choices are altered from pre- to post-exercise could ultimately allow individuals to pre-commit to healthier food choices by selecting food in a state which favors their choice of healthier options. Our primary hypothesis was that individuals are more likely to choose an "unhealthy," energy-dense snack in the post-exercise state when compared to choosing a snack for post-exercise consumption prior to exercising, supporting previous evidence of compensatory eating [11] as well as findings from behavioral studies of food choice [30]. To also account for previously reported reductions in appetite and hunger in the immediate post-exercise state, we further hypothesized that the number of participants who would decline a snack would also increase in the post-exercise state.

2. Materials and Methods

2.1. Study Design

The experiment was conducted at the University of Nebraska-Lincoln Recreational and Wellness Center and was focused on the effect of the timing of food choice—which was assigned by the researchers to take place before or after the participant exercised—on selection of a food item. The food item was received after the exercise was completed in both conditions. The experiment took place on randomly chosen weekdays between February 16 and April 30, 2018, and implementation of conditions was balanced across the study period and days of the week. Individuals were recruited to participate as they entered the recreational center. All participants had to be at least 19 years of age and to have come to the Recreational and Wellness Center to exercise. When individuals were invited to participate, the purpose of the study was presented as being focused on calibrating activity sensors to various exercise types. As an incentive for participation in this "calibration study," participants were offered a choice of food items upon completion of the study. In actuality, participants' choice of a food item was of central interest to the study, but this interest in participants' food choices was not highlighted to minimize experimenter demand effects [36] and social desirability biases [37,38]. Participants signed a written informed consent form prior to the experiment. The study was approved by the University of Nebraska-Lincoln Institutional Review Board.

2.2. Procedures

After participants signed the informed consent form, study staff measured their height and weight on a digital column scale (Seca, Hamburg, Germany). Next, participants were fitted with an accelerometer (GT3X, ActiGraph, Pensacola, FL, USA) on their non-dominant wrist, which they wore for the duration of their workout. Study staff instructed all participants to proceed with the workout that the participant had planned to complete at the Recreation and Wellness Center prior to study recruitment.

When subjects returned the accelerometers upon completion of their workout, additional information was collected, including each participant's date of birth, the type of activity or activities conducted during the workout, and whether participants ate or drank anything during the workout. Participants were told that this information was needed to personalize the accelerometer data.

2.3. Food Choice Paradigm

In order to examine changes in food choices over the course of a self-selected workout, participants were given a snack choice to be received upon completion of their workout as a reward for study

participation. In one condition, prior to beginning their workout ("before"), participants were asked to choose which snack item they wanted to receive. Upon completion of their workout and them returning the accelerometer, participants were given the food item they had selected. In the other condition ("after"), participants were asked to choose their snack right as they came back to return their accelerometer after their workout. In the "after" condition, participants received their snack immediately after making their choice. For logistical purposes, one condition ("before" or "after") was implemented per study day.

In order to present two snack options with distinguishable perceived health attributes but similar taste attributes (sweet), participants were able to choose between an apple (deemed "healthy") and a brownie (deemed "unhealthy"). Participants also had the option to decline both snacks ("neither"). Both snack options were visible to participants as they checked in ("before" condition) or as they checked out ("after" condition). Prior to giving the chosen snack to the participant, study staff inquired about food allergies and intolerances.

All food used in the experiment was purchased and prepared in a large batch by a food and beverage management specialist in a department certified kitchen and stored at appropriate conditions (temperature checks were performed twice per day) prior to presenting it to study participants. The apple variety offered in the study was Fuji with an average energy content of 121 kcal per medium-sized apple [39]. Brownies were prepared from a prepackaged commercial brownie mix (Ghiradelli, San Leandro, CA, USA) and had an energy content of 140 kcal per piece.

2.4. Data Analyses

Data were analyzed using R Statistical Software (R Core Team, R Foundation, Vienna, Austria). Prior to data analysis, data from individuals who participated in the study multiple times were eliminated from the dataset such that only the first study visit was included in the analyses. In addition, data from participants who reported food allergies or intolerances that could have affected the food choice were eliminated from the dataset. Participants' food item choices were analyzed to evaluate whether the proportion of choices of the different food items (including "neither") differed by condition using a chi-squared test. We then used multinomial, multivariate logistic regression models to examine the relationship between food choice and condition while controlling for potentially confounding variables comprising body mass index (BMI) category ($\leq$25 kg/m^2; >25 kg/m^2), age (in years), gender, workout duration (in minutes), whether the participant consumed any food during their workout, and mode of exercise (aerobic, resistance, and other). BMI categories were aggregated from four initial categories (underweight, normal weight, overweight, and obese) into two (underweight/normal weight, and overweight/obese) because few participants fell into the underweight (3 participants) and obese (18 participants) categories. Results of the multinomial logistic regression models are presented as odds ratios (OR) and 95-percent confidence intervals (95% CI) for independent variables. Statistical significance was considered for p-values < 0.05.

3. Results

A total of 299 data points were initially collected. Data from 31 participants (42 observations) were eliminated because individuals had participated in the study more than once. In addition, data from one participant who reported having celiac disease were omitted. The final dataset used for analysis contained observations from 256 unique participants. Of these, 137 participants (53.5%) completed the "before" condition and 119 completed the "after" condition (46.5%). On average, 54.7% of participants were male, 22.1 $\pm$ 3.1 years old, had an average BMI of 24.7 $\pm$ 3.7 kg/m^2, and exercised for 65.3 $\pm$ 22.5 min. There were no significant differences between conditions in gender distribution, age, BMI or BMI categories, workout duration, food consumption, or mode of exercise (Table 1).

Table 1. Characteristics of the study sample.

	All Participants (n = 256)	"Before" Condition (n = 137)	"After" Condition (n = 119)	p-Value *
Age	22.1 ± 3.1	22.0 ± 2.9	22.1 ± 3.4	0.72
Gender (Male)	135 (52.7%)	75 (54.7%)	60 (50.4%)	0.49
BMI (kg/m^2)	24.7 ± 3.7	24.8 ± 3.6	24.6 ± 3.8	0.55
Underweight/Normal weight	148 (57.8%)	75 (54.8%)	73 (62.3%)	0.29
Overweight/Obese	108 (42.2%)	62 (45.2%)	46 (38.7%)	0.29
Workout Duration (min)	65.3 ± 22.5	67.3 ± 25.5	63.0 ± 18.3	0.12
Food Consumption (Yes)	8 (3.1%)	5 (3.6%)	3 (2.5%)	0.60
Aerobic Exercise (Yes)	172 (67.2%)	87 (63.5%)	85 (73.4%)	0.18
Resistance Exercise (Yes)	192 (75%)	102 (74.5%)	90 (75.6%)	0.83
Other Exercise (Yes)	9 (3.5%)	7 (5.1%)	2 (1.7%)	0.13

* "before" vs. "after".

In the "before" condition, 101 participants (73.7%) selected an apple, 19 (13.9%) selected a brownie, and 17 (12.4%) declined a snack upon completion of their workout. In the "after" condition, 65 participants (54.6%) selected an apple, which is an ~20% decrease from the "before" condition. Twenty-four participants (20.2%) selected a brownie in the "after" condition, and 30 participants (25.2%) declined a snack option upon completion of their workout. The patterns of choices (Figure 1) differed significantly between the "before" and "after" condition (χ-squared = 26.578, df = 2; $p < 0.001$).

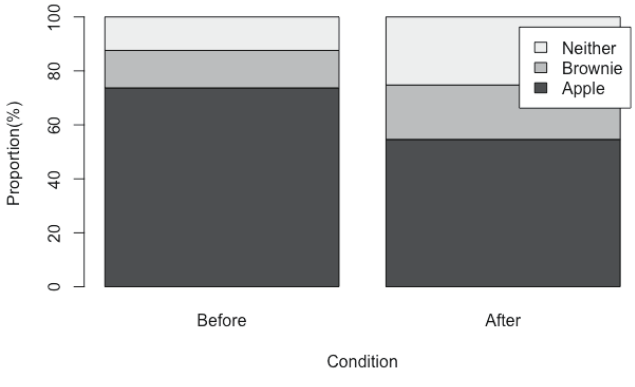

Figure 1. Proportion of snack choices (apple, brownie, or neither) for consumption after completion of a workout chosen either before the beginning of the workout ("before" condition) or after completion of the workout ("after" condition).

Results from the multinomial, multivariate logistic regression model are reported in Table 2. The omitted snack choice was "neither" in the regression model. Results confirm that the odds of a participant choosing an apple decreased significantly in the "after" condition relative to the choice of neither (OR: 0.33; 95% CI: 0.16–0.66), even after controlling for other variables. The estimated odds ratio for the effect of the "after" condition on apple choice remained unchanged regardless of other independent variables included in the regression model, including BMI status, age, gender, and workout duration. There were no significant differences in brownie choice (relative to choosing neither) based on condition (OR: 0.64; 95% CI: 0.26–1.55). In addition to the condition, only the BMI category significantly contributed to the regression model of snack choice. Individuals who were classified as overweight or obese (BMI > 25 kg/m^2) were significantly less likely to select a brownie than individuals with a BMI < 25 kg/m^2 (OR: 0.37; 95% CI: 0.15–0.92).

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) from multinomial logistic regression results of snack choice, relative to neither.

	Apple		Brownie	
	OR	**95% CI**	**OR**	**95% CI**
Condition: "After"	0.33	(0.16–0.66)	0.64	(0.26–1.55)
BMI Status (>25 kg/m^2)	0.61	(0.31–1.23)	0.37	(0.15–0.92)
Age	0.98	(0.87–1.10)	1.09	(0.96–1.24)
Gender (male)	1.17	(0.56–2.43)	1.81	(0.71–4.64)
Workout Duration (min)	1.00	(0.98–1.02)	1.00	(0.98–1.02)
Food Consumption (Yes)	1.51	(0.17–13.62)	1.16	(0.07–20.00)
Aerobic Exercise (Yes)	1.11	(0.48–2.59)	0.85	(0.30–2.40)
Resistance Exercise (Yes)	1.03	(0.43–2.43)	0.91	(0.30–2.77)
Other Exercise (Yes)	0.47	(0.04–1.56)	0.47	(0.05–4.45)
Intercept	10.65	(0.53–214.60)	0.31	(0.01–9.75)

Number of observations = 256, Akaike Information Criterion = 470.9.

4. Discussion

The goal of the present study was to assess whether the timing of snack choice would interact with exposure to a single exercise bout to alter food choices. Using a behavioral intervention approach, we show that a very simple modification—making a choice about a post-exercise snack either prior to or following the completion of the exercise bout—significantly alters the snack choice. Our findings indicate that the likelihood of choosing an apple, a food typically considered as "healthy", is about one third (33.5%) greater when the choice is presented prior to engaging in exercise; however, when the choice is presented following the exercise bout, individuals are approximately 39% more likely to choose a brownie, a food typically considered as "unhealthy", and 112% more likely to decline either snack option. These findings exhibit elements of two previously identified effects of exercise on food choice: compensatory eating, which refers to the increase in food intake following exercise of physical activity [11], and exercise-induced anorexia, which refers to a temporary reduction in appetite immediately following exercise [40]. They also correspond to patterns seen in previous behavioral research, in which individuals are more likely to make healthier choices if the food will be delivered in the future rather than immediately [30].

4.1. Interindividual Variation in Post-Exercise Food Choices: Compensatory Eating and Exercise-Induced Anorexia

The increase in the preference for a brownie, an energy-dense and palatable snack, can be seen as evidence for compensatory eating. Finlayson et al. speculated that hedonic processes are modulated by increased energy expended during exercise, thereby promoting overconsumption in individuals prone to compensatory eating [22]. Considering that a greater tendency for compensatory eating has been linked to attenuated weight loss during exercise interventions [21], our findings highlight the importance of timing of food choices for individuals who exercise to lose weight. In contrast, others have reported a reduced preference for energy-dense food following exercise. For example, an acute bout of resistance exercise decreased the preference for high-fat food [27], and 2 weeks of aerobic exercise were found to reduce the reinforcing value of high energy density foods [28]. However, it is noteworthy that there was a dose-dependent relationship between habitual exercise and the reduction in the reinforcing value of high energy density foods, which was most pronounced in individuals who exercised 5 days per week when compared to individuals who exercised less regularly [28].

In addition to an increased preference for an "unhealthy" option, we further observed an increase in the number of individuals who declined either food option among participants who were given a snack choice in the post-exercise state. While we acknowledge that there may be other reasons for this finding, this finding is in accordance with previous reports of exercise-induced anorexia. This phenomenon, which describes a transient reduction in appetite and hunger in the immediate post-exercise state, has been linked to reductions in ghrelin, an appetite-stimulating hormone, and increased concentrations of satiety hormones, including peptide YY and glucagon-like peptide 1 [17–19,41]. However, hunger

is not necessarily indicative of actual food intake, as shown in a study involving exposure to exercise message commercials, which resulted in higher ratings of hunger but lower caloric intake [42].

Taken together, increases in compensatory eating and exercise-induced anorexia result in a greater inter-individual variability in post-exercise food intake. These competing effects, i.e., an increase in consumption of an "unhealthy" option along with a greater number of individuals declining either food option, negate an overall group effect. In fact, when determining the caloric intake based on food choices made (brownie, apple or neither), average caloric intake did not change dramatically from "before" (109 kcal/participant) to "after" (94 kcal/participant), which is in support of a previous meta-analysis by Schubert et al. who failed to identify a definite effect of an exercise task on post-exercise caloric intake [10]. This finding is also in agreement with observations from our laboratory, according to which inter-individual differences in food choices are greater in the post-exercise state when compared to the rested state (Koehler, unpublished observation).

4.2. Impact of Gender and Body Mass Index

In contrast to previous studies, we failed to observe a significant gender effect in our food choice paradigm. Although most laboratory experiments addressing the impact of a single exercise bout on food intake were not specifically designed to detect gender differences, it is well established in the literature that food intake patterns differ between men and women and that women consistently make healthier choices than men [43]. Furthermore, the motivation to exercise tends to differ between genders, as women are more likely to exercise in attempts to lose weight [44]. As such, it is not surprising that when compared to their male counterparts, female athletes exhibit more self-control for high-calorie and sweet food options [45]. However, despite these gender differences, a recent meta-regression showed no impact of gender on post-exercise calorie intake [10], which is in agreement with the lack of a gender effect in our study. This lack of gender difference may be further explained by the fact that a greater proportion of our female participants conducted aerobic exercise (84% vs. 52% in males, $p < 0.001$), which is reflective of the general exercise motives of young adults [46]. It is possible that the lower energy expenditure of resistance exercise, when compared to aerobic exercise [47], reduced the likelihood of compensatory eating in the male participants. Furthermore, a recent study reported that resistance exercise, but not aerobic exercise, reduced explicit liking for high-fat food [27].

We did, however, find an effect of BMI on overall food choice, whereby individuals with a BMI indicative of overweight or obesity (>25 kg/m^2) were less likely to choose our "unhealthy" option. This finding is contrary to previous literature suggesting that obesity is associated with an increased drive to eat palatable foods [48], specifically in the absence of hunger [49], and greater impulsivity toward food reward [50]. However, it should be noted that the current study was selectively conducted in individuals who voluntarily exercised, a group not necessarily representative of individuals who are overweight and obese [51]. As such, our overweight or obese participants may have exhibited a greater motivation to lose weight compared to non-exercising individuals [52]. Furthermore, it is also possible that our measure of BMI overestimated levels of overweight and obesity in our group of exercisers, as it is well known that BMI fails to differentiate between individuals with excess adiposity vs. individuals with increased muscle mass [53].

4.3. Limitations

As the study presents a simple behavioral manipulation in a real-life setting, there are various limitations to our investigation. First, we failed to assess changes in ratings of appetite or hunger over the course of the exercise bout as well as appetite-regulating peptides, and more specifically whether inter-individual differences in these outcomes would explain differences in food choices from pre- to post-exercise. However, as previously mentioned, it is well known that hunger levels alone fail to explain differences in the food intake response following exercise [22].

In order to test the impact of exercise on food choice in a real-life scenario, we chose to allow participants to follow their regular exercise regimen. As such, exercise intensity was self-selected

and consequently varied among our participants. High-intensity exercise has been shown to favor a negative energy balance to a greater extent than low-intensity exercise [54]. Although each participant wore an accelerometer during their exercise bout, we chose not to include this data in the present analysis due to previous studies demonstrating that the Actigraph tends to perform poorly during vigorous activities [55], which we presumed to be the primary intensity range in the present study. Regardless, future studies should attempt to assess exercise intensity using objective (e.g., heart rate) or subjective (e.g., ratings of perceived exertion) measures. Another limitation was that we did not assess the prandial state, such as the time of the last meal or current hunger levels. While intriguing, these measures were not taken in order to avoid revealing the true purpose of this investigation. We did, however, assess food intake (in excess of water) during the workouts, but this variable did not have a significant effect on food choice as shown by our regression analysis (Table 2). While future studies should more carefully monitor food intake and hunger levels prior to the experiment, we are confident that using a relatively large sample and conducting all experimental trials at the same time of day minimized the impact of the prandial state on our outcomes.

Our findings clearly highlight that food choices change depending on whether a post-exercise snack is chosen prior to or following the exercise bout. Behavioral economic models of intertemporal choice provide another perspective on this pattern of choice [32]. Many people have inconsistent preferences for outcomes that occur at different time points relative to when the choice is made. While most of the evidence is for choices made over monetary outcomes, there is some evidence about food choice as well. When people make choices for foods that they will receive immediately, time-inconsistent preferences tend to lead them to be more indulgent—choosing less healthy foods—than if receipt of the selected food is in the future. This model predicts that people are more likely to make a healthy choice for their future self than they are for their current self [56]. While this phenomenon may have explained the increased preference for the "unhealthy" option (brownie) following the workout (immediately before the snack) compared to before the workout (on average 65 min before their snack), it fails to explain the increase in the number of participants who declined either snack. A second behavioral economic influence may shed light on increases in both unhealthy and neither food option: projection bias [57]. Projection bias refers to the failure of a decision-maker to correctly predict their preferences for outcomes that occur in the future. This bias is thought to be particularly likely to occur when the individual is making a choice in one state that will be experienced in another state [58]. It is well established that exercise induces changes in an individual's state by, for instance, suppressing appetite-inducing hormones [17–19,41]. Future studies can feature more sophisticated designs that separately identify these various influences on decision-making, such as including non-exercising control activities to disentangle the effects of time-inconsistent preferences from compensatory eating and exercise-induced anorexia and varying the state in which participants make food choices for immediate and future receipt.

5. Conclusions

Overall, our study demonstrates that the choice of a post-exercise snack can be shifted through a simple behavioral intervention, i.e., choosing the snack prior to or after the exercise bout. As such, our results provide support for both an increased preference for compensatory eating as well as an increased degree of exercise-induced anorexia. Our findings have important practical implications for individuals who are attempting to lose or control their weight through exercise, as well as for health professionals providing guidance and support to these individuals. Corroborating previous research [34,58], participants in this study who chose their snack before they exercised—the "before" condition—were more likely to select a healthier snack than participants who chose immediately prior to receipt of the snack (the "after" condition). A simple strategy such as encouraging individuals to make choices about foods that they will eat post-exercise prior to their workout may help those who are attempting to lose weight through diet and exercise pre-commit to healthier foods. Pre-commitment

can prevent individuals from offsetting the gains in exercise-related caloric expenditure through compensatory eating.

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3

Comparison of Pro-Regenerative Effects of Carbohydrates and Protein Administrated by Shake and Non-Macro-Nutrient Matched Food Items on the Skeletal Muscle after Acute Endurance Exercise

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Abstract: Physical performance and regeneration after exercise is enhanced by the ingestion of proteins and carbohydrates. These nutrients are generally consumed by athletes via whey protein and glucose-based shakes. In this study, effects of protein and carbohydrate on skeletal muscle regeneration, given either by shake or by a meal, were compared. 35 subjects performed a 10 km run. After exercise, they ingested nothing (control), a protein/glucose shake (shake) or a combination of white bread and sour milk cheese (food) in a randomized cross over design. Serum glucose (n = 35), serum insulin (n = 35), serum creatine kinase (n = 15) and myoglobin (n = 15), hematologic parameters, cortisol (n = 35), inflammation markers (n = 27) and leg strength (n = 15) as a functional marker were measured. Insulin secretion was significantly stimulated by shake and food. In contrast, only shake resulted in an increase of blood glucose. Food resulted in a decrease of pro, and stimulation of anti-inflammatory serum markers. The exercise induced skeletal muscle damage, indicated by serum creatine kinase and myoglobin, and exercise induced loss of leg strength was decreased by shake and food. Our data indicate that uptake of protein and carbohydrate by shake or food reduces exercise induced skeletal muscle damage and has pro-regenerative effects.

Keywords: endurance exercise; skeletal muscle damage; inflammation; protein; carbohydrates; protein shake; food

1. Introduction

Consumption of proteins and carbohydrates after exercise via whey protein and glucose shakes is a common strategy for the enhancement of regeneration and physical performance after training [1]. There are reports that isolated amino acids, mainly leucine, can increase strength after exercise and result in a stimulation of the recovery of skeletal muscle after exercise [2]. Damage of muscle fibers by physical activity modulates protein synthesis, but simultaneously protein degradation [3]. Disturbance of the balance of muscle fiber degradation and synthesis leads to fiber degeneration and muscle atrophy, reduction of strength, and increase in muscle soreness [4,5].

Molecular mechanisms involved in muscle recovery include a modulation of protein synthesis and protein breakdown [6], an inhibition of the inflammatory response, and the activation of satellite

cells [7]. Modulation of the balance of protein breakdown and protein synthesis is an important mechanism in skeletal muscle recovery and adaptation after exercise. It has been demonstrated that muscle protein breakdown rates are elevated in the days following resistance exercise [8]. In mouse knock out models, the inhibition of muscle protein breakdown impairs muscle recovery [9]. Some authors claim that modulation of protein breakdown is an important mechanism in muscle recovery in the period immediately after muscle damage [10]. In contrast, the stimulation of protein synthesis seems to be important for long term regeneration and hypertrophy. Therefore, all strategies affecting the balance between protein breakdown and synthesis in the period after damage will directly influence skeletal muscle recovery.

Protein metabolism in skeletal muscle has been shown to be stimulated by an ingestion of dietary proteins [11,12]. Whey protein supplementation results in a high availability of amino acids within the blood [13]. However, there are conflicting data about the effects of protein supplementation on physiological markers related to muscle recovery, like muscle strength, muscle soreness and serum creatine kinase (CK) concentrations after exercise [14,15]. These conflicting data are mainly caused by variables in the different studies. These variables include different amounts of nutritional feeding, timing, habitual food intake and type of exercise, volume of exercise, recovery measurements, and the timing of recovery measurement [16].

Apart from protein shakes, carbohydrate shakes are also frequently consumed to stimulate recovery after physical activity, containing protein/carbohydrates combinations. These drinks are recommended by the manufacturing companies to be ingested immediately after exercise, in order to have the most effective pro-regenerative effect. Research on this subject shows highly conflicting results. Some studies demonstrate that adding protein to carbohydrates improves the process of the recovery of endurance performance [17–21]. Others, focusing on the modulating of protein breakdown and synthesis, provide no evidence that carbohydrates increase exercise-induced protein secretion versus protein alone [22].

Mechanistically, it is believed that, beside the compensation of carbohydrate loss during exercise, carbohydrates also activate molecular mechanisms related to pro-regenerative effects. Here, insulin is claimed to be an important factor. It has been shown that a protein/carbohydrate combination leads to a higher increase in blood sugar and insulin concentration than just carbohydrate intake [23]. The molecular mechanism as to how proteins stimulate insulin secretion is unknown so far [24]. It also has been demonstrated that the amount of glycogen storage in skeletal muscle is higher with protein/carbohydrate combinations than with carbohydrates alone [23]. It is discussed that any uptake of carbohydrates immediately after exercise results in a strong increase of serum insulin, followed by a strong decrease of blood glucose. Binding of insulin to IGF-1 receptors should result in the activation of skeletal muscle specific signal transduction pathways, and via mTOR (Rapamycin), it should also result in a stimulation of protein synthesis in skeletal muscle [25,26]. Moreover, increasing insulin secretion also should stimulate amino acid uptake in skeletal muscle cells [26]. However, other investigations demonstrate that insulin does not stimulate muscle protein synthesis under physiological conditions in humans. It is very likely that the experimental design will have a huge impact on the results of the studies.

Beside ingestion of isolated amino acids, effects of protein/carbohydrate combinations on skeletal muscle could also be observed after the uptake of food containing protein and carbohydrates in a ratio of 70% to 30% [27]. In previous studies, we could observe pro-regenerative effects on the skeletal muscle after exercise, by eating combinations of dairy products and white bread [28]. Based on these observations, we concluded that eating food containing suitable concentrations of protein and carbohydrates may be an alternative strategy to promote skeletal muscle regeneration after exercise. Therefore, the aim of this follow up study was to compare the pro-regenerative effects of any protein/carbohydrate shake on the recovery of skeletal muscle after endurance exercise, with the ingestion of similar amounts of protein/carbohydrate combinations by eating a nearly iso-caloric meal.

Our hypothesis was that providing proteins/carbohydrate combinations will result in pro-regenerative effects, independent as to whether it was given by a shake or by eating a meal. Therefore, 35 nonspecifically trained subjects consumed either a whey protein/glucose shake, or a meal of white bread and sour milk cheese directly after endurance exercise. The following mechanistic read outs were determined: Serum glucose and serum insulin, serum creatine kinase (CK) and myoglobin (Myo) as muscle damage markers, hematologic parameters, cortisol, serum levels of the inflammation markers interleukin 6 (IL 6) and 10 (IL 10), as well as the macrophage migration inhibitory factor (MIF). In addition, leg strength as a functional marker for skeletal muscle regeneration was measured.

2. Methods

2.1. Participants

The study protocol has been approved by the local ethics committee (German Sports University, Cologne) and is in accord with the Declaration of Helsinki. It is registered in the German Clinical Trials Register under the title NUPROMU, and under the registration number DRKS-ID: DRKS00013359.

All participants provided written informed consent prior to their participation. The study excluded subjects who were currently taking any dietary supplements, sports drinks or functional food intended to enhance performance, or had taken any of these in the previous month. Moreover, subjects with known hypersensitivity to any of the constituents of the products under study (milk protein or lactose), were excluded. Throughout the study, subjects maintained their usual training routines and diets. Based on a statistical power analysis a total of 35 male participants (age: 23.2 $\pm$ 2.3 years; height: 181.5 $\pm$ 6 cm; weight: 77.9 $\pm$ 8.4 kg; mean $\pm$ standard deviation) were recruited for the study. Power was calculated with the program G *power (University of Düsseldorf, (Heinrich-Heine-Universität Düsseldorf (**HHU**)), Germany) based on effect sizes measured for the biological endpoints creatine kinase (CK) IL6 and IL10 in our pilot study [28]. Power analysis revealed that a number of 15 participants would be sufficient. Nevertheless, the group size was doubled, because additional parameters not measured in the pilot study were included. All participants were healthy and free of injury in the time period preceding the study. They were not specifically endurance trained, but they were physically active sports students. Anthropomorphic characteristics of the participants are indicated in Table 1.

Table 1. Compositions and calories of the standardized breakfast.

	Carbohydrates (g)	Protein (g)	Fat (g)	Calories (kcal)
30 g Cornflakes	25.2	2.1	0.3	111.9
250 mL milk (1.5% fat)	12.0	8.5	3.8	116.2
One banana (100 g)	27.4	1.3	0.4	118.4
Sum	64.6	11.9	4.5	346.5

2.2. Experimental Procedure

The aim of this study was to investigate the beneficial effects of a co-ingestion of protein and carbohydrate from a traditional food source in amateur sportsmen. For this purpose, 35 non-specific endurance trained male subjects performed a 10 km run with an intensity at 80% of their individual anaerobic threshold (IAT). Intensity was chosen based on previous investigations, demonstrating that 80% IAT and a distance of 10 km is a sufficient load for nonspecific endurance trained subjects to increase their serum CK [29]. The IAT was determined using an incremental field test with lactate determination according to a standard protocol [30]. Participants started with a speed of 2.5 m/s and distance of 800 m. After each successful termination of load level, the speed was increased by 0.5 m/s. The load duration was always between 5–6 min. Between the speed levels, 5 µL of capillary blood was taken from all participants. If a load level could not be successfully passed, the test was terminated.

The anaerobic threshold was used to calculate the meters per second and the resulting lap time in a track and field stadium (400 m lap length) for the 10 km run. Between the field test and the first investigation a wash out period of three weeks was used. Before running, the individuals were randomly divided into three intervention conditions—control, shake or food. Through a crossover design, each subject participated in each condition. However, the order was given after the first random assignment—control, shake, food.

Between the respective interventions there was a minimum wash out period of three weeks. The experimental design of the procedure at the intervention days is shown in Figure 1.

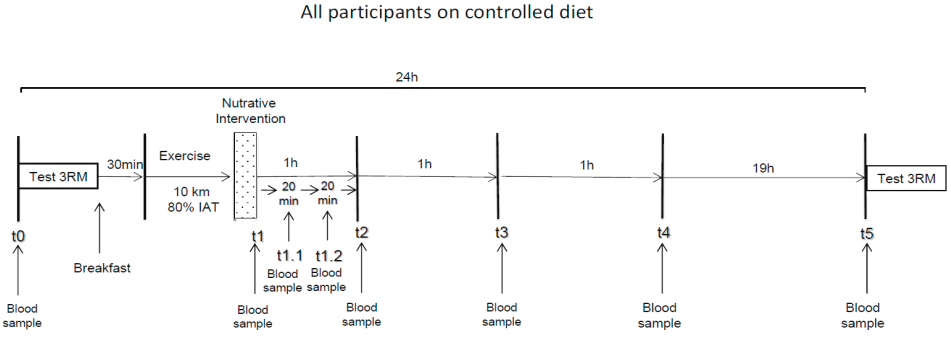

Figure 1. Experimental design of the study. IAT = individual anaerobic threshold. 3RM = leg strength.

Blood samples were determined for different time points (Figure 1). In the morning, blood sample t0 was collected from the overnight fasted participants. Leg strength was tested by maximum three repetitions in back squat (3-RM BS) followed by s a defined small breakfast (Table 1).

30 min after breakfast, all participants started with the 10 km run with 80% of IAT. Immediately after exercise, subjects ingested either nothing (control), a combination of carbohydrates by eating white bread (35.3 g) and 100 g of a sour milk cheese high in protein (36.1 g) but very low in fat (3.5 g)(Loose GmbH, Leppersdorf, Germany), or by drinking a whey protein (52 g)/glucose (45 g) shake. The composition of the shake used was taken from a published study [21] where effects regarding skeletal muscle regeneration have already been demonstrated. It serves as a standard. The nutritional values of the foodstuffs used in each intervention are shown in Table 2. As a consequence of using whole foods, it is not possible to match the macronutrient content of the shake and foods exactly. Whole foods, like dairy products, usually contain fat. Nevertheless, we have opted for low-fat protein and carbohydrate sources as indicated in Table 2. In addition we have tried as accurately as possible to be isocaloric. For practical reasons our participants got the food as a sandwich composed of two slices of white bread, and 100g of sour milk cheese. So, we differed from the shake with regard to the calories around 17% (Table 2).

Table 2. Compositions and calories of protein/carbohydrate interventions.

		Carbohydrates (g)	Protein (g)	Fat (g)	Calories (kcal)
Food	76 g white bread and 100 g sour milk cheese	35.3	36.1	3.5	321
Shake	45 g Glucose 52 g Whey protein	45	52	0	386

Immediately after ingestion, blood samples were determined at t1 (directly after exercise), followed by t.1.1 (+20 min after exercise), t1.2. (+40 min), t2 (+60 min), t3 (+120 h), t4 (+180 h) and t5 (+22 h). During the testing period, starting 12 h before exercise and 24 h after exercise, all participants were kept under a standardized nutrition to exclude additional effects by nutrition. All food for this 36 h period was provided to them.

12 h before exercise participants had a standardized dinner—spaghetti with tomato sauce (377 kcal, 15 g protein, 2.5 g fat, 72 g carbohydrates), and fasted until the standardized breakfast the next day. On the intervention day, participants were not allowed to eat until 3 h after exercise (t4; +180 min) except the provided nutritional compositions. After t4 they were allowed to eat the food provided in a period of 5 h after exercise until 8 pm. After 8 pm on the exercise day, participants fasted until the next day, when they were given the standardized breakfast. The standardized 24 h nutrition at the intervention day was calculated to fulfill all daily requirements regarding macro- and micronutrients, and provide sufficient calories.

Including the nutritive intervention, participants consumed a total of 3000 kcal a day. Daily macronutrient content was 146 g protein, 77 g fat and 411 g carbohydrates. Participants were allowed to drink water ad libitum.

3. Measurements

3.1. Determination of Serum Glucose and Serum Insulin

Samples were analyzed for glucose by oxidase method (COBAS Mira Plus; Roche Diagnostic Systems, Rotkreuz, Switzerland) and insulin by EIA (ALPCO diagnostics 1–2-3 Human Insulin EIA, Windham, NH, USA). Both serum concentrations were measured at all time points.

3.2. Determination of Serum Cortisol

Serum Cortisol concentrations were determined using the COBAS Mira Plus system (Roche Diagnostic Systems by ElectroChemiLumineszenz ImmunoAssay, ECLIA Rotkreuz, Switzerland). The serum cortisol concentration was measured at the time points t0, t1 (directly after exercise), t2 (+60 min), t3 (+120 min), t4 (+180 min) and t5 (+22 h).

3.3. Hematology

Blood samples were analyzed for routine CBC including total WBC count and differential for WBC sub fractions using a BC2300 hematology analyzer (Mindray Medical International Systems, Shenzhen, Peoples' Republic of China). The Hematology parameters were determined at t0, t4 (+180 min) and t5 (+22 h).

3.4. Skeletal Muscle Creatine Kinase (CK mm) and Myoglobin (Myo)

Skeletal muscle specific creatine kinase activity (CKmm) and myoglobin (Myo) concentrations in the serum were determined using the COBAS h 232 Point-of-Care-System (Roche Diagnostic Systems, Rotkreuz, Switzerland) at t0 and t5 (+22 h).

3.5. Serum Cytokine Levels

IL-6 concentrations of serum samples were analyzed using the Human IL-6 ELISA Kit High. Sensitivity (Abcam, Cambridge, UK). IL10 serum concentrations were analyzed using a human IL-10. ELISA Kit (Abcam, Cambridge, UK). MIF serum concentrations were analyzed using a human MIF. ELISA Kit (Abcam, Cambridge, UK). Cytokine concentrations were determined at t0 and t5.

3.6. Leg Strength—3-RM Back Squat

The strength test was performed t0 and t5 (+22 h). The strength protocol is based on the guidelines of NSCA [31]. All subjects performed three warm-up sets and started at 50% of their planned maximum power with 10 repetitions. In the following warm-up sets, the weight was increased by about 10–20% and the repetition number reduced to five and three repetitions. This was followed by four 3-RM tests, starting with about 90% of the planned 3-RM. Between the sets a four-minute break was taken. The subsequent increases were made individually. If a load level was not successfully mastered twice in succession, the test was stopped.

3.7. Statistical Analyses

Quantitative variables were presented as mean values and standard deviations (SD). All measurement parameters were tested for the Normal Distribution. As a result, the Wilcoxon sign rank test (repeated measurement) and the Kruskal-Wallis test were used for Myo, CK, IL6, IL10, glucose and insulin. For MIF and 3-RM BS, a 2-way Analysis of Variance (ANOVA) with time and condition effects, and a dependent (Student's) t-test, were used. The current version of SPSS (IBM SPSS Statistics 25.0, Ehningen, Germany) was used for statistical analysis, and $p < 0.05$ was taken as the level of statistical significance for all procedures. The images were created using GraphPad PRISM software (GraphPad Software, Inc. La Jolla, CA, USA).

4. Results

4.1. Effects of Exercise and Protein/Carbohydrate on Blood Glucose and Insulin Concentrations

It has been shown that the consumption of protein/carbohydrate combinations after exercise influences the blood sugar and insulin response, which is discussed to improve regeneration.

In Figure 2A, showing mean serum glucose concentrations of all participants ($n = 35$), a significant increase of serum glucose compared to t0 is detectable in the control and the shake condition at t1.1 (+20 min). A significant decrease compared to t0 is detectable in the shake condition and the food condition at t2 (+60 min).

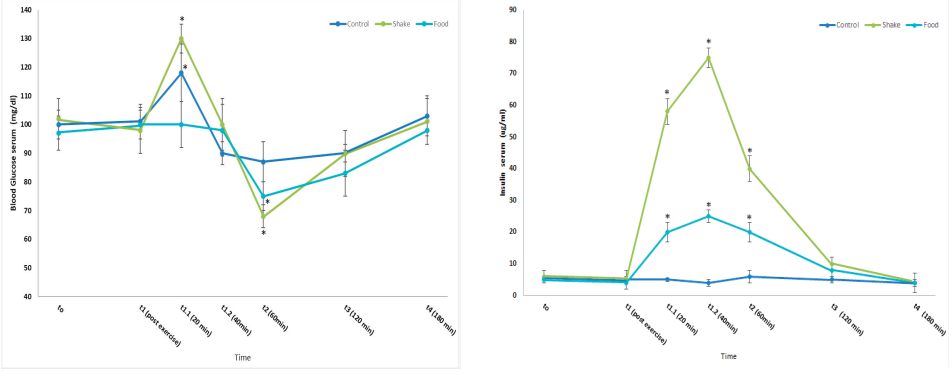

Figure 2. Effects of protein/carbohydrate uptake via food and shake, immediately after exercise, on serum glucose and serum insulin serum concentrations. Left: Mean serum blood glucose concentrations of all participants. All data are expressed as mean ± SD. * = $p \leq 0.05$. Statistically significant differences between marked time points and respective t0 value are observed. Right: Serum insulin concentrations of all participants (Mean ± SD). * = $p \leq 0.01$ show statistically significant differences between marked time point and respective t0 value in each condition.

In Figure 2B, mean serum insulin concentrations of all participants are shown. A significant increase of serum insulin compared to t0 is detectable in the food and the shake condition at t1.1 (+20 min), t1.2 (+40 min), t2 (+60 min) and t3 (+120 min).

4.2. Effects of Exercise and Protein/Carbohydrate on Hematopoietic Parameters

In agreement with published data [32] a significant decrease in the total leucocyte number in conditions could be observed 3 h (t4; +180 min) after exercise. This effect was not affected by any nutritive intervention (data not shown). After 24h (t5; +22 h), the total leucocyte number was again on the baseline in each condition. All other investigate hematopoietic parameters remained also unaffected by exercises and the nutritive intervention (data not shown).

4.3. Effects of Exercise and Protein/Carbohydrate on Blood Cortisol Levels

Serum Cortisol levels in athletes have been demonstrated to be influenced by physical activity and nutrition [33]. Figure 3 shows mean cortisol serum concentrations from all participants. The well described circadian rhythm of cortisol could be observed in all intervention conditions. Neither physical activity nor the nutritive interventions resulted in significant effects.

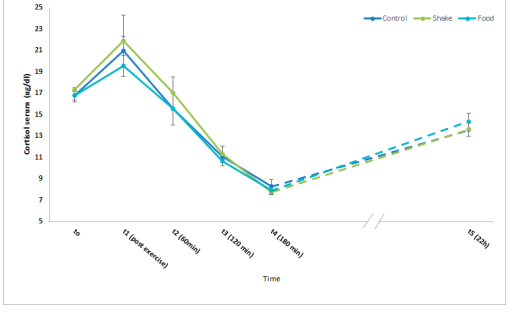

Figure 3. Effects of protein/carbohydrate uptake via food or shake, immediately after exercise, on serum cortisol concentrations. Mean serum cortisol concentrations of all participants. Mean ± SD. $p \leq 0.05$ show statistically significant differences between marked group and respective t0 value.

4.4. Effects of Exercise and Protein/Carbohydrate on Markers of Inflammation

Skeletal muscle damage results in an induction of inflammation. Interleukin 6 (IL 6), Interleukin 10 (IL 10), and Macrophage migration inhibitory factor (MIF) in the serum at the time point's t0 and t4 (+180 min) were measured after exercise as markers for inflammation.

MIF serum levels (n = 18) were significantly increased in all conditions, however, in the food and shake conditions, the increase was significantly lower, compared to the control. (Figure 4A) IL 6 serum levels (n = 27) were significantly increased by exercise (Figure 4B). No significant differences could be observed between the shake and the food condition. Il10 serum levels (n = 28) were significantly increased in all conditions after exercise; however, the increase in the shake and food condition was significantly higher (Figure 4C).

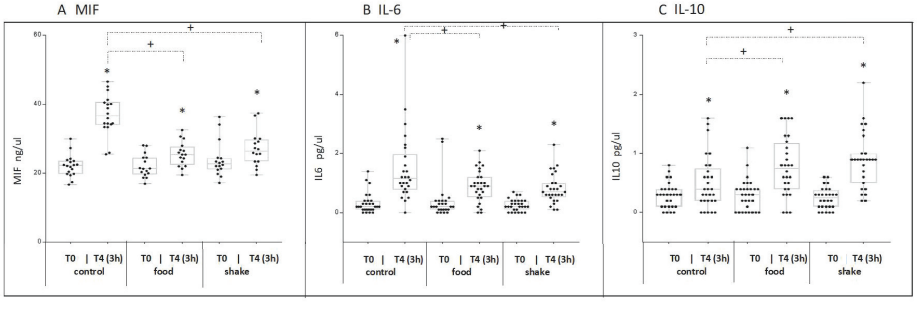

Figure 4. Effects of protein/carbohydrate uptake via food and shake, immediately after exercise, on serum levels of MIF (**A**) IL 6 (**B**) IL 10 (**C**) and 3h after exercise (t4; +180 min)). Shown are mean individual serum concentrations of the respective cytokines (A, B, C). Shown is mean ± SD. * = $p \leq 0.05$, statistically significant differences between marked time point and respective t0 value. + = $p \leq 0.05$ show statistically significant differences between marked groups.

4.5. Effects of Exercise and Protein/Carbohydrate on Serum Markers for Skeletal Muscle Damage

Skeletal muscle creatine kinase (CKmm) and myoglobin (Myo) are markers for skeletal muscle damage. Figure 5A shows the mean absolute Myo serum concentrations (n = 15) and Figure 5B shows the mean absolute of CKmm (n = 15).

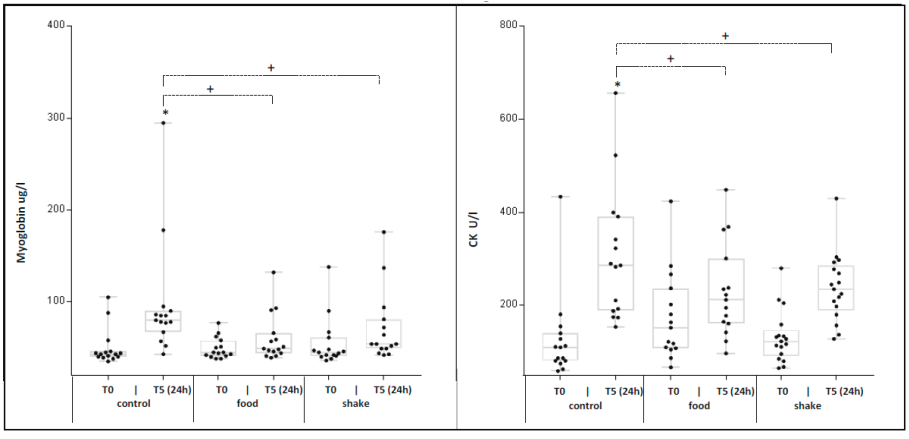

Figure 5. Effects of protein/carbohydrate uptake via food and shake on serum creatine kinase (CK) and myoglobin (Myo) concentrations, immediately after exercise, and 24 h after exercise. Shown are mean individual serum concentrations Myo (**A**) and CK (**B**). Mean of all individuals ± SD. * = $p \leq 0.05$ shows statistically significant differences between marked time point and respective t0 value. + = $p \leq 0.05$ show statistically significant differences between marked groups.

Exercise in the control condition resulted in a significant increase of CKmm and Myo in the blood at t5 (+22 h). In the shake but also in the food condition exercise induced increase of skeletal muscle damage markers and was significantly lower compared to the control.

4.6. Effects of Exercise and Protein/Carbohydrate on Leg Strength as a Functional Marker for Skeletal Muscle Regeneration

We postulated that a better regeneration after endurance exercise will result in a lower loss of muscle strength (n = 15). As a functional approach for leg strength the 3-repetition maximum back squat was used. As seen in Figure 6, the uptake of protein/carbohydrate resulted in no significant loss of leg strength after endurance exercise. Only in the control condition did endurance exercise resulte in significantly lower leg strength at t5 (+22 h) after exercise.

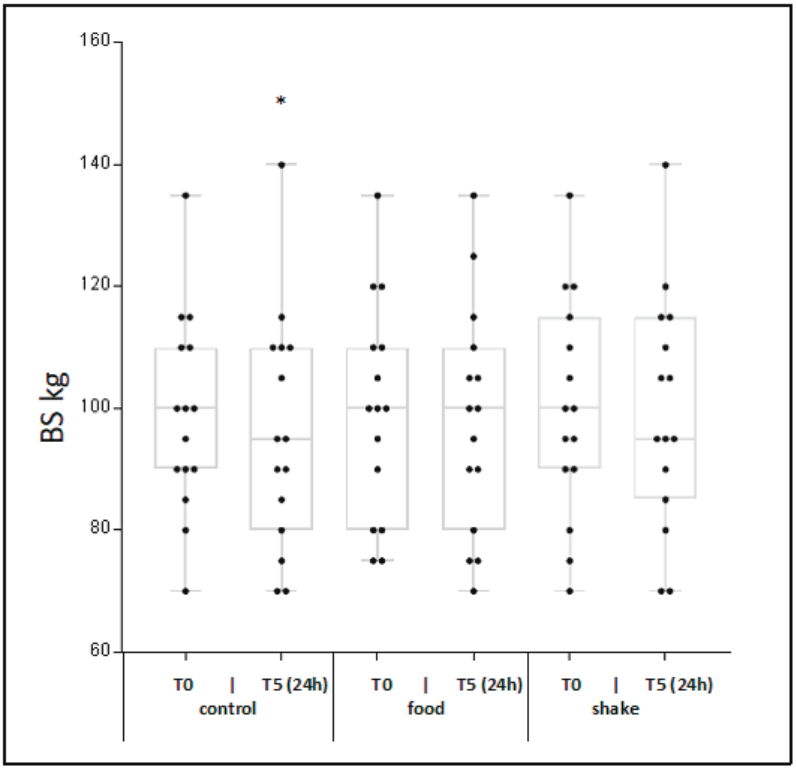

Figure 6. Effects of protein/carbohydrate uptake via food and shake on leg strength measured by the 3-repetition maximum back squat. In Figure 6 individual leg strength is shown Mean ± SD. * = $p \leq$ 0.01 show statistically significant differences between t5 and respective t0 value.

5. Discussion

In the present study, we compared pro-regenerative effects of protein and carbohydrates administration on the skeletal muscle regeneration after exercise, given either by shake or by a standardized meal. Pro-regenerative effects of protein/carbohydrate combinations after endurance exercise have been described and mechanistically linked to a stimulation of insulin secretion [21]. Therefore, in this investigation blood glucose levels and insulin levels were measured at different time points after exercise and subsequent protein/carbohydrate ingestion.

It was clearly visible that exercise results in an increase of blood glucose concentrations 20 min after exercise termination. This is in agreement to our previous investigations [28] and confirms observations that moderate exercise increases blood glucose concentrations in healthy non-diabetic persons when a required exercise volume is achieved [34]. As expected, blood glucose concentration after administration of the shake extended a maximum 40 min after ingestion. In contrast, after food administration, no significant increase of blood glucose concentration could be observed, although a comparable amount of carbohydrates was ingested (Table 2). Both shake and also food ingestion resulted in a significant increase of insulin serum concentration, starting 20 min after exercise/ingestion, and reaching a maximum after 40 min. The increase of insulin in the food condition is lower, compared to a shake, which could be explained by the lower content of total carbohydrates (35 g compared to 45 g in the shake condition). Nevertheless, the kinetics of insulin in both conditions is absolutely comparable.

A possible explanation for the missing increase of blood sugar levels in the food condition may be the direct uptake of the resorbed sugars in the skeletal muscle and the liver. In the shake condition, same mechanisms may be relevant, but the faster and higher load of glucose could not be compensated completely. In general, our observation is supported by data demonstrating that, combining protein and carbohydrate increases insulin levels, but does not improve glucose response [35]. Our observation is also in line with published data, showing that the uptake of specific amounts of carbohydrates results in a much stronger stimulation of insulin secretion when they are combined with proteins [17,21].

Hematopoietic factors and cortisol serum concentrations were not affected in our study by any ingestion of carbohydrates and proteins, but the response of pro- and anti-inflammatory serum markers to exercise was strongly influenced. Here it has to be mentioned that the role of inflammation in the skeletal muscle's adaptation to exercise is complex. Acute inflammatory response to exercise seems to promote skeletal muscle training adaptation and regeneration. In contrast, persistent, low-grade inflammation, as seen in a multitude of chronic diseases, is obviously detrimental [36]. Acute exercise has been described to induce an inflammatory response in the skeletal muscle. This can be detected by changes in the serum concentrations of inflammation-related cytokines [37,38]. Strenuous exercise results in changed serum concentrations of pro-inflammatory and anti-inflammatory cytokines like TNF alpha, IL-1, IL-6, IL-1 receptor antagonist, TNF receptors, IL-10, IL-8 and macrophage inflammatory protein-1 [38]. Increase of pro- and anti-inflammatory cytokines has been also described in animal models were skeletal muscle damage is induced by notoxin [39]. For these reasons the circulating levels of the pro-inflammatory cytokines IL6, and MIF and the anti-inflammatory cytokine IL10, were investigated 3 h after exercise in this study. We observed a significant increase of the serum concentrations of the pro-inflammatory cytokines IL6 and MIF after exercise in the control condition, which was significantly lower in both protein/carbohydrate conditions. In contrast, the serum concentrations of the anti-inflammatory cytokine IL10 were even more increased after exercise in both protein/carbohydrate conditions compared to the control condition. Remarkably, the effects after ingestion of the proteins by shake and by food are quantitatively and qualitatively comparable. These regulation patterns demonstrate that administration of protein/carbohydrate after exercise reduces the excretion of pro-inflammatory cytokines, but increases the excretion of anti-inflammatory proteins in the serum, which can be interpreted as an anti-inflammatory effect. Indeed, in an animal model where muscle damage was induced by administration of notoxin, accelerated skeletal muscle recovery correlated with increased IL10 expression and decreased TNF-alpha expression in the respective skeleton muscles [39]. As our study demonstrated, while a complete suppression of inflammation after exercise, e.g., after administration of glucocorticoids [40], promotes catabolic effects in the skeletal muscle, a modulation of the inflammatory response can be interpreted as an indication for pro-regenerative effects.

An important physiological endpoint investigated in this study was skeletal muscle damage and post-exercise recovery. Some studies show no effects of proteins/carbohydrates ingestion on skeletal muscle recovery [41] after exercise, while other investigations, however, have demonstrated a reduced post-exercise muscle soreness [42] and lower plasma concentrations of Myo [43] and CK [44] by administration of proteins/carbohydrates before, after, or during endurance exercise. Therefore, in our investigation CKmm and Myo concentrations were measured time-dependently after exercise, and a significant increase of the mean serum CK and Myo concentrations was detectable in the control condition 22h after training. The increase was not detectable in both protein/carbohydrate conditions. Our observation might be interpreted as an indication that skeletal muscle damage, induced by exercise, is lower after the uptake of protein/carbohydrate combinations. This is in line with the observation of Diel et al. [28] and previous described results [14]. Our data also indicate that administration of protein/carbohydrate combinations by food was as effective as administration by shake.

This hypothesis is further supported by our functional regeneration marker, the measurement of leg strength. The reduction of muscle strength especially after endurance training is well reported [45]. As molecular mechanisms for this reduction, reduced numbers of connective structures between actin

and myosin filaments, and a reduced sensitivity for calcium, are discussed [46–48]. We postulate that a better regeneration after endurance exercise will result in a lower loss of muscle strength. As a functional approach, we have chosen the 3-repetition maximum back squat assay, which is believed to be one of the most effective training approaches based on the interaction of several muscle groups in the leg [49]. We found that the loss of leg strength after endurance is significantly lower in the nutritive intervention conditions, compared to the control condition. This is an obvious functional indication that uptake of protein/carbohydrate results in pro-regenerative effects. Again, food and shake are of comparable effectiveness.

Our study has several limitations. One limitation is that the food and the shake in our study do not match perfectly for macronutrient content and calories. However, for calories both groups differ only by 17%. With respect to macronutrient content, a perfect match is not possible because protein/carbohydrate shakes do not contain fat. Moreover, in our study, although there is no perfect match, food is lower in calories, protein and glucose compared to shake, but nevertheless we could observe comparable effects. Another limitation of our study is that important parameters related to skeletal muscle recovery, protein synthesis and protein breakdown, were not investigated. These are of course important endpoints which should be addressed in future studies and in a similar design.

Our study also has strength. As biological markers for skeletal muscle damage, we did not only focus on CK. CK is highly debated as a suitable parameter [50]. Therefore we have also analyzed Myo and used two independent markers for skeletal muscle damage in comparison. Looking for effects on the immune response, we have investigated a panel of different cytokines, as well pro- and anti-inflammatory ones. This also strengthens the interpretation of our results.

In summary, our results demonstrate that ingestion of protein and carbohydrate combinations by a shake, but also by a nearly isocaloric meal immediately after endurance exercise, affects a variety of physiological endpoints. In our study, uptake of protein and carbohydrate combinations by shake and meal resulted in a strong increase of insulin serum concentrations of the participants. Moreover, a modulation of any inflammatory response of the skeletal muscle towards exercise, indicated by reduced concentrations of pro-inflammatory markers, and an increase in anti-inflammatory markers, could be observed. The endurance training induced an increase of serum CKmm and Myo, markers of skeletal muscle damage, and as a functional marker for regeneration, the loss of leg strength was observed. All this could be antagonized by protein/carbohydrate combinations, given either by a shake or a meal, respectively. Mechanistically our data provide evidence that a combined uptake of protein and carbohydrates appears to reduce skeletal muscle damage after endurance exercise via a modulation of the immune response of the skeletal muscle. Also, insulin seems to be involved in initiation and mediation of the pro-regenerative effects. The most important part our observation is that all these beneficial effects can be achieved, either by the ingestion of food containing sufficient concentrations of carbohydrates and protein, or with the same efficiency as consuming a shake. This finding demonstrates again that it is possible to develop concepts to support training by a suitable diet without the need to consume nutrition supplements or isolated proteins.

Author Contributions: E.I. = main investigator; F.B., D.A.B., V.H., S.P., L.S. = supporting investigators; S.S., W.S. = statistics, P.D. = study design.

Abbreviations

ANS	anaerobic threshold
CK	Creatine kinase
CKmm	Creatine kinase skeletal muscle
Myo	Myoglobin
IL 10	Interleukin 10
IL 6	Interleukin 6

MIF	Macrophage migration inhibitor factor
SD	standard deviations
SEM	standard error of the mean
TNF alpha	Tumor necrosis receptor alpha
km	kilometer
IGF 1	insulin like growth factor 1
mTOR	mechanistic Target of Rapamycin

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The Effectiveness of Nutrition Education for Overweight/Obese Mother with Stunted Children (NEO-MOM) in Reducing the Double Burden of Malnutrition

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Abstract: (1) Background: In households experiencing the double burden of malnutrition, stunted children are in a better position for growth improvement when parents are able to direct their resources to support nutrition requirements. This study assesses the effectiveness of maternal nutrition education to reduce child stunting. (2) Methods: This was a Randomized Controlled Trial involving pairs of overweight/obese mothers with stunted children aged 2 to 5 years old in urban Indonesia. Methods: Seventy-one mother-child pairs were randomly assigned to receive either a 12-week nutrition education or printed educational materials. Mixed factorial ANOVA was used to test for between-group differences over time in relation to child's height, weight, maternal self-efficacy, outcome expectation, and caloric intake. (3) Results: Across groups, there was a significant effect of time on child height and weight but no significant differences were observed between-groups. Maternal self-efficacy, outcome expectations in providing animal protein for the children (p-value = 0.025) and mother's total caloric intake (p-value = 0.017) favored the intervention group over the comparison group. (4) Conclusions: The behavioral intervention produced strong improvement in maternal self-efficacy to engage in physical activity, eat fruits and vegetables and to provide children with growth-promoting animal protein, but did not significantly influence child height gain.

Keywords: nutrition education; health promotion; behavioral intervention; self-efficacy; stunting; overweight; obesity; physical activity; dual burden of malnutrition

1. Introduction

In developing countries, one fourth of children under the age of five fail to grow normally because of a condition known as stunting [1]. Stunting is a condition where the child is shorter than their normal peers as measured using the height-for-age z-score (HAZ) of less than minus two according to the child growth standard from the WHO-Anthro 2005. Child stunting is a public health nutrition

problem that hinders the development of future generations. Compared to their non-stunted peer, stunted children have shown to be more susceptible to gain more fat mass than lean mass in a cohort in Brazil [2]. After 7 to 9 years follow up, previously stunted children at 2 years of age were significantly shorter and lighter but their body mass index (BMI) or centralization of body fat was not significantly different from non-stunted South African children [3]. Beyond physiological effects, stunting may limit a child's cognitive abilities and productivity [4]. In light of these damaging consequences, the WHO and its member countries are working to achieve a 40% reduction in child stunting by 2025 through the Scaling-Up Nutrition (SUN) program [4].

Effective community-based interventions must be developed to ameliorate child stunting and support WHO and UNICEF programs to combat child growth problems worldwide. A systematic review of the literature to explore the impact of education and complementary feeding on growth of children under 2 years of age in developing countries showed positive results [5]. In a subgroup analysis of the food secure population, child-feeding education alone yielded a significant improvement in height gain in children under the age of 2 years [5]. A previous study in Bangladesh that assessed a 3-month nutrition education intervention along with complementary feeding showed promising results for height gain [6,7]. The effect of providing complementary food and intensive nutrition education on height gain (cm) in Bangladesh was 0.80 (95% Confidence Interval (CI) = 0.007–1.53) [7]. A systematic review of community-based nutrition education programs revealed significant results when community leaders met with caregivers twice a week in their home, to deliver nutrition education programs and cooking demonstrations [8].

A demographic shift in conjunction with an epidemiological and nutrition transition has created an unusual situation in which both over- and under-nutrition occur within the same population. Child stunting is a persistent feature of this problem, known as the double burden of malnutrition. A study in a Guatemalan population informed our hypothesis that in households suffering from the double burden of malnutrition, stunted children are less likely to experience food insecurity. Results of that study revealed that the prevalence of coexistence of under-nutrition (child stunting) and over-nutrition (maternal overweight/obesity) was highest (22.7%) among those in the middle (third) quintile of socioeconomic status (SES) [9]. The study showed that maternal overweight was positively related to higher economic status while child stunting was negatively associated with higher household economic status. Lack of access to food geared to the fulfilment of dietary energy was influential for the high prevalence of child stunting but not playing the major role to double burden of malnutrition as mothers exceeded their energy consumption. Households that was suffering from double burden of malnutrition did not necessarily lacking in food access in term of energy intake. It is believed that the difference was coming from unequal food distribution in terms among household's member. Larger number of family member or having extended family would increase the change of unmet nutrient requirement among member of the household as it varies across age groups. Top with low level of maternal nutritional literacy the problem of children having less nutrient intake resulted in their failure to grow (stunted) but the adults having excess energy intake ending up with overweight and obesity. This evidence suggests that in this socioeconomic group, relative to the others, in the absence of food insecurity and economic deprivation, modifiable factors such as food distribution and dietary diversity within the household were associated with the double burden of malnutrition. Furthermore, these households appeared to lack the capacity to direct resources properly to prevent child stunting. More specifically, we hypothesize that mothers were unable to make healthy food choices and manage food supply and distribution within the household.

A behavioral intervention was developed to target modifiable behaviors related to the double burden of malnutrition and to equip mothers with skills necessary to overcome these problems. The study was conducted in an urban setting in Indonesia through the Nutrition Education for Overweight/Obese Mother with Stunted Children (NEO-MOM) intervention. Drawing on concepts from Social Cognitive Theory (SCT), participants were prompted to set goals for themselves to improve

their dietary habits and child feeding behaviors, with a focus on self-efficacy, nutrition literacy and dietary diversity.

This study was designed to test the hypothesis that for households facing the double burden of malnutrition in urban Indonesia, a behavioral intervention, coupled with a government food supplementation program, would be more effective than standard care combined with print educational materials for improving child outcomes for height and height-for-age *z*-score, maternal outcomes for weight, waist circumference, BMI, dietary diversity, dietary intake, self-efficacy, outcome expectations and nutrition literacy.

2. Materials and Methods

This randomized controlled trial (RCT) assessed the effectiveness of a behavioral intervention aimed at empowering mothers to address the double burden of malnutrition within the household.

2.1. Theory

The intervention was based on concepts of Bandura's Social Cognitive Theory (SCT) [10], which are briefly mentioned here. Following the constructs of Social Cognitive Theory, we measured the mother's self-efficacy, outcome expectations and knowledge measured as nutrition literacy. We developed eight measures of maternal self-efficacy, according to Bandura's guidelines for constructing self-efficacy scales [11].

2.2. Sample Size and Allocation

Based on the previous study in Bangladesh [7], which found 0.8 effect size of a three months length nutrition education intervention accompanied with complementary feeding and using 90% power, a minimum of 66 total samples were required to detect changes in height gain with two tailed alphas of 0.05. At baseline this study involved 71 eligible samples that was randomly allocated to 35 in the intervention group and 36 in the comparison group/usual care. Details about the methodology and protocols of the study can be found elsewhere [12]. This study did not compare the effect of the intervention group (NEO-MOM group) with a true control group, but with a comparison group that received printed educational materials (PRINT group) plus government supplementation on child stunting and maternal overweight/obesity (see Figure 1).

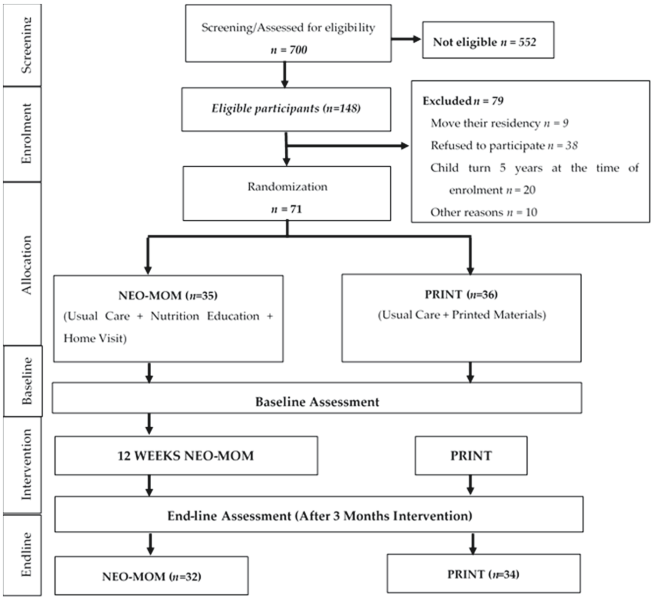

Figure 1. Adapted CONSORT diagram of the Nutrition Education for Overweight/Obese Mother with Stunted Children (NEO-MOM) study.

2.3. Dietary Data

We collected two 24-h dietary food recalls per mother at set times, baseline, and at the end of intervention (three months after baseline). A portion-size guide and food models were provided to parents to assist them in estimating portion sizes. Dietary data was analyzed using NutriSurvey, a software that draws on a database containing nutrition information on typical Indonesian Food. The database is updated yearly by the Department of Nutrition, Universitas Airlangga (UA)–Indonesia. Dietary diversity was calculated following the guidance developed by the Food and Agriculture Organization of the United Nations (FAO) where mothers were asked to recall dietary consumption in the past 24 h. The answer was then aggregated into 12 food groups to create the household's dietary diversity score (HDDS). The 12 food groups were cereals, tubers/roots, vegetables, fruits, fish, meat and poultry, eggs, nuts and seeds, dairy products, spices, oils and fats, and sweets. The dietary diversity score was ranging from 0 to 12.

2.4. Behavioral Measures

We measure maternal self-efficacy based on the Likert-scale ranged from 0 to 100 and covered barriers and tasks for mothers related to being physically active, to eating fruits and vegetables and providing children with animal protein in their meals. The animal protein in question was referring to any source of protein coming from animal-based products that include fish, meat and poultry, eggs, and dairy products. Outcome expectations were measured with a series of questions for the same tasks rated on a scale from 1 to 5, with 1 representing a strong disagreement and 5 representing a strong agreement. Nutrition literacy was measured in three domains: knowledge of macronutrients, skill in household food measures, and skills in grouping food in categories. There were 6-item of close-ended questions in the macronutrient's domain, and also there were 6-item questionnaire in the household food measure domain that was reflecting the common household measurements used in Indonesia. The food groups domain was adapted from the original American "*MyPlate*" to the Indonesian version of MyPlate known in Indonesian language as "*Piring Makanku*" or recently promoted as "*Isi Piringku*".

2.5. Statistical Analysis

For all variables that were normally distributed or transformed to normality, we analyzed the difference in the outcome from control and intervention group using a mixed factorial ANOVA. The within-subjects variables were the outcome variables in this research, and the between-subject variable was the group of intervention (NEO-MOM and PRINT). We used the household food insecurity access scale (HFIAS) score as covariates in the analysis. Furthermore, we conducted the ANCOVA test to see the difference in changes of primary and secondary outcome adjusted for its baseline value and the HFIAS score. For nonparametric statistics, we employed the two related-samples Mann Whitney *U* test and analyzed the data separately for the NEO-MOM group and the PRINT group with Bonferroni correction. All data analyses were performed in IBM SPSS Statistics 22 (Armonk, NY, USA). The statistical significance for all the tests was set at an alpha level of 0.05.

2.6. Ethical Clearance

This study was approved by The Institutional Review Board (IRB) at Kansas State University (reference number: 7894) as well as approved by the Surabaya City Review Board (Bakesbangpol No: 1366/LIT/2015) in Indonesia. All participants were explained about the study and signed the informed consent following the World Health Organization procedure, this study obtained the Universal Trial Number (UTN) U1111-1175-5834 and also registered in the Australian New Zealand Clinical Trials Registry (ANZCTR) and allocated the registration number: ACTRN12615001243505.

3. Results

3.1. Characteristics of Participants and Groups

Table 1 summarizes characteristics of the participants (children, mother, and household) at baseline in the NEO-MOM and PRINT groups. Almost all of the variables were similar and did not have significant different with the exception of child's weight (p-value = 0.045) and monthly food expenditure (p-value = 0.010). The average of child's weight in the NEO-MOM group was significantly lower (mean = 11.32 kg) compare to the PRINT group (mean = 12.25 kg). Monthly food expenditure in the NEO-MOM group was also around IDR 500,000 lower than those in PRINT group.

Table 1. Participants characteristics at baseline (n = 71).

Variable	NEO-MOM (n = 35)		PRINT (n = 36)		p-Value
	Mean	(SD)	Mean	(SD)	
Child characteristics					
Age (months)	39.57	7.82	40.24	8.11	0.679
Weight (kg)	11.32	1.92	12.25	2.44	0.045 *
Height (cm)	86.84	6.34	89.43	5.34	0.060
Height-for-age Z-score	−2.998	0.85	−2.674	0.57	0.071
Maternal characteristics					
Age (years)	34.09	6.86	31.47	6.76	0.123
Education (years)	7.29	7.75	7.89	3.62	0.417
Weight (kg)	64.59	8.83	67.99	10.15	0.233
Height (cm)	147.43	5.11	148.23	4.67	0.441
Waist circumference (cm)	92.61	8.30	93.91	9.72	0.364
Household characteristics					
Dietary diversity score	7.29	1.86	7.22	1.80	0.737
HFIAS score	8.94	5.75	5.92	5.49	0.061
Monthly Income (IDR)	1,532,857	512,769	2,116,666	1,448,562	0.054
Monthly Food expenditure (IDR)	974,074	343,726	1,217,307	316,418	0.010 *
Maternal physical activity					
Average daily step	3156	2134	2899	2356	0.196

Note. Significance based on $\alpha = 0.05$; * p-value < 0.05. The analysis is based on Independent t-test.

3.2. Intervention Effect on Outcomes and Mediators

We used the HFIAS score as covariates when testing for an intervention effect in a mixed repeated measure ANOVA.

3.2.1. Child's Outcomes

There were no significant effects observed in the group-by-linear-time trend interaction for any of the child health outcomes, but we observed a significant time effect for child weight (p-value = 0.023) and child height (p-value = 0.001). There were significant increases in weight (p-value < 0.001) and child height (p-value < 0.001) for all groups in a pairwise comparison from baseline to 3-month after baseline evaluation (Figure 2). The mean group difference for child height was 2.47 cm (95% CI = 1.55 to 3.39) and for child weight it was 0.58 kg (95% CI = 0.32 to 0.85). The ANCOVA test showed that the change in child's height and weight was not significantly different between NEO-MOM and PRINT group (p-value = 0.526 and p-value = 0.431 respectively). In terms of child height-for-age z-score (HAZ), the observed improved value was not statistically significant using the related-samples Mann Whitney U test for both the NEO-MOM and PRINT groups (p-value = 0.183 and p-value = 0.051, respectively).

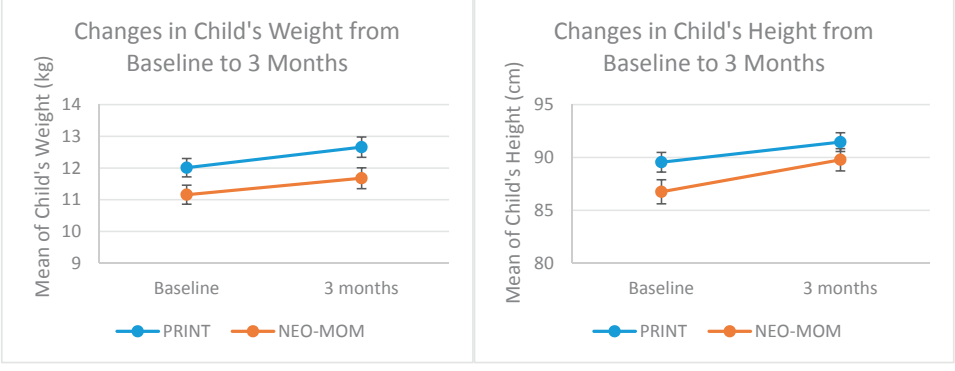

Figure 2. Profile plot of child's weight and height change from baseline to three months evaluation.

3.2.2. Maternal Outcomes

There were no significant effects in the group-by-linear-time trend interaction for maternal anthropometric outcomes such as weight, waist circumference, and the BMI. Similarly, there were no significant mean group differences from baseline to three months after the baseline evaluation for maternal weight (p-value = 0.223), waist circumference (p-value = 0.929), and the BMI (p-value = 0.066). In this analysis, to achieve normal distribution of the data, BMI was transformed using logistic transformation. There was no significant difference in the effect of study condition for any of the maternal outcome measures. As seen in Table 2, after three months intervention, the ANCOVA test revealed that the change in mother's weight and waist circumference was not significantly different between NEO-MOM and PRINT group (p-value = 0.871 and p-value = 0.397 respectively).

3.2.3. Household Dietary Diversity

The household dietary diversity score decreased for both NEO-MOM and PRINT group after the 3-month period. In the non-parametric related samples Mann Whitney U test, the results showed statistical significance at Z = −2,847 (p-value = 0.004) and Z = −3.380 (p-value < 0.001). The decline in the dietary diversity score was steeper in the PRINT group (from 7.29 at baseline to 5.68 after three months) than in the NEO-MOM group (from 7.44 at baseline to 6.50 after the 3-month intervention).

Table 2. The ANCOVA test results on primary outcomes & maternal self-efficacy (n = 66).

Variable	F	p-Value	Partial Eta Squared	Adjusted R Squared
Child's outcomes				
Weight (kg)	0.629	0.431	0.010	0.151
Height (cm)	0.407	0.526	0.007	−0.022
Maternal outcomes				
Weight (kg)	0.027	0.871	0.000	−0.028
Waist circumference (mm)	0.726	0.397	0.012	−0.025
BMI #	0.115	0.736	0.002	−0.023
Maternal self-efficacy (Barrier)				
Being physically active	2.035	0.159	0.032	0.323
Eating fruit	10.011	0.002 *	0.139	0.404
Eating vegetables	10.238	0.002 *	0.142	0.236
Providing animal protein for kids	5.224	0.026 *	0.078	0.474
Maternal self-efficacy (Task)				
Being physically active	3.922	0.052	0.059	0.276
Eating fruit	4.624	0.036 *	0.070	0.096
Eating vegetables	3.137	0.081	0.048	0.179
Providing animal protein for kids	4.468	0.039 *	0.067	0.081

Note. Significance based on $\alpha = 0.05$; * p-value < 0.05. # The analysis is based on log-transformed variable.

3.2.4. Maternal Self-Efficacy

We measured maternal self-efficacy in terms of barriers and task performance of four behaviors: being physically active, eating fruit, eating vegetables, and providing children with animal protein. All measures of maternal self-efficacy were having good internal consistency indicated by having Cronbach alpha > 0.65. The group by time interaction on maternal self-efficacy in dealing with barriers was significant for all measures, with rates of increase being more strongly positive in the NEO-MOM group than in the PRINT group (Table 3). The group by time interaction on maternal self-efficacy barriers for being physically active, eating fruits, eating vegetables, and providing the child with animal protein were all statistically significant (p-value = 0.030, 0.006, 0.002, and 0.042, respectively). As seen in Figure 3 the improvement in maternal self-efficacy barriers to provide their child with animal protein was in the right direction for the NEO-MOM group (from 60.36 at baseline to 67.24 after the 3-month evaluation) in contrast to the PRINT group, which showed a decrease (from 63.47 at baseline to 57.78 after a 3-month evaluation). There was a significant time effect for the maternal self-efficacy barrier of eating vegetables (p-value < 0.001). However, a similar time effect was not observed within subjects for the other three measures. There was no significant result in the between-subjects test. The group by linear time trend interaction effects on maternal self-efficacy to perform certain tasks was only significant for the task of eating fruit (p-value = 0.043) and for the task of providing animal protein for the child (p-value = 0.032) (See Figures 4 and 5). The rate of increase in the maternal self-efficacy in the task of eating fruit was strongly positive in the intervention condition (from 49.16 at baseline to 58.19 after the 3-month evaluation) than the comparison condition, which showed a negative trend (from 50.08 at baseline to 47.66 after a 3-month evaluation).

Table 3. Group means and test of within- and between- subject effect (n = 66).

Outcome/Mediator	Mean (SE)				Test of Within-Subject Effect		Test of Between-Subject Effect
	Intervention (n = 32)		Comparison (n = 34)		Partial Eta Square (p)		
	Baseline	3 Month	Baseline	3 Month	Time	Time by Group Interaction	Group Difference
Child's outcomes							
Weight (kg)	11.19 (0.31)	11.69 (0.34)	11.98 (0.29)	12.64 (0.33)	0.080 (0.023) *	0.005 (0.578)	0.059 (0.050) *
Height (cm)	86.80 (1.07)	89.90 (1.01)	89.49 (1.04)	91.34 (0.98)	0.173 (0.001) **	0.027 (0.189)	0.034 (0.143)
Maternal outcomes							
Weight (kg)	65.17 (1.49)	64.87 (1.54)	68.35 (1.44)	67.91 (1.49)	0.034 (0.139)	0.001 (0.819)	0.033 (0.146)
Waist circumference (mm)	92.18 (1.60)	91.73 (1.80)	95.08 (1.56)	95.67 (1.74)	0.000 (0.992)	0.008 (0.505)	0.034 (0.141)
BMI [a]	30.13 (0.62)	29.95 (0.59)	31.01 (0.66)	30.63 (0.75)	0.054 (0.062)	0.006 (0.543)	0.022 (0.235)
Maternal self-efficacy (Barrier)							
Being physically active			50.55 (2.53)				
Eating fruit	43.95 (2.61)	49.11 (2.88)	55.66 (3.69)	45.34 (2.79)	0.008 (0.472)	0.072 (0.030) *	0.003 (0.650)
Eating vegetables	53.04 (3.80)	62.49 (3.41)	38.03 (2.54)	49.82 (3.30)	0.025 (0.207)	0.115 (0.006) *	0.021 (0.251)
Providing animal	35.29 (2.62)	61.13 (3.39)	63.47 (3.83)	48.54 (3.28)	0.376 (<0.001) ***	0.146 (0.002) **	0.029 (0.174)
protein for kids	60.36 (3.95)	67.24 (3.31)		57.78 (3.21)	0.008 (0.491)	0.064 (0.042) *	0.009 (0.448)
Maternal self-efficacy (Task)							
Being physically active	34.71 (3.99)	56.19 (4.17)	30.53 (3.86)	43.62 (4.04)			
Eating fruit	49.16 (2.12)	58.19 (3.73)	50.08 (2.09)	47.66 (3.67)	0.203 (<0.001) ***	0.026 (0.199)	0.047 (0.083)
Eating vegetables	55.17 (2.19)	58.57 (3.48)	55.30 (2.12)	49.98 (3.38)	0.028 (0.185)	0.071 (0.034) *	0.031 (0.167)
Providing animal	63.51 (1.83)	63.18 (3.42)	64.78 (1.78)	54.20 (3.32)	0.014 (0.355)	0.041 (0.105)	0.028 (0.182)
protein for kids					0.002 (0.717)	0.071 (0.032) *	0.024 (0.217)

Note. Significance based on $\alpha = 0.05$; *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05. [a] The analysis is based on log-transformed variable.

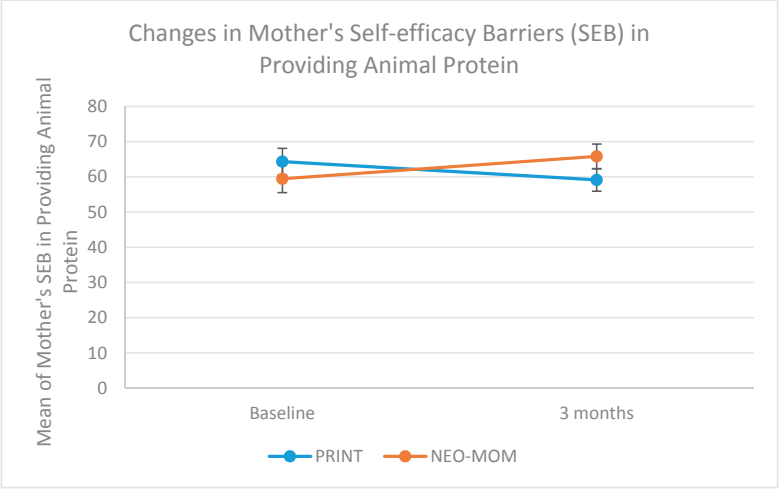

Figure 3. Profile plot of maternal self-efficacy (barriers) for providing animal protein.

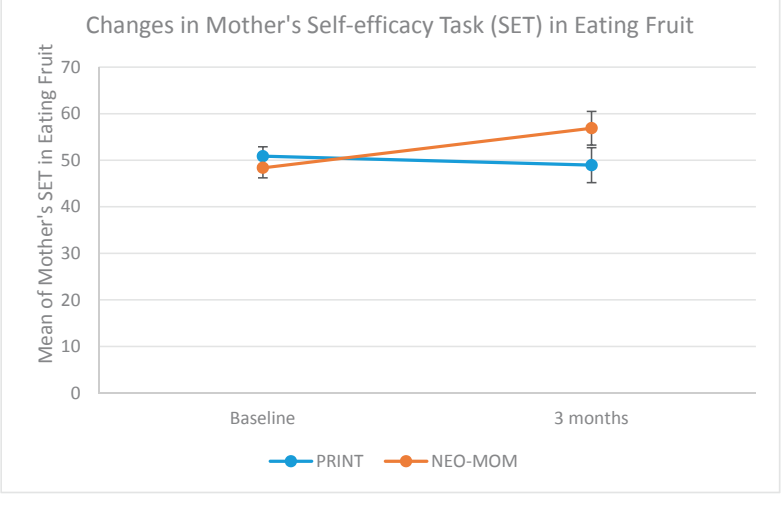

Figure 4. Profile plot of maternal self-efficacy (task) for eating fruit.

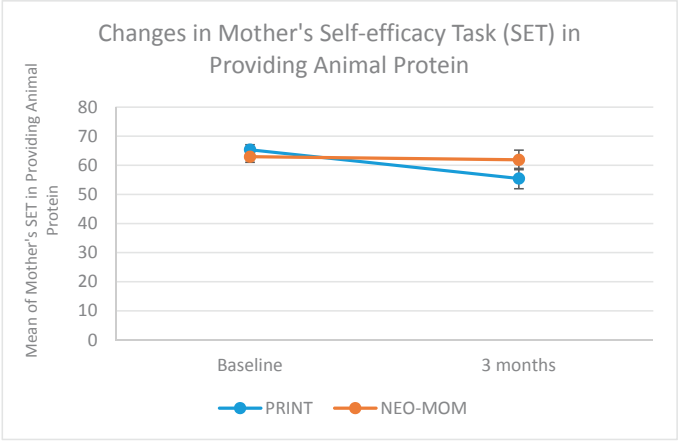

Figure 5. Profile plot of maternal self-efficacy (task) for providing animal protein.

The mother's self-efficacy (task) in providing animal protein for their children, showed a negative trend for both the NEO-MOM and PRINT group with a steeper rate of decline in the latter group (Figure 5). In the NEO-MOM group, maternal self-efficacy (task) for providing animal protein was 63.51 at baseline and was 63.18 after the 3-month evaluation, while in the PRINT group it declined from 64.78 at baseline to 54.20 after 3-months. There was also significant time effect for maternal self-efficacy (task) for being physically active (p-value < 0.001). No significant between-subjects' effects were revealed for any of the maternal self-efficacy tasks. As seen in Table 2, after three months intervention, the ANCOVA test revealed that the change in mother's self-efficacy was shown to be significantly different between NEO-MOM and PRINT group especially in the barriers self-efficacy in eating fruit (p-value = 0.002), barriers self-efficacy in eating vegetables (p-value = 0.002), barriers self-efficacy in serving the children with animal protein (p-value = 0.026), task self-efficacy in eating fruit (p-value = 0.036), task self-efficacy in providing their children with animal protein (p-value = 0.039).

3.2.5. Maternal Outcome Expectation

All measures of maternal outcome expectation were having good internal consistency such as: being physically active (Cronbach alpha = 0.86), eating fruit and vegetables (Cronbach alpha = 0.84), providing animal protein for their kids (Cronbach alpha = 0.89). Measures of maternal outcome expectation were not normally distributed. Even though all measures showed positive increase in the NEO-MOM group relative to the PRINT group (Table 4), there was only one measure, providing animal protein for the child that increased significantly in the NEO-MOM group ($Z = -2.242$; p-value = 0.025). Maternal outcome expectation for providing the children with animal protein was increasing for both NEO-MOM group (from 5.12 at baseline to 5.33 at 3-month evaluation) and PRINT group (from 5.13 at baseline to 5.17 at three months) even though it was not statistically significant.

Table 4. Group means and differences between group means for all outcomes in non-parametric statistics (n = 66).

Outcome/Mediator	Intervention				Comparison			
	Mean (SD)		Z	*p*-Value	Mean (SD)		Z	*p*-Value
	Baseline (n = 32)	3 Month (n = 32)			Baseline (n = 34)	3 Month (n = 34)		
Child's HAZ	−2.99 (0.85)	−2.85 (0.79)	−1.333	0.183	−2.67 (0.57)	−2.55 (0.61)	−1.951	0.051
Mother's BMI	30.13 (3.52)	29.95 (3.34)	−0.895	0.371	31.01 (3.84)	30.63 (4.36)	−1.646	0.100
Household dietary diversity	7.44 (1.70)	6.50 (2.11)	−2.847	0.004 *	7.29 (1.75)	5.68 (1.61)	−3.380	<0.001 **
Maternal outcome expectation								
Being physically active	5.04 (0.65)	5.21 (0.48)	−1.381	0.167	5.01 (0.72)	4.86 (0.72)	−1.082	0.279
Eating fruit & vegetables	5.06 (0.56)	5.18 (0.49)	−1.312	0.190	4.98 (0.54)	4.97 (0.57)	−0.152	0.879
Providing animal protein for kids	5.12 (0.54)	5.33 (0.48)	−2.242	0.025 *	5.13 (0.59)	5.17 (0.56)	−0.320	0.749
Maternal nutrition literacy								
Macronutrient	2.63 (1.62)	2.47 (1.48)	-0.630	0.529	3.15 (1.50)	3.03 (1.62)	−0.367	0.714
Household food measures	1.78 (1.01)	1.75 (0.76)	−0.339	0.735	1.53 (1.11)	1.32 (0.98)	−0.920	0.358
MyPlate categorization	12.72 (1.78)	13.19 (2.39)	−1.442	0.149	12.79 (1.90)	12.82 (1.96)	−0.039	0.969
Maternal dietary intake								
Energy	1075 (538)	845 (559)	−2.393	0.017 *	1029 (774)	840 (502)	−1.135	0.257
Protein	57.29 (42.11)	44.69 (39.59)	−1.627	0.104	46.50 (38.99)	39.28 (32.47)	−1.027	0.304
Fat	49.95 (51.99)	37.82 (48.80)	−1.646	0.100	56.21 (62.00)	43.29 (48.23)	−0.652	0.514
Carbohydrate	103.18 (74.50)	90.75 (69.79)	−0.926	0.355	91.71 (59.06)	77.71 (39.49)	−1.349	0.177
Iron	6.62 (8.04)	6.58 (11.54)	−1.197	0.231	6.32 (7.69)	5.22 (3.40)	−0.527	0.598
Zinc	3.93 (2.13)	3.18 (2.27)	−1.833	0.067	3.62 (2.51)	3.35 (1.89)	−0.225	0.822
Calcium	140.17 (96.21)	144.31 (200.88)	−1.141	0.254	157.05 (190.80)	159.81 (186.58)	−0.527	0.698
Vitamin A	842 (559)	680 (1466)	−1.496	0.135	982 (2441)	503 (738)	−1.930	0.054
Fiber	6.77 (7.29)	6.75 (10.60)	−1.029	0.303	8.47 (14.88)	5.45 (5.22)	−1.524	0.127

Note. The test was based on repeated measures non-parametric statistic of Mann Whitney U. Note. Significance based on $\alpha = 0.05$; ** p-value < 0.001, * p-value < 0.05.

3.2.6. Maternal Nutrition Literacy

All measures of maternal nutrition literacy were not showing good internal consistency with Cronbach alpha <0.65. At baseline, Cronbach alpha obtained for maternal nutrition literacy for macronutrient domain was 0.56, for household food measures was 0.14, and for grouping foods according to Indonesian version of MyPlate was 0.41. After three months intervention, the internal consistency was also not good for all domains of nutrition literacy: macronutrient (Cronbach alpha = 0.53), household food measures (Cronbach alpha = 0.33), grouping foods (Cronbach alpha = 0.64).

Because the data for maternal literacy was not normally distributed, we employed repeated Mann Whitney test for statistical analysis. Results showed no significant effect of the intervention on the mother's nutrition literacy measures (Table 4). The greatest change in the nutrition literacy was observed in the NEO-MOM group for the literacy test for food group categorization test using the Indonesian version of MyPlate called "*Piring Makanku*" ($Z = -1.442$; p-value = 0.149).

3.2.7. Maternal Dietary Intake

Based on the results of the Mann Whitney test, almost all measures of maternal dietary intake showed no significant effect with only total energy (caloric) intake that was statistically significant in the NEO-MOM group (Table 4). Mother's total energy intake in the NEO-MOM group decreased from 1075 kcal at baseline to 845 kcal at 3 months ($Z = -2.393$; p-value = 0.017) and declined in the PRINT group from 1029 kcal at baseline to 840 at 3 months ($Z = -1.135$; p-value = 0.257).

3.2.8. Moderation of Intervention Effects

For all variables that passed the normality assumption we used the household food insecurity access scale (HFIAS) score as the covariate. Our results showed that the HFIAS score was a significant moderator of some of the significant outcome variables measures. Treated as a covariate in the mixed method ANOVA analysis, the HFIAS score revealed significant between-subjects effects for maternal self-efficacy (barriers) in providing children with animal protein ($F(1, 64) = 5.534$, p-value = 0.022), self-efficacy (task) in eating fruit ($F(1, 64) = 4.943$, p-value = 0.030), self-efficacy (task) in eating vegetables ($F(1, 64) = 4.781$, p-value = 0.033), and self-efficacy (task) in providing their child with animal protein ($F(1, 64) = 6.802$, p-value = 0.011).

4. Discussion

The goal of this study was to empower and equip overweight or obese mothers to overcome the double burden of malnutrition. Mothers received training on strategies in overcoming the double burden of malnutrition. They were trained through behavioral strategies such as mastery experience, vicarious experience, goal setting, and verbal motivation to achieve better health outcomes for themselves, as well as for their children. The hypothesis of this randomized controlled trial was that applying behavioral intervention strategies based on Bandura's Social Cognitive Theory for three months would be effective in improving child growth to address the issue of child stunting in a household facing the double burden of malnutrition. Results revealed that after a 3-month intervention there was a positive increase in child height in both groups but no catch-up in terms of the HAZ score. The lack of significance in the time and group interactions as well as the test of the between-subjects effect indicated that the significant time effect observed could well be attributable to the natural growth of the child and not the intervention.

Another possible explanation for the observed results might be related to the compliance of the mothers in implementing the Indonesian government food supplementation program for the underweight children. Compared to the previous study in Bangladesh that included food supplementation as a part of the intervention [6,7], in our study we relied on the food supplementation part from the government program and use it as an inclusion criterion for eligible participants. Hence, the amount of effort in ensuring participant's compliance in the use of food supplementation

in the Bangladeshi study was likely to be more rigorous than this one. But, because we did not measure the compliance rate related to the consumption of food supplementation from the government, a comparison with previous study in Bangladesh was not possible.

Furthermore, children 2 to 5 years old have passed the optimum time for rapid linear growth that occurs during the first 1000 days of life. However, stunted children in this age range needed an intervention to catch up with their normal peers. In terms of the height-for-age *z*-score (HAZ) measure, we did not find a significant effect in both the intervention and comparison group. The fact that our intervention targeted children from the age of 24 months to less than 5 years old might have hindered the effect in comparison to one targeting children during the first 1000 days of life or from the womb up to 24 months old when they have the best opportunity for growth improvement. Results of a meta-analysis on interventions aimed at improving child nutritional status revealed that interventions were generally more effective for children under the age of 2 years, and for those who were nutritionally deprived [13].

We saw a significant increase in child's weight overtime for both group (p-value = 0.023), and there was borderline significant difference in the weight change from baseline between intervention and comparison group (p-value = 0.050). While for child's height, no significant difference was observed between the effect of intervention and comparison condition (p-value = 0.143). This supports a previous study in a developing country that reported greater effect size in increasing a child's weight than in improving a child's linear growth [6].

All of the maternal primary outcome measures showed no significant improvement after a 3-month intervention for both groups. For anthropometric outcomes such as weight, waist circumference and BMI it might take longer for the intervention to have significant effects. The length of time employed in the current study was calculated based on the time needed to improve child's height from a previous study and was not based on the maternal anthropometric measures. Therefore, the lack of significant effects in our study for maternal primary outcomes might be due to the insufficient length of the intervention.

However, we saw significant improvement on almost all measures of maternal self-efficacy for both tasks and barriers. There was significant increase for all four maternal self-efficacy (barriers) and two of the maternal self-efficacy (tasks) overtime. This result aligned with a study in Australia that showed maternal self-efficacy was a good predictor for the quality of diet among children aged 3 to 5 years [14]. Mother's child feeding behavior was indirectly related to child vegetable intake through maternal feeding self-efficacy in an Australian population [15]. Results from qualitative studies also support the importance of self-efficacy in influencing healthy eating decision-making [16] and food preparation behavior [17]. Even though we did not directly measure mother's behavior towards child feeding practices, we found significant effect of the intervention for both NEO-MOM and PRINT group in household's dietary diversity as an indirect measure of maternal healthy food choice behavior.

We measured behavior in terms of dietary diversity as an indication of healthy food choice in the household. The results revealed a significant effect but in a negative direction. After the 3-month intervention, the average household dietary diversity score significantly decreased for both the NEO-MOM group and comparison group. This may have been affected by the time the interview was conducted between baseline and the 3-month evaluation. Baseline data were collected at the beginning of the month, while the evaluation data was collected at the end of the month. Results may have been influenced by food budget availability and the fact that most Indonesian people receive their salary at the beginning of the month and may have had more money to spend on food at that time, as compared to the end of the month [18].

The effect of our intervention on maternal dietary intake was significant only for total energy (caloric) intake in the intervention group. These results align with previous RCTs on weight loss that suggest that as the first step in trying to lose weight, women tend to reduce their caloric intake. Other nutrient intake did not show significant results, perhaps because our two times in 24 h dietary recall

may not have been sufficient to capture the variability of micronutrient intake as compared to total energy [19].

There was no significant effect revealed in the three domains of maternal nutrition literacy used in this study. These results might be related to the fact that our intervention was designed based on the behavioral strategies that followed the tenets of Social Cognitive Theory. Even though we provided six weeks (over 600 h) of nutrition education classes to improve mothers' knowledge, the content might not necessarily fit with the questions included in the validated nutrition literacy questionnaire [20]. The tools for nutrition literacy developed in the more highly educated settings in the U.S. might be too difficult to for mothers with less education in developing countries such as Indonesia.

Strengths and Limitation

To the best of our knowledge, this study was the first to conduct a randomized controlled trial (RCT) on households experiencing the problem of double burden of malnutrition in the form of the coexistence of stunted children and overweight/obese mother pairs (SCOWT). The strength of the study was a solid methodological approach and RCT design, a small attrition rate of around 5.5% in each group, and the high participation from local community health workers to deliver the intervention that promote adoption of our strategies. However, with limited resources, we could not incorporate supplementary feeding as part of our intervention, but we made use of the Indonesian government's 3-month supplementary feeding program to overcome severely underweight children as inclusion criteria. Therefore, a limitation of the study was the absence of a true control group. Without it, it is impossible to know whether the observed time effect was significantly different from natural growth following the age increase. For this reason, we may have underestimated the effect of print educational materials and ongoing food supplementation by the Indonesian government. Our observed effect could have been higher if we did not use the HFIAS score (measure of food insecurity) as covariates in the analysis. In this study we also assume that the mother was the focal member of the household responsible for purchasing food and its distribution in the family, which might not always be true. Other concerns might arise from the use of relatively high effect size from previous study [7] in calculating the sample size that might be attributable to under power the study. However, we minimize the effect by using fairly substantial bigger power relatively compared to the traditional 80%. The application of our results might be limited to an urban population in a developing country setting.

5. Conclusions

The behavioral-based nutrition education intervention produced strong improvement in maternal self-efficacy to engage in physical activity, eat fruits and vegetables, and to provide children with growth-promoting animal protein, but did not significantly influence child height gain. Although both of our interventions (NEO-MOM and PRINT) allowed significant increases in child growth overtime, no catch-up growth was observed in either group. Relative to the PRINT comparison group, our intervention improved almost all maternal self-efficacy measures, which are viewed as necessary steps for engaging in healthy behaviors. The behavioral intervention in this study was deemed feasible and it had a good retention rate. This study provides a basis for potential strategies to reduce the rate of child stunting in households undergoing double burden of malnutrition.

Author Contributions: T.M. was responsible for overall and/or sectional scientific management, formulating research question, making concept and design of the study, preparation of draft manuscript, doing revisions. A.A.M. was responsible for data cleaning, statistical analysis, providing critiques and revision of the manuscript. T.S.N. lead the data collection, coordinate the participants, setting up ground work for nutrition education session, H.M. develop hands on activities for nutrition education sessions and supervise data collection. D.R.A. was responsible in managing data input and preliminary cleaning of the data and writing the first draft of manuscript in Bahasa Indonesia. R.R.R. responsible for substantial contributions in design and conception of the study, and was involved in data analysis, manuscript preparation, providing critique, revision of the manuscript, and supervises the data collection. T.M. gave final approval of the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have given approval of the final manuscript.

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5

Whole Body Protein Oxidation Unaffected after a Protein Restricted Diet in Healthy Young Males

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Abstract: Protein oxidation may play a role in the balance between anabolism and catabolism. We assessed the effect of a protein restricted diet on protein oxidation as a possible reflection of whole body protein metabolism. Sixteen healthy males (23 ± 3 years) were instructed to use a 4-day isocaloric protein restricted diet (0.25 g protein/kg body weight/day). Their habitual dietary intake was assessed by a 4-day food diary. After an overnight fast, a 30 g ^{13}C-milk protein test drink was administered, followed by 330 min breath sample collection. Protein oxidation was measured by Isotope Ratio Mass Spectrometry. To assess actual change in protein intake from 24-h urea excretion, 24-h urine was collected. During the 4-day protein restricted diet, the urinary urea:creatinine ratio decreased by 56 ± 9%, which is comparable to a protein intake of ~0.65 g protein/kg body weight/day. After the protein restricted diet, 30.5 ± 7.3% of the 30 g ^{13}C-milk protein was oxidized over 330 min, compared to 31.5 ± 6.4% (NS) after the subject's habitual diet (1.3 ± 0.3 g protein/kg body weight/day). A large range in the effect of the diet on protein oxidation (−43.2% vs. +44.0%) was observed. The residual standard deviation of the measurements was very small (0.601 ± 0.167). This suggests that in healthy males, protein oxidation is unaffected after a protein restricted diet. It is uncertain how important the role of fluctuations in short-term protein oxidation is within whole body protein metabolism.

Keywords: Protein; oxidation; anabolic competence; breath test; naturally enriched ^{13}C-milk proteins

1. Introduction

Adequate protein intake and subsequent utilization of protein is of great importance for health. The recommended daily protein intake for healthy adults is 0.8 g protein/kg body weight/day, and is suited for maintaining normal body composition and meeting metabolic demand [1]. Patients with disease-related malnutrition (DRM) have an absolute or relative deficiency and inadequate utilization of energy, protein, and other nutrients caused by a concomitant disease. Compromised outcomes, such as impaired clinical outcome from disease, and diminished physical and mental function have

been described in relation to DRM [2–4]. Nutrition, exercise, and the hormonal milieu are essential to reach a state which optimally supports protein synthesis and lean body mass (LBM), global aspects of muscle and organ function, and the immune response, a paradigm also known as "anabolic competence" [5].

Prevention and treatment of LBM loss could benefit from direct measurement and monitoring of disturbed protein metabolism. Current methods to measure protein metabolism focus on protein synthesis, which requires blood sampling, muscle biopsies, and/or the use of expensive synthetic labelled amino acids [6–8]. Therefore, these methods are not suitable for the clinical setting. A non-invasive bedside method to measure protein metabolism would be more suitable, as direct measurements of the metabolic state could lead to more insight in optimal protein intake and optimal physical activity, which then enables tailored improved treatment for each patient, resulting in improved outcomes of disease.

Measuring protein oxidation is a feasible and non-invasive technique and can be performed with naturally labelled ^{13}C-protein, which is relatively inexpensive. All oxidized ^{13}C-protein will be exhaled as $^{13}CO_2$ [9]. However, it is unknown to what extent variations in protein oxidation occur under various physiological conditions, such as changes in protein intake. Generally, after the ingestion of protein, the protein derived amino acids will be incorporated into new proteins until protein synthesis requirements are met. The lack of protein storage leads to the oxidation of surplus amino acids [10,11]. Accordingly, an altered protein intake could modify protein oxidation under normal conditions. Thus, we hypothesized that restriction in protein intake in healthy subjects leads to decreased activity of the oxidation pathway, as assessed by the $^{13}CO_2$ breath test.

To test this hypothesis, in the current study, we aimed to measure the effect of a four-day protein restricted diet, compared to their habitual diet, on protein oxidation, as assessed by the $^{13}CO_2$ breath test in healthy subjects.

2. Materials and Methods

2.1. Subjects

Healthy young males were included as being a representative group for healthy subjects. The decision to recruit young subjects versus older subjects was based on logistics, as the pool of young healthy subjects is more easily accessible for study. Women were excluded to rule out possible effects of the menstrual cycle on protein metabolism, and to exclude possible effects of differences in body composition between women and men. Furthermore, subjects having a disease and/or undergoing or starting medical treatment were excluded. Sixteen healthy young male subjects were recruited via local advertising. To obtain a homogeneous group of subjects, reducing the possible influence of covariates, the following inclusion criteria were applied: Age between 18–30 years, body mass index (BMI) between 20–25 kg/m^2, and being able to fast overnight. Exclusion criteria were: Having a disease and/or being medically treated, milk protein allergy or intolerance, smoking, use of drugs, drinking on average more than 2 glasses of alcohol per day, waist circumference larger than 102 cm, and using a vegetarian diet. This design was chosen to minimize possible confounding effects of subject characteristics over the protein restricted diet intervention.

The study was approved by the local Medical Ethical Committee at the University Medical Center Groningen (NL56982.042.16, METc 2016.144), conducted in accordance with the Helsinki Declaration of 2013, and registered in the Dutch Trial Register under the registration number, NTR6101. After receiving an information letter about the purpose and practical procedures of the study, and an informative meeting with the researcher, every subject gave his written informed consent prior to participation.

2.2. Study Protocol

In each subject, age (year), height (cm), waist circumference (cm), bodyweight (kg), BMI (kg/m^2), and LBM (kg) were measured. LBM was measured by bioelectrical impedance analysis (Quadscan 4000, Bodystat Ltd., Isle of Man, British Isles). After these measurements, subjects were instructed to keep a four-day food diary with respect to their habitual food intake to calculate the average daily intake of energy (kcal), protein (g), protein, en%, animal protein (g), plant protein (g), carbohydrates (g), carbohydrates, en%, fat (g), and fat, en%. The calculations on dietary intake were performed with Evry (Evry BV), which uses the NEVO 2013, RIVM database [12].

On each subject, at two separate days, two breath tests were performed; one after the subject's habitual diet and one after an isocaloric protein restricted diet (0.25 g protein/kg body weight/day). Between the breath tests, there was a washout period of at least a week to return to baseline $^{13}CO_2$ levels. On the evening before the breath test, subjects were instructed to start fasting overnight (only consumption of water and tea, or coffee without milk and sugar was allowed) from 22:00 p.m. onwards to arrive sober the next morning at 08:45 a.m. During each test, 3 basal breath samples were collected and averaged to establish the subject's baseline $^{13}CO_2$:$^{12}CO_2$ ratio. At 09:15 a.m., 30 g naturally enriched ^{13}C-milk protein dissolved in 500 mL water was consumed within 5 minutes. The isotope, ^{13}C, is a stable isotope. From 09:25 a.m. until 14:45 p.m. (5.5 h), a breath sample was collected every 10 minutes. During this period, subjects were instructed to remain seated in upright position and not to eat or drink during the remainder of the breath test. Subjects were allowed to work on a laptop, to read, and to write.

The four-day protein restricted diet was given as a food menu, which described in detail what and when to eat, to facilitate energy and protein intake as prescribed. The subjects were instructed to use the food menus. The food menu was tailored to each subject's habitual energy intake, which was calculated from the four-day food diaries. Therefore, the created protein restricted diet was isocaloric to the habitual diet. Consequently, by both reducing protein intake and keeping the protein restricted diet isocaloric, the macronutrient composition of the diet changed, as the energy lost from protein intake was replaced by mainly an increase in carbohydrates and to a lesser extent with an increase in fat, as food which contains fat also contains protein. Each subject underwent the breath tests in the same order: Starting the first after habitual diet and second after the protein restricted diet. The four-day protein restricted diet was tailored to each subject.

During five days, 24-h urine was collected, on the fourth day of the subject's habitual diet and, next, every day during the four-day isocaloric protein restricted diet. From each 24-h urine sample, urea and creatinine concentrations were measured to calculate the subject's actual protein intake [13]. The urea:creatinine ratio was calculated to assess the compliance to the isocaloric protein restricted diet. A change to a protein restricted diet will reduce 24-h urinary urea production, while 24-h urinary creatinine production, which depends almost exclusively on muscle mass, will remain steady, and therefore the urea:creatinine ratio will decrease.

2.3. Calculations and Statistical Analysis

Disturbances in short-term protein oxidation can be measured by pulse-labelling with the use of naturally enriched ^{13}C-proteins. After ingestion of ^{13}C-proteins, the protein oxidation can be quantified by measuring the $^{13}CO_2$:$^{12}CO_2$ ratio in exhaled breath over time. To calculate the amount of CO_2 produced on each timepoint, the CO_2 production in rest was calculated by the following regression formula: 300 mmol CO_2/hour $\times$ body surface area (BSA), which was calculated with the Haycock formula [14]:

$$\text{BSA (m\^2)} = \text{weight (kg)\^0.5378} \times \text{height (cm)\^0.3964} \times 0.024265 \quad (1)$$

The breath samples were measured for their $^{13}CO_2$:$^{12}CO_2$ ratio with an isotope ratio mass spectrometer (IRMS) and compared to a high ^{13}C-enriched international standard, Pee Dee Belemnite

(PDB), which has an accepted absolute $^{13}C/^{12}C$ ratio of 0.0112372. The differences (delta, δ) between the breath samples and the standard is expressed in parts per 1000 (:‰) as [15] follows:

$$\delta\ 13C\ \text{sample} = ((13C/12C\text{sample})/(13C/12C\text{standard}) - 1) \times 1000 \quad (2)$$

The PDB standard $^{13}C/^{12}C$ ratio is defined as 0:‰. To calculate the $^{13}C/^{12}C$ ratio from the IRMS delta values, the following inversion formula was used [10]:

$$13C/12C\text{ratio} = ((\text{deltavalue}/1000) + 1) \times 0.0112372 \quad (3)$$

Next, the $^{13}C/^{12}C$ ratio of each breath sample is used to calculate the $\%^{13}C$ [16] by:

$$\%13C = ((13C/12C\text{ratio})/(13C/12C\text{ratio} + 1)) \times 100 \quad (4)$$

The baseline (t = 0) breath sample $^{13}C/^{12}C$ ratio was subtracted from each subsequent breath sample to acquire the change from the subject's baseline. The estimated CO_2 production, together with the delta value on each timepoint, and the enrichment of the ^{13}C-milk protein was used to calculate the protein oxidation rate of the subject at each timepoint with 10 minutes in between.

The change in the $^{13}CO_2$:$^{12}CO_2$ ratio over time has been described [17,18] by the general concentration model as follows:

$$y(t) = a \times\ t\hat{}b \times e\hat{}(-kt)\ +\ \varepsilon\ (\text{normally distributed}) \quad (5)$$

The model function was fitted to the measurement data for each subject over time, with t ranging from zero to 330 minutes. That is, the parameters, a, b, and k, were determined by fitting the oxidation rate curves to the measurement data per subject per day, with the goal of finding optimal parameters per subject per day. Total protein oxidation was calculated as the integral over the curve representing the area under the curve (AUC). The term, ε, is normally distributed with a zero mean, and its variance is estimated by the residual error variance. Each resulting curve starts at the natural amount, y = 0, as no ^{13}C-milk protein has been ingested at timepoint, t = 0. After ingestion, the stomach releases the ^{13}C-milk protein into the digestive track where the proteins are digested and taken up by the gut. The digested proteins are circulated and become available to the cells for protein synthesis or oxidation. The process of protein oxidation is reflected by the oxidation curve. From t = 0 onwards, the oxidation curve ascends, reaching its maximum and thereafter, the oxidation rate of ^{13}C-amino acids descends towards the subject's baseline over multiple hours.

Per breath test, the values of the fitted parameters, a, b, and k, differ over persons and type of diet. For larger values of a and b, the ascending slope becomes steeper, whereas a higher value for the constant, k, leads to a steeper descending slope after the maximum was reached.

The difference in the average AUC after a habitual diet and protein restricted diet within subjects was tested with the paired t-test. Two other important characteristics directly calculated from the parameters of each fitted curve per subject were the timepoint (t_{max}) in minutes at which the maximum oxidation rate was reached, as well as the corresponding maximum oxidation rate, $y(t_{max})$, where $t_{max} = b/k$. The latter was expected to be similar within subjects in both experimental conditions, as t_{max} is mainly determined by the rate of stomach emptying [19], which is dependent on the test drink. The latter was identical for both breath tests. The standard error of the t_{max} per person is computed by the delta method [20]. Both the maximum oxidation rate, $y(t_{max})$, and the total oxidation (AUC) were expected to be lower after the protein restricted diet compared to the habitual diet, as a deficit of amino acids in the body is hypothesized to lead to less oxidation of the 30 g ingested milk protein. From each concentration model, the timepoint at which 1% oxidation/hour was reached was calculated using the above formula and a numerical intersection method.

All statistical analyses were performed by the statistical programming language, R (R Core Team, 2017), with the package, "car" [21], specifically using the non-linear least squares function [22] to fit the

concentration curve to the measurements over time and the delta method to determine the standard error of t_{max} [20]. The difference in the urea:creatinine ratio between the habitual diet and day four of the protein restricted diet was investigated with the paired Student's *t*-test. The associations between total protein oxidation and the demographic characteristics, i.e., age, bodyweight, BMI, LBM, habitual protein intake, habitual energy intake, and baseline urea:creatinine ratio, were investigated with the Pearson correlation coefficient, *r*. All data are represented as mean ± standard deviation (SD).

3. Results

Baseline characteristics of the 16 male subjects are presented in Table 1.

Table 1. Baseline characteristics of the subjects (n = 16).

	Mean	SD
Age (years)	23.0	3.1
Height (cm)	185.4	8.6
Body weight (kg)	77.1	9.5
Body Mass Index (kg/m^2)	22.3	1.1
Lean Body Mass (%)	88.3	2.7
Habitual diet		
Protein intake (g protein/kg body weight/day)	1.3	0.3
Protein intake (g protein/day)	102	25
En% protein (%)	17	4
En% carbohydrates (%)	47	5
En% mono- and disaccharides (%)	20	8
En% fat (%)	35	6
En% saturated fat (%)	13	4
En% unsaturated fat (%)	19	7
Protein restricted diet		
En% protein (%)	3	1
En% carbohydrates (%)	73	7
En% mono- and disaccharides (%)	53	8
En% fat (%)	22	7
En% saturated fat (%)	9	5
En% unsaturated fat (%)	12	6
Baseline breath $^{13}CO_2$ enrichment (delta value)	−26.18	0.50

During the four-day protein restricted diet, the urea:creatinine ratio in 24-h urine decreased with an average of 56 ± 9%, as compared to the habitual diet from day 0 to day 4 (Figure 1). The mean difference in the urea:creatinine ratio between the habitual diet day 0 and protein restricted diet day 4 was statistically significant ($p < 0.001$, $t = 12.837$, $df = 15$). Based on the change in the urea:creatinine ratio, the protein intake decreased to 0.65 g protein/kg body weight/day, compared to the habitual protein intake of 1.3 g protein/kg body weight/day.

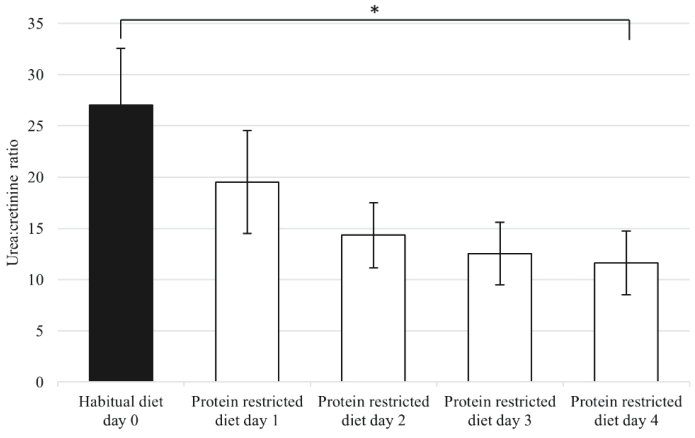

Figure 1. Urea:creatinine ratio calculated from 24-h urine collections (n = 16). The symbol "*" denotes the statistically significant change from day 0 to day 4 with $p < 0.001$. Protein intake during the habitual diet was 1.3 g protein/kg body weight/day ± 0.3 g; the prescribed four-day protein restricted diet was 0.25 g protein/kg body weight/day

The average protein oxidation kinetics of all subjects during the 330 minute breath tests, separated by the habitual diet and the protein restricted diet, are shown in Figure 2. Total oxidation (AUC) after the habitual diet and the protein restricted diet was 31.5 ± 6.4% and 30.5 ± 7.3%, respectively. The difference in the mean total oxidation between the habitual and protein restricted diet was not statistically significant (p = 0.530, t = 0.643, df = 15). The mean total protein oxidation of ~30%, corresponds to ~10 g oxidized. Time to t_{max}, after the habitual and protein restricted diet, was 137 minutes ± 24 and 138 minutes ± 18, respectively (p = 0.854, t = −0.188, df = 15). The maximum %oxidation rate per hour, after the habitual diet and the protein restricted diet, was 8.05 ± 1.27% and 8.11 ± 1.62% (p = 0.868, t = −0.170, df = 15).

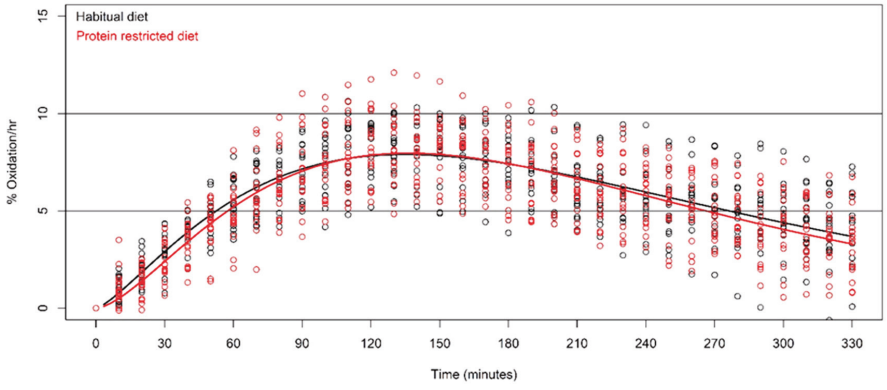

Figure 2. Protein oxidation kinetics after the habitual diet (black) and protein restricted diet (red) (n = 16).

The following means and confidence intervals were calculated with all 32 breath tests (16 subjects × 2 breath tests). From the curve fitting with the function, $y(t) = a \times t\hat{}b \times e\hat{}(-kt)$, the mean of constant, a, was 0.037 ± 0.041. For the constant, b, the mean was 1.636 ± 0.458. For the constant, k, the mean was 0.012 ± 0.004. The concentration curve fitted well with the breath test measurements with a mean R^2 of 0.930 ± 0.033. The timepoint of maximum oxidation (t_{max}) was obtained with a

mean 137 ± 21 minutes. The mean proportionate decrease from the maximum oxidation rate (mean 8.1% oxidation/hour) to the final timepoint (mean 3.5% oxidation/hour) over all 32 breath tests was −57 ± 16%. The mean time to reach 1% oxidation/hour was 502 ± 119 minutes. The total oxidation was positively correlated with the maximal oxidation rate (0.95). Residual standard deviation of the measurements to the fitted curve was 0.601 ± 0.167. The associations between the total protein oxidation and demographic characteristics, i.e., age, body weight, BMI, LBM, habitual protein intake, habitual energy intake, and baseline urea:creatinine ratio, were fair to poor ($r < 0.4$) [23].

Differences in the total protein oxidation after each subject's habitual diet (1.3 ± 0.3 g protein/kg body weight/day), and after a prescribed isocaloric protein restricted diet (0.25 g/kg body weight/day) are shown in Figure 3. The results of the subjects are ordered based on the strongest relative decrease in protein oxidation from their habitual diet to the protein restricted diet, towards the strongest relative increase. A large range in the effect on the total protein oxidation (relative change −43.2% vs. +44.0%) was observed, however, the large range of the increase and decrease in the total protein oxidation canceled each other out, as shown in Figure 2.

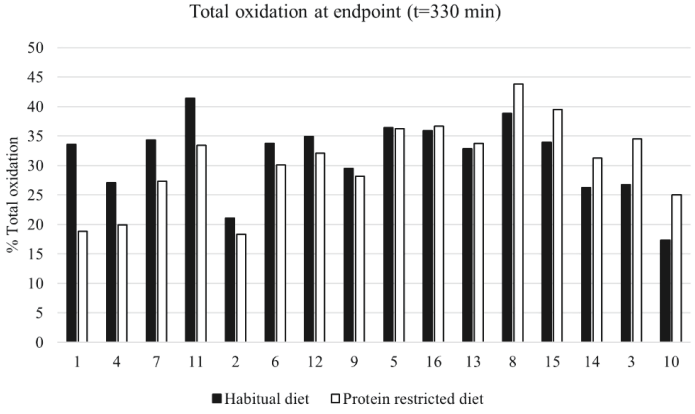

Figure 3. Total protein oxidation (% of given 30 g dose) measured with the breath test after a habitual diet (black bars) versus the protein restricted diet (0.25 g/kg body weight/day)(white bars) ($n = 16$). Subjects are ordered from left to right, based on the strongest relative reduction in protein oxidation from their habitual diet to the protein restricted diet, towards the strongest relative increase.

4. Discussion

In the current study, we assessed the effect of a protein restricted diet on protein oxidation, as assessed by the $^{13}CO_2$ breath test in healthy young males, as a possible reflection of whole body protein metabolism. On the group level, the total protein oxidation was not affected by the two-fold reduction in mean protein intake, which decreased from 1.3 to 0.65 g protein/kg body weight/day. We found a large variation in the total protein oxidation response after the protein restricted diet compared to the habitual diet, which ranged from a decrease of 43.2% to an increase of 44.0%. Combined with the precision of the breath test, this implies that the range in effects on the oxidation rate may have a biological background.

This is the second study that has assessed the overall protein oxidation with naturally ^{13}C-enriched milk protein during different states of protein intake. An explorative investigation was performed with a three-day protein restricted isocaloric diet, in which the protein intake was reduced to ~10 g of protein per day, which corresponds to ~0.15 g protein/kg body weight/day [9]. In that study, the total protein oxidation after the protein restricted diet had a lower mean than after the habitual diet, but the difference in the mean was not significant ($p = 0.142$). Further comparison of our results with those of other studies, which either measured the effect of several conditions or the effect of different exercise

regimens combined with protein ingestion on the protein synthetic response, is difficult. However, our finding that ingestion of 30 g of ^{13}C-milk protein was associated with an overall oxidation of ~30%, corresponding to ~10 g oxidation over 5.5 hours, seems in line with the study by Moore et al. [8]. In that study, in which protein muscle synthesis was measured with primed ^{13}C-leucine infusion over 4 hours, muscle protein synthesis after resistance exercise was maximally stimulated with 20 g of ingested whole egg protein and dietary protein ingested in excess of 20 g stimulated ^{13}C-leucine oxidation. Therefore, Moore et al. imply that they would have found ~10 g of whole egg protein oxidized if they had tested a 30 g dose of whole egg protein, which is comparable to the amount of ^{13}C-milk protein oxidized in our study. Our findings did not confirm our hypothesis that a change in protein intake would be associated with a change in overall protein oxidation. This could mean that a change in protein intake in the range investigated here does indeed not affect protein oxidation in healthy males, or, alternatively, that there were methodological limitations in detecting such an alleged change in the current methodological design of our study.

The first limitation that needs to be taken into account is that there could be a pre-meal effect of the non-standardized evening meal, prior to the breath test, on the utilization and oxidation subsequent to the 30 g protein test drink. However, no relationship between the evening meal energy intake, en% carbohydrates, and en% protein intake on the subsequent protein oxidation was found, which could in part be related to the small sample size. Second, during the protein restricted diet, the subjects did not reach the intended target of 0.25 g protein/kg body weight/day intake, as the mean level of protein intake decreased to only 0.65 g protein/kg body weight/day as measured by the urea:creatinine ratio. This level of intake is only slightly lower than the general protein intake recommendation of 0.80 g/kg body weight/day for healthy adults set by the World Health Organization (WHO) [1]. It would seem that in this study, the subjects were probably still within the range of adequate protein intake to maintain protein homeostasis. On the other hand, whether the subjects in this study were in steady state is uncertain, as they acutely adjusted their protein intake from habitual to the four-day protein restricted diet. The use of 24-hour urinary urea for the estimation of dietary protein intake is most reliable in subjects who are in a steady state [24,25]. Therefore, it could be argued that in these experimental circumstances, the change in the urea:creatinine ratio does not accurately reflect protein homeostasis and also the actual protein intake. However, it does at least underline a clear reduction in protein intake. Third, the replacement of protein with carbohydrates to obtain an isocaloric protein restricted diet changed the macronutrient ratios within the diet, and might have resulted in a decreased uptake of amino acids into tissue and, consequently, an increased amino acid oxidation in the splanchnic area due to a possible altered insulin response [26,27]. Fourth, the current protocol includes an overnight fast, to forgo breakfast, and after consumption of the test drink, the subjects did not eat or drink for 5.5 hours. This was in order to minimize the influence of differences in starting conditions, such as the stomach emptying rate, between the subjects. These requirements enable better interpretable measurements and are unlikely to harm healthy subjects. However, fasting can further deteriorate the condition of clinical populations. For future studies in clinical populations, adaptations to the protocol should be made to minimize the burden. Potential targets to reduce the burden are a standardized breakfast and reduction in the collection of breath samples over time.

The major strengths of this study were the well-controlled design, with subjects as their own control, and the precise protein oxidation measurements. First, in the current tests, natural enriched ^{13}C-milk protein was used, which implies that all amino acids are enriched with ^{13}C and therefore the exhaled $^{13}CO_2$ represents the oxidation of all amino acids, which reflects the total body protein oxidation [10]. Oxidation studies with specific amino acids, like ^{13}C-leucine, most likely do not reflect overall amino acid oxidation, as all the amino acids have various biological functions, aside from being building blocks for synthesizing protein [28]. Second, the breath test is reliable as it measures the protein oxidation process well, with the concentration function fitting well to all breath test measurements, as demonstrated by a mean R^2 of 0.930 ± 0.033. The formula of each curve provided estimated parameters per subject. These parameters, such as the timepoint of the maximum, and the

oxidation rate at the timepoint of the maximum, had small standard errors. Moreover, the residual standard deviation of the oxidation rate was very small (0.601 ± 0.167). As the breath test is reliable, the personal parameters found have a biological basis. Finding potentially important biological factors involved in protein oxidation and protein utilization are a next step in understanding whole body protein metabolism.

In conclusion, this study has shown that on the group level, the total protein oxidation was not affected by a short-term reduction in protein intake in healthy subjects. This suggests that over the range of protein intake investigated here, the overall protein metabolism is robust against challenges. However, due to large variations found on the individual level with respect to the change in total protein oxidation between the habitual and protein restricted diet, and the poor to fair associations of total protein oxidation with demographic characteristics, it is uncertain how important the role of fluctuations in short-term protein oxidation is within whole body protein metabolism.

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6

Association of Sports Participation and Diet with Motor Competence in Austrian Middle School Students

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Abstract: Physical activity and diet are important contributors to overall health and development in adolescents. There remains, however, limited research on the combined association of sports participation and dietary pattern on motor competence, which is crucial for an active lifestyle during and beyond adolescence. The present study, therefore, examined the association between sports participation, dietary pattern, and motor competence in 165 middle school students (55% male) between 11 and 14 years of age. Body weight and height were measured, and motor competence was determined via the German motor test during regular Physical Education (PE). Further, participants completed a food frequency questionnaire and reported their engagement in club sports. Of the total sample 20% were overweight/obese and 49% reported participation in club sports, with no differences between boys and girls. Interaction effects of sports participation and dietary pattern on motor competence were limited, but sports participation and healthy diet were independently associated with higher motor performance. Healthy dietary choices, along with participation in club sports, therefore, should be promoted in adolescents in order to facilitate motor development. As adolescence is a crucial time for the establishment of lifelong behaviors, such efforts could facilitate a healthy lifestyle throughout adulthood.

Keywords: physical activity; food intake; adolescents; movement skills; fitness

1. Introduction

Low levels of physical activity (PA) and poor dietary choices are considered key health risk factors in youth [1]. In fact, 70% of premature deaths are attributed to behavioral choices begun during adolescent years [2]. Accordingly, adolescence provides a crucial window of opportunity for sustainable health promotion, including sufficient PA and healthy dietary choices [3]. Current guidelines emphasize a minimum of 60 min of moderate-to-vigorous PA [4], along with a diverse dietary intake that includes high consumption of leafy greens, fruits and vegetables, poultry, fish, and dairy while the consumption of fat and sugars should be limited [5]. Nevertheless, sedentary choices during leisure time, along with prolonged sitting times during school time are common in youth [6–8], and many children and adolescents do not meet current dietary recommendations [9,10]. Low PA levels most likely also contributed to a decline in physical fitness and motor competence in children and adolescents [11,12], which is a crucial contributor to a sustainable active lifestyle, as well as overall health and well-being [13,14].

In light of these trends, organized PA, including club sports, provides important opportunities for PA and the establishment of healthy behaviors [15,16]. In fact, a study in Australian adolescents

between 10 and 16 years of age showed that they accrued the majority of their total PA during organized PA such as club sports [17]. Participation in sports during childhood and adolescence has also been associated with higher PA levels during adulthood [18,19]. With more than two-thirds of children and adolescents in various European countries participating in club sports, this may also be a viable setting for interventions targeting an active and healthy lifestyle [20]. In addition to beneficial associations with overall PA [21–23], participation in club sports has been associated with increased physical fitness and motor performance [24,25], as well as beneficial socio-emotional outcomes [26] and higher academic achievement [27,28]. A recent study further indicates beneficial associations between club sports participation and food intake [29], but the overall evidence on this relationship remains equivocal [30]. This may, at least partially, be attributed to higher energy needs in more active youth [31]. Accordingly, there appears to be a positive association between physical fitness and energy intake in children and adolescents [31,32]. Nevertheless, higher physical fitness has been associated with healthier dietary choices in adolescents, while this association is less clear in children [33–35].

Limited research, however, is available on the combined association of dietary pattern and sports participation with motor competence. Given the complex interaction between behavioral choices (i.e., diet and sports) and motor development, and the importance of motor competence in the promotion of an active lifestyle [36], such information may help with the refinement of current interventions, as well as the development of new strategies for the promotion of motor development in youth. The present study, therefore, examines the combined and independent association of club sports participation and dietary habits with motor competence in Austrian middle-school students. It was hypothesized that club sports participation, as well as healthy dietary patterns, are positively associated with motor competence. Further, it was hypothesized that the association between dietary pattern and motor competence is more pronounced in adolescents not participating in club sports.

2. Materials and Methods

A convenience sample of nine classes between grades 6 and 8 from middle schools in the Federal State of Tyrol, Austria, were selected for participation, resulting in 172 eligible participants between 11 and 14 years of age. Parents were informed about the nature of the study via mail, and provided written informed consent. Oral assent was obtained from the participants at the time of data collection, which occurred during May and June of 2018. The study was performed according to the ethical standards of the 2008 Declaration of Helsinki, with the study protocol being approved by the Institutional Review Board of the University of Innsbruck, the school board of the Federal State of Tyrol and the principals of the participating schools (approval number: 16/2017).

Anthropometric measurements. Body weight (kg) and height (cm) were measured according to standard procedures during a physical education class, by trained technicians, with participants wearing gym clothes and being barefoot. Body weight was measured to the nearest 0.1 kg with a gauged body scale (SECA® 803, Seca, Hamburg, Germany), and height was measured to the nearest 0.1 cm with a mobile stadiometer (SECA® 217, Seca, Hamburg, Germany). Body mass index (BMI, kg/m^2) was calculated and converted to BMI percentiles based on the German reference system [37]. Subsequently, participants were classified as non-overweight/obese or overweight/obese using the 90th percentile.

Motor Competence. Following anthropometric measurements, motor competence was assessed via the German motor test (Deutscher Motorik Test, DMT6-18), which consists of eight test items to evaluate endurance, power, speed, coordination, and agility [38]. Specifically, the DMT6-18 consists of a 20 m sprint, sideways jumping, standing long jump, sit ups, push-ups, backwards balancing, a stand and reach test, and a 6 min run. After a standardized 5 min warm-up, participants started the test with the 20 m sprint. Other tests were completed in random order, except for the 6 min run, which was completed at the end of the session. All tests, including practice trials, were administered in accordance with the specifications provided by the test manual [38]. In addition to raw performance scores, age- and sex-specific standardized values were calculated based on a German reference sample,

which were used in the statistical analyses. The mean of these standardized scores was further used to calculate an overall motor competence score.

Dietary assessment. Dietary information was obtained via a standardized food frequency questionnaire that has been used previously with Austrian adolescents [39]. The questionnaire was administered by a trained technician during regular class time. Participants reported the frequency (days/week) of the consumption of 42 foods that were subsequently summarized into food groups. Principal component analysis was used to identify dietary patterns. The analysis revealed three factors with an Eigenvalue >1, which explained 55.9% of the total variance of dietary intake. Specifically, factor 1 was characterized by high loadings of meat, fish, bread (white and/or wholemeal), pasta and sweets consumption (meat/carbohydrates (CHO)); factor 2 was characterized by high loadings of milk, cereal, nuts and fruits (milk/cereal); and factor 3 was characterized by high consumption of water and vegetables, as well as low consumption of fast food (FF) and soft drinks (water/low FF).

Club sports participation. Participants also reported whether they participated in club sports, and how much time was spent in organized PA. Due to the limited variability in time spent in club sports, participants were stratified into club sports or no club sports for subsequent analyses.

Statistical Analysis. Descriptive statistics were calculated, and data was checked for normal distribution. Sex differences for club sports participation and weight status were determined via Chi-square tests, while differences in motor competence and dietary pattern were examined via multivariate analysis of variance (MANOVA). Tertiles of diet factor scores were used to examine the interaction and the main effects of club sports participation and dietary pattern on motor competence and body weight via 2 × 3 MANOVA (sports participation × diet factor tertiles). In a secondary multivariate analysis of covariance (MANCOVA), sex and BMI percentiles were included as covariates (2 × 3 MANCOVA).

3. Results

A total of 165 middle school students (55% male) provided complete data on food intake and motor competence. Descriptive data of the sample are shown in Table 1. Boys were significantly older than girls, and accordingly, were taller and heavier. There was, however, no sex difference in BMI percentile and the prevalence of overweight/obesity (girls: 17.1%, boys: 23.2%, $p = 0.358$). Almost half of the sample (48.5%) reported participation in club sports, with no difference in the sports participation rate between boys and girls (boys: 46.2%, girls: 51.4%, $p = 0.506$). Based on the absolute performance scores, boys performed significantly better than girls in the standing long jump and sit ups ($p < 0.001$) while girls performed better than boys in the stand and reach test ($p < 0.001$). Using age- and sex-normalized values, girls displayed higher scores compared to boys in the stand and reach test, sit ups, and 6-min run (Table 1). Boys, on the other hand, performed significantly better than girls at the 20 m sprint when using age- and sex-normalized values. Sex differences in total motor competence were borderline significant ($p = 0.056$), with girls having higher values than boys.

Dietary pattern also differed between boys and girls. Specifically, girls reported less frequent consumption of meat and soft drinks compared to boys, while their consumption of fruits and vegetables was more frequent (Table 2). This resulted in significantly lower scores on the meat/CHO factor ($p = 0.020$) in girls, while their score was higher for the water/low FF factor, compared to boys ($p = 0.006$). No significant sex difference occurred for the milk/cereal factor ($p = 0.225$).

Table 1. Anthropometric characteristics and motor competence for the total sample and separately for girls and boys. Values are mean ± SD.

	Total Sample (*N* = 165)	Girls (*N* = 74)	Boys (*N* = 91)	*p* *
Age (years)	12.9 ± 1.2	12.6 ± 1.1	13.1 ± 1.2	0.009
Height (cm)	161.3 ± 8.9	158.8 ± 6.8	163.4 ± 9.9	0.001
Weight (kg)	53.8 ± 14.3	51.3 ± 9.9	55.9 ± 16.9	0.043
BMI percentile	59.4 ± 29.4	60.9 ± 27.5	58.2 ± 31.0	0.563
20 m sprint (sec)	3.8 ± 0.4	3.9 ± 0.4	3.7 ± 0.4	0.023
Balance (steps)	38.2 ± 9.1	39.5 ± 7.5	37.2 ± 10.2	0.755
Sideways jump (# in 15 s)	42.1 ± 7.5	41.4 ± 7.0	42.6 ± 7.9	0.554
Stand and reach (cm)	−0.3 ± 9.7	3.8 ± 8.5	−3.6 ± 9.3	0.011
Push-ups (# in 40 s)	15.6 ± 3.9	15.6 ± 3.4	15.6 ± 4.2	0.067
Sit ups (# in 40 s)	23.9 ± 4.6	22.4 ± 3.8	25.1 ± 4.9	0.011
Standing long jump (cm)	171.0 ± 29.7	159.5 ± 25.2	180.3 ± 29.8	0.648
6 min run (m)	997 ± 157	988 ± 119	1005 ± 183	<0.001
Total motor competence	103.3 ± 7.7	104.6 ± 6.9	102.3 ± 8.1	0.056

* *p*-value based on age- and sex-normalized values. # number of repetitions performed. BMI: Body mass index.

Table 2. Frequency of consumption of food groups (days/week). Values are Means ± SD.

	Total Sample	Girls	Boys	*p*
Meat	2.5 ± 1.2	2.1 ± 1.1	2.7 ± 1.2	0.002
Fish & Eggs	1.8 ± 1.2	1.6 ± 1.0	2.0 ± 1.3	0.051
Milk	2.2 ± 1.0	2.2 ± 1.1	2.3 ± 1.0	0.458
Carbs	2.2 ± 1.1	2.2 ± 1.0	2.2 ± 1.1	0.702
Bread	3.2 ± 0.9	3.3 ± 0.8	3.1 ± 1.0	0.371
Nuts & Seeds	1.2 ± 0.9	1.2 ± 1.0	1.2 ± 0.9	0.662
Fast Food	1.6 ± 0.9	1.5 ± 0.8	1.7 ± 0.9	0.105
Sweets	2.6 ± 1.1	2.5 ± 1.1	2.7 ± 1.2	0.420
Fruits	2.7 ± 1.2	2.9 ± 1.3	2.6 ± 1.1	0.044
Veggie	2.5 ± 1.5	2.9 ± 1.6	2.2 ± 1.4	0.003
Soft Drink	2.3 ± 1.3	2.0 ± 1.1	2.6 ± 1.3	0.001
Water	4.4 ± 1.6	4.6 ± 1.7	4.2 ± 1.5	0.126

Dietary patterns did not differ by sports participation. The association between sports participation and meat/CHO intake, however, reached borderline significance ($p = 0.057$), with lower values occurring in adolescents participating in club sports. There was no difference in the prevalence of overweight/obesity by club sports participation, and across tertiles of diet factors. Also, no interaction effects of diet pattern and club sports participation on BMI percentile were observed.

Motor competence, however, was significantly associated with club sports participation and dietary pattern. Combined associations of diet and sports participation with motor competence, however, were limited. The only significant combined association was observed between club sports participation and milk/cereal consumption on backwards balancing ($p = 0.008$), with a stronger association between dietary pattern and motor competence in adolescents not participating in club sports (Table 3).

Several independent associations of club sports participation and dietary pattern on motor performance were observed. Specifically, club sports participation was associated with better performance on all individual motor competence test items ($p < 0.050$), except for the stand and reach test, resulting in better overall motor competence in participants reporting club sports, compared to those not reporting club sports (Figure 1). Regarding dietary patterns, higher water/low FF consumption was associated with better performance on sideways jumping (p for trend = 0.022), push-ups (p for trend = 0.020), sit ups (p for trend = 0.030), and 6-min run (p for trend = 0.032) (Figure 2). Further, lower scores on the milk/cereal factor were associated with better standing long jump performance and total motor competence (p for trend < 0.050). There was no significant

association between meat/CHO consumption and motor competence. All previously reported results remained essentially unchanged after adjusting for sex and BMI percentile.

Table 3. Motor competence by milk/veggie consumption and sports participation. Values are sex-and age-normalized Means † ± SD.

	Low Milk/Cereal		Moderate Milk/Cereal		High Milk/Cereal	
	Club Sports	**No Club Sports**	**Club Sports**	**No Club Sports**	**Club Sports**	**No Club Sports**
20 m sprint [1]	108.9 ± 13.0	107.5 ± 9.5	106.9 ± 10.7	103.8 ± 14.1	108.1 ± 12.5	99.5 ± 13.0
Balance [1,3,*]	104.6 ± 9.6	107.6 ± 8.2	107.8 ± 8.8	99.4 ± 10.2	108.4 ± 9.8	103.6 ± 11.1
Sideways jump [1,*]	118.0 ± 6.9	111.6 ± 9.7	116.7 ± 7.9	111.7 ± 12.6	117.6 ± 7.9	110.1 ± 12.3
Stand and reach	101.5 ± 12.1	102.0 ± 9.3	101.9 ± 12.0	97.8 ± 12.3	100.3 ± 11.8	98.7 ± 11.1
Push-ups [1,*]	111.2 ± 9.6	109.1 ± 7.9	110.1 ± 11.5	104.3 ± 13.3	111.5 ± 10.8	102.2 ± 10.9
Sit ups [1]	97.3 ± 8.7	96.9 ± 7.7	96.0 ± 9.2	92.1 ± 10.4	97.0 ± 7.4	91.8 ± 7.5
Standing long jump [1,*,2]	107.2 ± 9.2	104.2 ± 9.2	105.5 ± 12.6	100.6 ± 13.7	105.1 ± 11.0	97.4 ± 11.7
6 min run [1,*]	97.0 ± 10.1	94.1 ± 11.4	98.3 ± 9.1	89.3 ± 12.2	97.0 ± 9.6	89.8 ± 11.6
Total motor competence † [1,*,2]	105.8 ± 6.4	104.2 ± 5.8	105.5 ± 6.3	100.0 ± 9.4	105.8 ± 7.1	98.4 ± 7.5

† Values >100 indicate above-average performance, while values <100 indicate below-average performance. [1] Significant main effect for club sport ($p < 0.050$; * $p < 0.010$). [2] Significant main effect for milk/veggie consumption (p for trend < 0.050; * $p < 0.010$). [3] Significant interaction effect of milk/veggie consumption and club sport participation ($p < 0.050$; * $p < 0.010$).

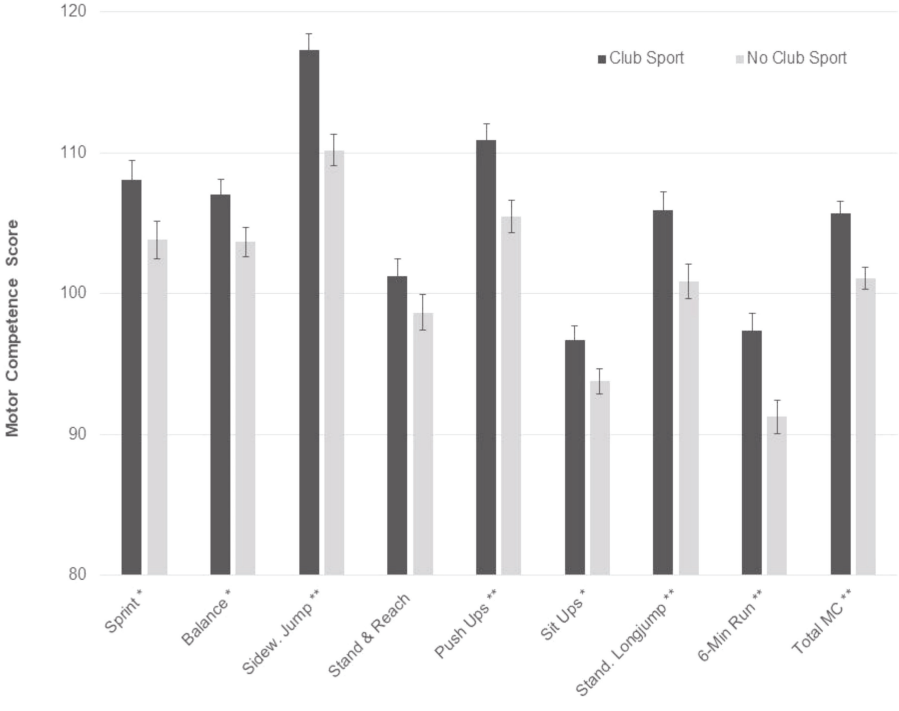

Figure 1. Main effects of club sports participation on motor competence based on 2 × 3 multivariate analysis of variance (MANOVA) (club sports by Water/low fast food (FF)). Values are sex-and age-normalized means with S.E.; * $p < 0.050$ ** $p < 0.010$. MC: motor competence.

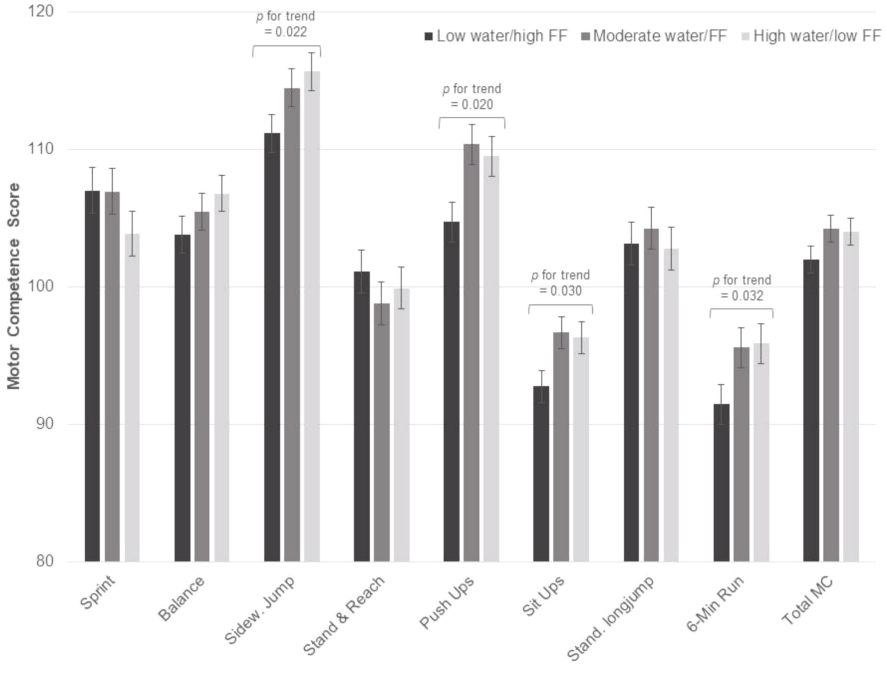

Figure 2. Main effects of club sports participation on motor competence based on 2 × 3 MANOVA (club sports by Water/low FF). Values are sex-and age-normalized means with S.E.

4. Discussion

Even though several studies have examined the association between sports/PA and motor competence [40–43], there exists limited research on the association between motor competence and dietary pattern. To the authors' knowledge, this was also the first study that examined the combined association of dietary pattern and club sports participation with motor competence in Austrian adolescents. While there were no significant associations between dietary patterns and club sports participation, the present study showed independent associations of dietary pattern, as well as club sports participation with motor competence in middle school students. Specifically, club sports participation and healthier dietary choices (i.e., high water and low fast food/soft drink consumption) were associated with higher motor competence. High milk/cereal consumption, on the other hand, was associated with lower motor competence, particularly in participants not reporting club sports. These associations were independent of body weight, and neither club sports participation nor dietary pattern was associated with body weight in the present study.

The positive association between club sports participation and motor competence is consistent with previous research [43–45]. Longitudinal studies further indicate that the strength and directionality of the association between motor competence and club sports participation, as well as PA change over time [46,47]. Particularly during adolescence, motor competence appears to be an important facilitator and precursor for participation in sports clubs, while high PA and sports participation may be a prerequisite for motor development during childhood [45–47]. Accordingly, children and adolescents enter either a positive spiral of high PA, including sports participation and increased motor competence, or a vicious cycle of low motor competence and disengagement from sports. The importance of motor competence for sustainable participation in sports during adolescence may be attributed to an easier acquisition of sport-specific skills in children with higher motor competence. Higher motor competence also enhances self-efficacy, which facilitates participation

in PA and sports [46]. In addition to actual motor competence, perceived competence appears to play an important role in the motivation for participation in sports [48], which is an important component to continuous engagement in various forms of PA.

Participation in organized sports has also been associated with other healthy lifestyle choices, including diet [29,49–51]. The present study, however, did not show healthier dietary patterns in club sports participants. Other studies also reported inconsistent results for the association between club sports participation and dietary pattern [29,52,53]. Even though sports participation has been associated with higher intake of fruits and vegetables, club sports participants also have been shown to consume high amounts of fast food and sugar-sweetened beverages [52]. In fact, it has been argued that sports participation during middle school is a strong risk factor for high fast food consumption during high-school years [54]. The higher fast food consumption in sports participants may be attributed to a more irregular eating pattern and a lower amount of meals consumed at home. The higher energy needs of more active adolescents may also contribute to the consumption of more energy dense foods, including fast foods. The results of the present study, nevertheless, indicate beneficial associations of a healthy dietary pattern with motor competence, independent of club sports participation. Specifically, healthier dietary choices were associated with better performance on agility, strength, and endurance tests. Previous studies also reported increased cardiorespiratory fitness with healthier dietary choices, particularly during adolescence [33,35,55]. While this may, at least partially, be attributed to an indirect association between body weight and motor competence [56], there was no difference in motor competence between overweight/obese and normal weight adolescents in the present study. Another possible explanation, therefore, could be that healthier dietary patterns indicate a greater parental support for a healthy lifestyle in general, including the facilitation of PA. Accordingly, parents may facilitate exposure to diverse movement experiences, which would facilitate motor development, even in the absence of club sports participation. Further, dietary pattern has been associated with sedentary choices, which also affect motor development [47]. Specifically, high media time has been associated with poorer diet quality [57,58] as well as low motor competence [47,59].

Sedentary behavior and total PA, rather than participation in club sports, are also crucial correlates of body weight [60]. Neither sports participation nor dietary pattern, however, was associated with body weight in the present study. Results on the association between club sports and body weight have generally been inconsistent [52], which may emphasize the importance of total PA rather than sports in weight management. In fact, it has been argued that a large amount of time in youth sport is spent sedentary or in only light PA [61,62]. Similarly, controversy remains on the relationship between diet and adiposity in youth [30]. Even though several studies showed an inverse association between dietary intake and body weight [63,64], there are also studies that did not show any association [65], or even direct associations between diet an body weight [66,67]. At least partially, this may be attributed to problems in obtaining accurate dietary data, particularly in youth [32]. It should, however, also be considered that more active youth have higher energy needs [31]. Accordingly, children and adolescents with high caloric intake may be able to maintain a healthy body weight as long as they are sufficiently active.

Several limitations of this study, however, need to be considered when interpreting the results. There was no objective measurement of total PA in the present study. Due to the reliance on self-reporting, club sports participation was used as an indicator for PA, as this may be reported more accurately than total PA. Previous research also indicated that participation in club sports is directly associated with total PA [52]. The sample distribution, however, did not allow for a differentiation by the amount of participation in club sports (e.g., hours, days); rather, only participants vs. non-participants could be analyzed. An additional limitation is that participants reported frequency rather than total amount of foods consumed, which provides only limited information on the total energy content of the diet. There is also an inherent risk of selective over- or under-reporting, due to social desirability and social approval with any form of diet report. Participants may have difficulties remembering all the foods, and some foods that they consumed may not have been listed on the

questionnaire, which could have affected the reported dietary pattern. The cross-sectional nature of the study further does not allow for the establishment of causal relationships and temporal trends between sports participation, dietary pattern, and motor competence. In addition, the generalizability of the results may be limited, due to the small sample size and homogeneity of the study population. The objective assessment of various components of motor competence with a widely used and previously validated test, on the other hand, should be considered a strength of the study.

5. Conclusions

PA and healthy dietary habits play a crucial role in the development and general health of children and adolescents [1]. The present study also showed that both behaviors are independently associated with motor competence, which is an important component in the facilitation of an active lifestyle [36]. The facilitation of participation in sports, along with the promotion of healthy dietary choices may be particularly important during adolescence, as this is a critical time for the development of future lifestyle choices [3]. Accordingly, coaches, parents, and youth need to be educated on the importance of adequate nutrition in addition to participation in various forms of PA, including sports, for optimal motor development. Even though this may require additional efforts and resources, it may be a worthwhile investment to enhance the health and well-being of future generations.

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Health Status of Female and Male Vegetarian and Vegan Endurance Runners Compared to Omnivores—Results from the NURMI Study (Step 2)

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Abstract: Health effects of vegetarian and vegan diets are well known. However, data is sparse in terms of their appropriateness for the special nutritional demands of endurance runners. Therefore, the aim of this study was to investigate the health status of vegetarian (VER) and vegan endurance runners (VGR) and compare it to omnivorous endurance runners (OR). A total of 245 female and male recreational runners completed an online survey. Health status was assessed by measuring health-related indicators (body weight, mental health, chronic diseases, and hypersensitivity reactions, medication intake) and health-related behavior (smoking habits, supplement intake, food choice, healthcare utilization). Data analysis was performed by using non-parametric ANOVA and MANOVA. There were 109 OR, 45 VER and 91 VGR. Significant differences ($p < 0.05$) were determined for the following findings: (i) body weight for VER and VGR was less than for OR, (ii) VGR had highest *food choice* scores, and (iii) VGR reported the lowest prevalences of allergies. There was no association ($p > 0.05$) between diet and mental health, medication intake, smoking habits, supplement intake, and healthcare utilization. These findings support the notion that adhering to vegetarian kinds of diet, in particular to a vegan diet, is associated with a good health status and, thus, at least an equal alternative to an omnivorous diet for endurance runners.

Keywords: vegetarian; vegan; half-marathon; marathon; running; health conscious; recreational athlete

1. Introduction

During an endurance event, such as a marathon running, body and mind are challenged to an extremely high degree. Athletes are exposed to several physiological and psychological challenges, in particular with regard to energy metabolism, body temperature and fluid balance [1–3]. A study by Hausswirth and Lehénaff highlighted the importance of fat metabolism, since an increase in free fatty acids and glycerol at the end of long-distance races crucially affects running economy and, thus, performance when the athlete is almost at the finish line [3]. Further important parameters with regard to running economy are maximal oxygen consumption, lactate-threshold, and metabolic efficacy [2]. Moreover, completing a long-distance race is a psychological challenge which requires favorable character traits, such as inhibitory control, the ability not only to inhibit motor response, but also to

suppress processing of irrelevant information, and the ability to protect cognitive performance so that it is less influenced by emotional stimuli [1]. In order to meet all these requirements, a good health status and a strong mind are necessary and will contribute to good exercise performance [1,2].

An essential requirement for a good health status is the choice of an appropriate, healthy, and sustainable diet [4,5]. As endurance running is known as a kind of sport with high energy expenditure and, thus, consumption, an endurance athlete's need for vitamins, trace elements and other valuable food ingredients besides macronutrient requirements is very high [4]. Therefore, a well-balanced energy turnover is crucial [4], resulting in the creation of a well-planned and reasonable nutrition strategy [5]. Current evidence suggests that one strategy could be adhering to a meatless diet rich in vegetables and fruits, such as a vegetarian kind of diet [6–8] (pp. 419–437). Vegetarian kind of diet is an umbrella term which subsumes four main dietary patterns: lacto-ovo-vegetarian, lacto-vegetarian, ovo-vegetarian, and vegan. Lacto-ovo vegetarians consume dairy products and eggs but no meat, poultry or seafood. Lacto-vegetarians eat dairy products but avoid eggs, meat, poultry, and seafood. Ovo-vegetarians eat eggs, but no dairy products, meat, poultry, or seafood. A vegan diet is characterized by the rejection of all products from animal sources, such as meat, fish/shellfish, milk and dairy products, eggs, and honey. A dietary pattern without any restriction is referred to as an omnivorous kind of diet [7].

Healthy vegetarian kinds of diet usually include complex carbohydrates, fiber, fruits, vegetables, and antioxidants [9]. Although potentially lower in some nutrients, such as zinc and vitamin B12 [9], carefully planned vegetarian kinds of diet meet or even exceed the nutritional requirements of athletes, in particular with regard to the intake of proteins, fatty acids and iron [6–8] (pp. 419–437). More than this, vegetarian kinds of diet are known to have further beneficial effects on health than just energy intake, in particular in terms of body weight control [10,11], the prevention of diabetes mellitus type 2 [12,13], ischemic heart disease [11,14], and protection against depression [15]. In addition, a vegetarian diet has been found to reduce the risk for some types of cancer, such as colon and prostate cancer [9,16]. Despite immediate health-related effects due to the consumption of healthy foods, being a vegetarian or vegan is often associated with a healthy lifestyle characterized by the avoidance of adverse health behavior, such as smoking and alcohol consumption, a high level of physical activity, and time for relaxation [8] (p. 393).

To date, little is known about the health status and health-related behavior of vegetarian and vegan endurance runners [17,18]. Most researchers have not classified their subjects by dietary subgroup [19]. Beyond that, these studies usually dealt with athletes in general, so that data in terms of endurance runners is sparse. A well-founded comparison of health characteristics between vegetarian, vegan and omnivorous endurance runners is lacking. Specific knowledge about the interconnectedness of diet choice and health could provide a better basis for athletes and their coaches, physicians, and nutritionists/dietitians, in order to optimize training and treatment strategies.

The aim of the study, therefore, was to investigate the health status of endurance runners and to compare athletes who adhere to a vegetarian or vegan diet to those who follow an omnivorous diet. Since a good state of health of non-active vegetarians and vegans is sound and compares favorably to that of omnivores [8] (p. 411), it was hypothesized that vegetarian and vegan endurance runners would have a better health status than omnivorous endurance runners.

2. Materials and Methods

2.1. Study Protocol and Ethics Approval

The study protocol [20] was approved by the ethics board of St. Gallen, Switzerland on 6 May 2015 (EKSG 14/145). The trial registration number is ISRCTN73074080.

2.2. Participants

The NURMI (Nutrition and Running High Mileage) Study was conducted in three steps following a cross-sectional design. Endurance runners, mainly from German-speaking countries including

Germany, Austria, and Switzerland, were recruited. In addition, people from around the world were addressed. Participants were contacted mainly via social media, websites of the organizers of marathon events, online running communities, email lists, and runners' magazines, as well as via magazines for health, vegetarian, and/or vegan nutrition and lifestyle, sports fairs, trade fairs on vegetarian and vegan nutrition and lifestyle, and through personal contacts. The characteristics of the subjects are presented in Table 1.

Table 1. Anthropometric and demographic characteristics of the subjects displayed by diet group.

		Omnivorous	Vegetarian	Vegan
Number of Subjects		100% (109)	100% (45)	100% (91)
Sex	Female	47% (51)	58% (26)	70% (64)
	Male	53% (58)	42% (19)	30% (27)
Age (years) (median)		43 (IQR 18)	39 (IQR 16)	37 (IQR 15)
Body Weight (kg) (median)		68 (IQR 16.7)	62 (IQR 11.3)	64 (IQR 10)
BMI_{CALC} (kg/m^2)	≤18.49	4% (4)	7% (3)	9% (8)
	18.50–24.99	80% (87)	87% (39)	82% (75)
	≥25–29.99	17% (18)	7% (3)	9% (8)
Race Distance	10 km	34% (37)	33% (15)	43% (39)
	Half-marathon	36% (39)	44% (20)	33% (30)
	Marathon/Ultramarathon	30% (33)	22% (10)	24% (22)
Academic Qualification	No Qualification	0% (0)	0% (0)	1% (1)
	Upper Secondary Education/Technical Qualification/GCSE or Equivalent	38% (41)	38% (17)	27% (25)
	A Levels or Equivalent	24% (26)	16% (7)	22% (20)
	University Degree/Higher Degree (i.e., doctorate)	30% (33)	38% (17)	36% (33)
	No Answer	8% (9)	9% (4)	13% (12)
Marital Status	Divorced/Separated	3% (3)	4% (2)	11% (10)
	Married/Living with Partner	75% (82)	58% (26)	62% (56)
	Single	22% (24)	38% (17)	27% (25)
Country of Residence	Austria	21% (23)	18% (8)	14% (13)
	Germany	70% (76)	76% (34)	74% (67)
	Switzerland	7% (8)	4% (2)	3% (3)
	Other	2% (2)	2% (1)	9% (8)
Motive for Diet Choice	Health, wellbeing	81% (21)	85% (28)	90% (79)
	Sporting performance	54% (14)	33% (11)	59% (52)
	Food scandals	15% (4)	55% (18)	32% (28)
	Animal welfare	46% (12)	79% (26)	90% (79)
	Ecological aspects	50% (13)	76% (25)	83% (73)
	Social aspects	35% (9)	55% (18)	57% (50)
	Economic aspects	8% (2)	12% (4)	22% (19)
	Religion/spirituality	0% (0)	12% (4)	7% (6)
	Custom/tradition	15% (4)	0% (0)	2% (2)
	Taste/enjoyment	42% (11)	33% (11)	44% (39)
	No specific reason	4% (1)	0% (0)	0% (0)

10 km = 10-km control group. BMI_{CALC} = Body Mass Index (calculated). IQR = interquartile range.

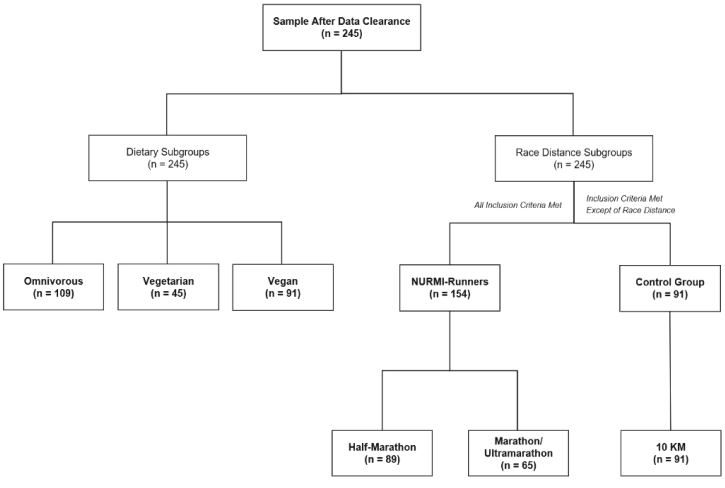
2.3. Procedures

2.3.1. Experimental Approach

Participants completed an online survey within the NURMI Study Step 2, which was available in German and English at www.nurmi-study.com from 1 February 2015 to 31 December 2015. Prior to completing the questionnaires on physical and psychological health, participants were provided with a written description of the procedures and gave their informed consent to take part in the study.

For successful participation in the study, the following inclusion criteria were required: (1) written informed consent, (2) at least 18 years of age, (3) completed questionnaire, (4) successful participation

in a running event of at least half-marathon distance in the past two years. Participants were classified into three dietary subgroups (Scheme 1): omnivorous (commonly known as Western diet, no dietary restrictions) diet; vegetarian (no meat); and vegan (no products from animal sources) [7]. In addition, they were categorized according to race distance: 10-km, half-marathon, and marathon/ultramarathon. Marathoners and ultramarathoners were pooled together since the marathon distance is included in an ultramarathon. A total of 91 highly-motivated runners provided accurate and useful answers with plenty of high-quality data. However, they had not successfully participated in either a half-marathon or marathon, but rather in a 10-km race. In order to avoid an irreversible loss of these valuable datasets, those who met all inclusion criteria but named a 10-km race as their running event were kept as the control group.



Scheme 1. Categorization of participants.

According to the WHO [21,22] the goal for individuals should be to maintain a BMI in the range 18.5–24.9 kg/m^2 (BMI_{NORM}) in order to achieve optimum health. They point to an increased risk of co-morbidities for a BMI 25.0–29.9 kg/m^2, and moderate to severe risk of co-morbidities for a BMI > 30 kg/m^2 [21,22]. Therefore, the calculated Body Mass Index (BMI_{CALC}) was classified into three categories of body weight-to-height ratio (kg/m^2): $\leq 18.49 < BMI_{NORM}$: 18.50–24.99 kg/m^2 ≥ 25. Since the BMI of active runners could be below BMI_{NORM} [23], but in addition people with a higher BMI might start running in order to achieve and maintain a stable, healthy body weight, participants with BMI < 30 were included. BMI has been shown to be a significant performance-determining parameter for speed improvement in running over various distances, with a continuous increase in BMI from 19.57 (1.29) kg/m^2 in marathoners to 23.3 (1.67) kg/m^2 over the 100 m distance [24]. An optimal BMI for high running pace, reported for the best performers over 10 km and marathon distance, was found to be between 19–20 kg/m^2.

2.3.2. Data Clearance

In order to control for measures of (1) diet and (2) running, two groups of control questions were included, each within different sections of the survey. In order to control for a minimal status of health linked to a minimum level of fitness and to further enhance the reliability of datasets, the BMI approach following the WHO [21,22] was used. With a BMI $\geq$ 30 other health-protecting and/or weight loss strategies than running would be necessary to safely reduce body weight. Therefore, three participants with a BMI $\geq$ 30 were excluded from the data analysis.

A total of 317 endurance runners completed the survey. Incomplete, inconsistent, and conflicting datasets were excluded from the data analysis. After data clearance a total of 245 runners with complete datasets were included for descriptive statistical analysis (Scheme 2).

Scheme 2. Flow chart of participants' enrollment.

2.4. Measures

Health status (latent variable) was derived by using both the two clusters 'Health-related Indicators' and 'Health-related Behavior'. Each cluster pooled four dimensions, with each defined by specific items based on manifest measures. An overview of the variables is presented in Table 2.

The following health-related indicators described health outcomes: (1) body weight/BMI, (2) mental health (stress perception), (3) chronic diseases and hypersensitivity reactions: prevalence of chronic diseases (heart disease, state after heart attack, cancer), prevalence of metabolic diseases (diabetes mellitus 1, diabetes mellitus 2, hyperthyroidism, hypothyroidism), prevalence of hypersensitivity reactions (allergies, intolerances), and (4) medication intake (for thyroid disease, for hypertension, for cholesterol level, for contraception).

Table 2. Overview of the variables in order to derive health status of endurance runners.

Cluster	Dimension	Indicator	Item	Measure
Health-related Indicators	**Body weight**/BMI	BMI_{calc} [1]	Your current body weight (kg)? Your height (m)?	Body weight (kg) Height (m)
	Mental health	Stress perception	Are you under pressure and/or are you suffering from stress?	Yes No
	Chronic diseases and hypersensitivity reactions	Cardiovascular diseases and Cancer	Are you currently suffering from the following chronic diseases or their direct consequences?	Heart disease requiring treatment Heart attack Cancer (now or in the past)?
		Metabolic diseases	Are you currently suffering from one of the following metabolic disease(s)?	Diabetes mellitus type 1 Diabetes mellitus type 2 Hyperthyroidism Hypothyroidism
		Hypersensitivity reactions	Are you currently suffering from ...?	Allergies Intolerances
	Medication intake		Do you take medicaments regularly (every day), for example, ...?	Thyroid High blood pressure Cholesterol and/or other blood serum lipid values contraceptive pill
Health-related Behavior	**Smoking habits**	Current consumption of cigarettes	Do you currently smoke?	Yes No
		Former consumption of cigarettes	Have you ever smoked?	Yes No
	Supplement intake	Substance intake for medical reasons	Do you take supplements prescribed by a doctor regularly (everyday)?	Yes No
		Intake of Performance enhancement substance	Do you take anything to boost your performance in your daily life, at work or while doing sport (e.g., energy drinks)?	Yes, regularly every day Yes, occasionally No
		Substance intake for stress coping	Do you take anything to help you cope with stress in your daily life, at work or while doing sport?	Yes, regularly every day Yes, occasionally No
	Food choice	Motivation for food choice	Do you choose ingredients and food on the basis Of the following (e.g., in view of the disease mentioned above or other illnesses)?	Healthy (e.g., if you are ill) Health-promoting (e.g., to prevent ill-health) Good for maintaining health (e.g., wholefoods)

Table 2. *Cont.*

Cluster	Dimension	Indicator	Item	Measure
		Avoided ingredients	Do you choose food in order to avoid particular ingredients or nutrients (e.g., in view of the diseases mentioned above or other illnesses or effects on health)?	Refined sugar Sweetener Fat in general Saturated fats Cholesterol Products made with white flour Sweet things (e.g., Jelly beans, chocolate drops, cream cakes) Nibbles (e.g., crisps, salted peanuts) Alcohol Caffeine or other stimulants (e.g., in coffee or energy drinks)
		Desired ingredients	Do you choose food because of particular valuable ingredients or nutrients (e.g., to prevent the diseases mentioned above or other illnesses or effects on health)?	Vitamins Minerals/trace elements Antioxidants Phytochemicals Fiber Other
	Healthcare utilization	Frequency of doctor consultations	How often have you seen a doctor in the last 12 months (except dentist and for routine check-ups)?	Never Once a month Every 2 months Every 3 months (four times a year) Every 6 months (twice a year) Once a year
		Utilization of regular health check-ups	Do you go for regular check-ups or routine health checks?	Yes No

[1] BMI_{CALC} = Body Mass Index calculated.

The following variables of health-related behavior described health outcomes: (1) smoking habits (current and former smoking), (2) supplement intake (supplements prescribed by a doctor, supplements for performance enhancement, supplements to cope with stress), (3) food choice (motivation, desired ingredients, avoided ingredients), and (4) healthcare utilization (regular check-ups). Resulting from this, eight domain scores (body weight/BMI, mental health, chronic diseases, and hypersensitivity reactions, medication intake, smoking, supplement intake, food choice, healthcare utilization) were derived, which generated scores between 0 and 1. Low scores indicate detrimental health effects, while higher scores indicate beneficial health effects (given as mean scores plus standard deviation, and percentage (%)).

2.5. Statistical Analysis

The statistical software R version 3.5.0 Core Team 2018 (R Foundation for Statistical Computing, Vienna, Austria) performed all statistical analyses. Exploratory analysis was performed by descriptive statistics (median and interquartile range (IQR)). Significant differences between dietary subgroups and domain scores to describe health status were calculated by using a non-parametric ANOVA. Chi-square test and Kruskal-Wallis test were used to examine the association between dietary subgroups and domain scores with nominal scale variables, and Wilcoxon test and Kruskal-Wallis test (ordinal and metric scale) approximated by using the F distributions.

Statistical modeling. State of health as the latent variable was derived by manifest variables (e.g., body weight, cancer, smoking, etc.). In order to scale the health status displayed by measures, items and dimensions, a heuristic index between 0 and 1 was defined (equivalence in all items). To test the statistical hypothesis considering significant differences between dietary subgroups, race distance and sex for each dimension a MANOVA was performed to define health status. The assumptions of the ANOVA were verified by residual analysis.

The level of statistical significance was set at $p \leq 0.05$.

3. Results

A total of 317 endurance runners completed the survey, of whom 245 (141 women and 104 men) remained after data clearance with a mean age of 39 (IQR 17) years, from Germany (n = 177), Switzerland (n = 13), Austria (n = 44) and from other countries (n = 11; Belgium, Brazil, Canada, Italy, Luxemburg, Netherlands, Poland, Spain, UK).

A total of 109 participants followed an omnivorous diet, 45 reported to adhere to a vegetarian diet, and 91 to a vegan diet. In addition, there were a total of 91 10-km runners, 89 half-marathoners, and 65 marathoners/ultramarathoners.

3.1. Cluster 'Health-Related Indicators'

3.1.1. Dimension of Body Weight/BMI

There was a significant difference in body weight between dietary subgroups ($F_{(2, 242)} = 6.86$, $p = 0.001$), with vegetarians and vegans showing lower body weight than omnivores. However, there was no difference in the health-related item BMI between dietary subgroups ($\chi^2_{(4)} = 6.08$, $p = 0.193$) (Table 3). Moreover, vegans had the highest counts for the health-related indicator *body weight/BMI* (0.69 (0.40), $F_{(2, 242)} = 0.41$, $p = 0.662$) (Figure 1a).

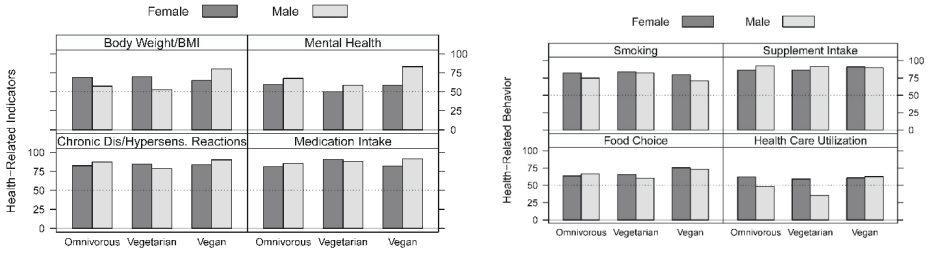
3.1.2. Dimension of Mental Health

There was no significant association between diet group and stress perception ($\chi^2_{(2)} = 1.78$, $p = 0.412$) (Table 3). However, vegans had the highest score with regard to *mental health* (0.66 (0.48), $F_{(2, 219)} = 0.88$, $p = 0.415$) (Figure 1a).

Table 3. Descriptive results and ANOVA of the 'Health-Related Indicators' cluster.

Dimension	Omnivorous	Vegetarian	Vegan	Statistics
Body Weight/BMI				
Body Weight (kg) (median)	68.00 (IQR 16.70)	62.00 (IQR 11.30)	64.00 (IQR 10.00)	$F_{(2, 242)} = 6.86$, $p = 0.001$
BMI_{CALC}				$\chi^2_{(4)} = 6.08$, $p = 0.193$
≤18.49	4% (4)	7% (3)	9% (8)	
18.50–24.99	80% (87)	87% (39)	82% (75)	
≥25–29.99	17% (18)	7% (3)	9% (8)	
Mental Health				$\chi^2_{(2)} = 1.78$, $p = 0.412$
Stress Perception				
Yes	36% (35)	46% (18)	34% (29)	
No	64% (63)	54% (21)	66% (56)	
Chronic Diseases and Hypersensitivity Reactions				
Prevalence of Chronic Diseases				$\chi^2_{(4)} = 2.88$, $p = 0.578$
Heart Disease	1% (1)			
Heart Attack				
Cancer			1% (1)	
No Diseases	99% (97)	100% (39)	99% (84)	
Prevalence of Metabolic Diseases				$\chi^2_{(10)} = 7.14$, $p = 0.713$
Diabetes Mellitus 1	1% (1)	3% (1)		
Diabetes Mellitus 2	2% (2)			
Hyperthyroidism	1% (1)	3% (1)	1% (1)	
Hypothyroidism	5% (5)	3% (1)	8% (7)	
Other Diseases	1% (1)		1% (1)	
No Diseases	90% (88)	92% (36)	89% (76)	
Prevalence of Hypersensitivity Reactions				$\chi^2_{(4)} = 12.87$, $p = 0.012$
Allergies	32% (31)	36% (14)	20% (17)	
Intolerances	1% (1)	10% (4)	12% (10)	
No Reactions	67% (66)	54% (21)	68% (58)	
Medication Intake (regularly)				$\chi^2_{(6)} = 7.58$, $p = 0.271$
Thyroid Disease	6% (6)	8% (3)	11% (9)	
Hypertension	5% (5)	3% (1)		
Cholesterol Level				
Other Medication	5% (5)		5% (4)	
No Medication	84% (82)	90% (35)	85% (72)	
Contraceptives	12% (12)	10% (4)	15% (13)	$\chi^2_{(2)} = 0.70$, $p = 0.704$

BMI_{CALC} = Body Mass Index (calculated). IQR = interquartile range.



(**a**) 'Health-Related Indicators' (**b**) 'Health-Related Behavior'

Figure 1. Indices of both clusters 'Health-Related Indicators' and 'Health-Related Behavior' of female and male endurance runners, displayed by dietary subgroups (as percentage, %). Low scores indicate detrimental health effects, high scores indicate beneficial health effects.

3.1.3. Dimension of Chronic Diseases and Hypersensitivity Reactions

There was no significant association between diet and the prevalence of cardiovascular diseases and cancer ($\chi^2_{(4)} = 2.88$, $p = 0.578$), and even between diet and prevalence of metabolic diseases ($\chi^2_{(10)} = 7.14$, $p = 0.713$). However, there was a significant difference between the prevalence of hypersensitivity reactions and diet ($\chi^2_{(4)} = 12.87$, $p = 0.012$), where vegan endurance runners stated least often that they had at least one allergy. In addition, omnivores reported having a food intolerance least often (Table 3). Omnivorous, vegan, and vegetarian runners scored similarly with regard to the

health-related indicator *chronic diseases and hypersensitivity reactions* (respectively, 0.85 (0.20), 0.82 (0.20), and 0.85 (0.18), $F_{(2, 219)} = 0.58$, $p = 0.562$) (Figure 1a).

3.1.4. Dimension of Medication Intake

There was no significant association between medication intake and dietary subgroup ($\chi^2_{(6)} = 7.58$, $p = 0.271$) (Table 3). Furthermore, there was no significant effect diet on the use of contraceptives ($\chi^2_{(2)} = 0.70$, $p = 0.704$). However, vegetarians had the highest scores with regard to medication intake, even though all dietary subgroups had similar scores (respectively, 0.84 (0.37), 0.90 (0.31), and 0.85 (0.36), $F_{(2, 219)} = 0.41$, $p = 0.663$) (Figure 1a).

3.2. Cluster 'Health-Related Behavior'

3.2.1. Dimension of Smoking Habits

Diet and current or former smoking were not significantly associated ($\chi^2_{(4)} = 8.96$, $p = 0.062$) (Table 4). Vegetarians showed the best health-related behavior with regard to *smoking habits* (0.83 (0.29), $F_{(2, 219)} = 1.30$, $p = 0.275$) (Figure 1b).

Table 4. Descriptive results and ANOVA of the 'Health-Related Behavior' cluster.

Dimension	Omnivorous	Vegetarian	Vegan	Statistics
Smoking Habits				$\chi^2_{(4)} = 8.96$, $p = 0.062$
Non-Smoker	58% (57)	72% (28)	54% (46)	
Ex-Smoker	40% (39)	23% (9)	46% (39)	
Smoker	2% (2)	5% (2)		
Supplement Intake				
prescribed by doctor	8% (8)	10% (4)	6% (5)	$\chi^2_{(2)} = 0.79$, $p = 0.675$
to boost your performance (occasionally)	10% (10)	21% (8)	11% (9)	$\chi^2_{(4)} = 4.09$, $p = 0.394$
to boost your performance (regularly)	3% (3)	0 % (0)	2% (2)	
to cope with stress (occasionally)	5% (5)	5% (2)	8% (7)	$\chi^2_{(4)} = 1.79$, $p = 0.774$
to cope with stress (regularly)	2% (2)		2% (2)	
Food Choice				
Motivation				
because it is healthy	67% (66)	74% (29)	75% (64)	$\chi^2_{(2)} = 1.59$, $p = 0.452$
because it is health-promoting	81% (79)	79% (31)	88% (75)	$\chi^2_{(2)} = 2.41$, $p = 0.300$
because it is good for maintaining health	85% (83)	92% (36)	95% (81)	$\chi^2_{(2)} = 5.99$, $p = 0.050$
Avoided Ingredients				
Refined Sugar	62% (61)	56% (22)	73% (62)	$\chi^2_{(2)} = 3.95$, $p = 0.138$
Sweetener	74% (73)	59% (23)	80% (68)	$\chi^2_{(2)} = 6.16$, $p = 0.046$
Fat in General	39% (38)	46% (18)	49% (42)	$\chi^2_{(2)} = 2.17$, $p = 0.339$
Saturated Fats	53% (52)	46% (18)	72% (61)	$\chi^2_{(2)} = 9.82$, $p = 0.007$
Cholesterol	34% (33)	31% (12)	65% (55)	$\chi^2_{(2)} = 21.60$, $p < 0.001$
White Flour	64% (63)	59% (23)	74% (63)	$\chi^2_{(2)} = 3.42$, $p = 0.181$
Sweets	58% (57)	62% (24)	69% (59)	$\chi^2_{(2)} = 2.52$, $p = 0.284$
Nibbles	62% (61)	59% (23)	62% (53)	$\chi^2_{(2)} = 0.15$, $p = 0.928$
Alcohol	55% (54)	51% (20)	56% (48)	$\chi^2_{(2)} = 0.29$, $p = 0.864$
Caffeine	26% (25)	36% (14)	46% (39)	$\chi^2_{(2)} = 8.30$, $p = 0.016$
Desired Ingredients				
Vitamins	81% (79)	72% (28)	86% (73)	$\chi^2_{(2)} = 3.48$, $p = 0.175$
Minerals/Trace Elements	70% (69)	72% (28)	75% (64)	$\chi^2_{(2)} = 0.56$, $p = 0.757$
Antioxidants	47% (46)	44% (17)	60% (51)	$\chi^2_{(2)} = 4.25$, $p = 0.119$
Phytochemicals	42% (41)	31% (12)	59% (50)	$\chi^2_{(2)} = 9.93$, $p = 0.007$
Fiber	68% (67)	62% (24)	75% (64)	$\chi^2_{(2)} = 2.58$, $p = 0.276$
Health Care Utilization				
Regular check-ups or routine health checks	54% (53)	49% (19)	61% (52)	$\chi^2_{(2)} = 1.91$, $p = 0.385$

3.2.2. Dimension of Supplement Intake

There was no significant association between diet and supplement intake prescribed by a doctor ($\chi^2_{(2)} = 0.79$, $p = 0.675$), the consumption of performance-enhancing substances ($\chi^2_{(4)} = 4.09$, $p = 0.394$) or the intake of substances to cope with stress ($\chi^2_{(4)} = 1.79$, $p = 0.774$) (Table 4). Vegans showed the

best health-related behavior with regard to *supplement intake* (0.91 (0.19), $F_{(2, 219)} = 0.35$, $p = 0.708$) (Figure 1b).

3.2.3. Dimension of Food Choice

There was no significant association between diet and food choice (i) because it is healthy ($\chi^2_{(2)} = 1.59$, $p = 0.452$) and health-promoting ($\chi^2_{(2)} = 2.41$, $p = 0.300$); or (ii) in order to obtain vitamins ($\chi^2_{(2)} = 3.48$, $p = 0.175$), minerals/trace elements ($\chi^2_{(2)} = 0.56$, $p = 0.757$), antioxidants ($\chi^2_{(2)} = 4.25$, $p = 0.119$) and fiber ($\chi^2_{(2)} = 2.58$, $p = 0.276$) (Table 4). Moreover, there was no significant association between diet and the avoidance of the following ingredients (Table 4): refined sugar ($\chi^2_{(2)} = 3.95$, $p = 0.138$), fat in general ($\chi^2_{(2)} = 2.17$, $p = 0.339$), white flour ($\chi^2_{(2)} = 3.42$, $p = 0.181$), sweets ($\chi^2_{(2)} = 2.52$, $p = 0.284$), nibbles ($\chi^2_{(2)} = 0.15$, $p = 0.928$), and alcohol ($\chi^2_{(2)} = 0.29$, $p = 0.864$).

However, there was a significant effect of diet on *food choice,* both (i) because it is good for maintaining health ($\chi^2_{(2)} = 5.99$, $p = 0.050$), with vegetarians and vegans reporting doing so more often; and (ii) in order to obtain phytochemicals ($\chi^2_{(2)} = 9.93$, $p = 0.007$), with vegans reporting doing so more often. Moreover, there was a significant association between diet and the avoidance of the following ingredients (Table 4): sweetener ($\chi^2_{(2)} = 6.16$, $p = 0.046$), saturated fats ($\chi^2_{(2)} = 9.82$, $p = 0.007$), cholesterol ($\chi^2_{(2)} = 21.60$, $p < 0.001$), and caffeine ($\chi^2_{(2)} = 8.30$, $p = 0.016$). Vegans were more likely to report considering avoiding these ingredients in their food choice than vegetarians and omnivores.

Vegan athletes had the highest scores in *food choice* compared to the other dietary subgroups (0.75 (0.20), $F_{(2, 219)} = 6.76$, $p = 0.001$) (Figure 1b).

3.2.4. Dimension of Healthcare Utilization

There was no significant association between the use of regular health check-ups and diet ($\chi^2_{(2)} = 1.91$, $p = 0.385$) (Table 4). Vegan athletes had the highest scores with regard to *healthcare utilization* (0.61 (0.49), $F_{(2, 219)} = 0.95$, $p = 0.389$) (Figure 1b).

3.3. Results of the MANOVA

The findings of the MANOVA considering state of health are presented in Table 5, indicating significant differences ($p < 0.05$) for the following results: (i) race distance (F = 3.39, Df = 2, $p = 0.036$) and sex (F = 4.06, Df = 1, $p = 0.045$) had an effect on *mental health*, (ii) race distance had an impact on *chronic diseases* and *hypersensitivity reactions* (F = 3.27, Df = 2, $p = 0.040$), (iii) an association between sex and *smoking habits* (F = 4.22, Df = 1, $p = 0.041$), and (iv) an association between *food choice* and diet (F = 6.10, Df = 2, $p = 0.003$), with vegans having the highest scores (0.75).

Table 5. Results of the MANOVA considering health status.

Cluster	Dimension	Subgroup	F	Df	*p*
Health-related Indicators	**Body weight/BMI**	Diet	0.75	2	0.475
		Race Distance	0.49	2	0.613
		Sex	0.62	1	0.432
	Mental health	Diet	0.91	2	0.402
		Race Distance	3.39	2	0.036
		Sex	4.06	1	0.045
	Chronic diseases and hypersensitivity reactions	Diet	0.49	2	0.611
		Race Distance	3.27	2	0.040
		Sex	0.77	1	0.381
	Medication intake	Diet	0.41	2	0.665
		Race Distance	0.15	2	0.859
		Sex	1.06	1	0.304

Table 5. *Cont.*

Cluster	Dimension	Subgroup	F	Df	*p*
Health-related Behavior	**Smoking habits**	Diet	0.80	2	0.451
		Race Distance	1.78	2	0.172
		Sex	4.22	1	0.041
	Supplement intake	Diet	0.14	2	0.866
		Race Distance	0.93	2	0.395
		Sex	1.91	1	0.168
	Food choice	Diet	6.10	2	0.003
		Race Distance	1.11	2	0.331
		Sex	0.08	1	0.779
	Healthcare utilization	Diet	0.96	2	0.385
		Race Distance	1.52	2	0.222
		Sex	2.14	1	0.145

F = F-value. Df = Degrees of freedom. *p* = *p*-value for difference among groups.

However, the overall health status derived from all dimensions showed differences between race distances with statistical trend (F = 1.83, Df = 2, $p = 0.71$), but no significant differences were found for either diet or sex.

4. Discussion

This study intended to investigate the health status of vegetarian and vegan endurance runners and to compare it to omnivorous athletes, regarding potential differences in body weight, smoking habits, stress perception, the prevalence of chronic and metabolic diseases, the prevalence of allergies and food intolerances, medication and supplement intake, food choice, consumption of performance-enhancing substances, and healthcare utilization. In terms of assessing the state of health of endurance runners, it is generally accepted, that body weight, BMI and smoking behavior were known to affect running performance.

The main findings were: (i) vegetarians and vegans weighed significantly less than omnivores, (ii) vegans had the highest *food choice* scores, (iii) vegans reported choosing food because it is good for maintaining health more often, (iv) vegans reported avoiding sweeteners, saturated fats, cholesterol, and caffeine when choosing food more often, (v) vegans reported choosing food in order to obtain phytochemicals more often, and (vi) vegans reported the lowest prevalence of allergies.

4.1. Body Weight and BMI

A first important finding was that both vegetarians and vegans had lower body weight (62.00 kg (IQR 11.30) and 64.00 (IQR 10.00) kg, respectively) than omnivores (68.00 kg (IQR 16.70). At the same time, the majority of all participants had a BMI which was within the normal range of 18.50–24.99 kg/m^2 (80 % in omnivores vs. 87 % in vegetarians vs. 82 % in vegans) [21,22,24], with vegans having the best *body weight/BMI* health scores.

BMI is a relevant parameter, since it is associated with an increased risk for diseases, such as cardiovascular diseases, if it is higher than BMI_{NORM}, and with a couple of other disorders, such as anorexia nervosa, if it is below BMI_{NORM} [21,22]. In addition, it is a key factor with regard to running performance [24]. However, careful use and interpretation of the BMI is required. For example, the BMI of active runners could be below the normal range without being pathological [23].

In the light of this, the findings of the present study were in line with previous literature, where vegetarians and vegans also had lower BMI than meat-eaters [25–27]. Spencer et al. [28] attributed these differences in body weight and BMI mainly to differences in macronutrient intake between vegetarians, vegans, and omnivores. High protein and low fiber intakes were the factors most strongly associated with increasing BMI. Considering the fact that running speed and endurance performance

are significantly associated with body mass and BMI [24], vegetarian kinds of diet are known to be a good basis for body weight control strategies for endurance athletes [7,16,29]. Meanwhile, athletes, as well as their coaches, have to be particularly aware of unintended body weight loss [30], which is why regular monitoring of body weight is recommended [27]. Beyond athletic concerns, vegetarian, but in particular vegan, dietary patterns are known as to be useful for body weight control for people who suffer from obesity and diabetes mellitus type 2 and hypercholesterinemia [12,13].

4.2. Vegetarians' and Vegans' Attitudes Towards Food Choice

While only the dimension *food choice* showed significant differences between dietary subgroups, overall the vegan dietary subgroup displayed the highest health scores from all dimensions (except for *medication intake*) and contributed to runners' good state of health, ranging from 61%–91%.

A main result was that vegans showed the highest score (75%) in endurance runners in the dimension *food choice* to contribute most beneficially to the overall state of health. This means that they reported choosing food ingredients because they are good for maintaining health. This finding was consistent with available scientific literature.

Studies of vegetarians and vegans have identified a range of motivations for dietary choices [8] (p. 395), although personal health and animal welfare were predominant motives [31–33] (pp. 24–28). It has also been shown that vegetarians and vegans usually have healthier lifestyles than omnivores [8, 34] (p. 393). Their healthy lifestyle is characterized by the avoidance of adverse health behaviors, such as smoking and alcohol consumption, a high level of physical activity, and time for relaxation. Moreover, vegetarians and vegans are usually well-educated, have a certain degree of intellectual curiosity, and are open to new experiences [8] (p. 393). These findings match the results from the present study and support the characterization of vegetarians, but vegans in particular, as being health-conscious. However, all participants, meaning vegetarian, vegan, and omnivorous endurance runners, reported health-reasons as being important for food and ingredients choice. This supports the notion that athletes in general are health-conscious [35], but vegan athletes are supposed to be those who care most about this specific health-related strategy [8] (p. 393).

However, there was no significant major effect of dietary subgroups on whether food or ingredients had been chosen because they were healthy or health-promoting, even though there was a slight predominance of vegetarian and vegan runners. This was not entirely in line with current scientific evidence, as it has been shown that vegetarians and vegans are usually more health-conscious than omnivores [33,34,36]. Notwithstanding this, the contradiction might be explained by the composition of the sample. As all participants were endurance runners, who are known to be health-conscious compared to non-active people of the general population [35], the predominance of vegetarians and vegans might have been compensated for in this regard. Furthermore, the survey was based on self-reporting, so the definition of what is healthy or health-promoting in terms of food ingredients would depend on individual definitions based on personal suggestions and beliefs. Therefore, the results might have been biased to a certain degree. However, as the majority of all runners reported considering health aspects when choosing food, the findings support the characterization of the participants as being health-conscious.

A further main result was that vegan participants reported choosing food ingredients in order to avoid cholesterol, caffeine, sweetener and saturated fats more often. This finding was in line with the literature as well [37] and supports the fact that vegans in particular are supposed to be health-conscious.

Even though caffeine and cholesterol do not have detrimental health effects or may even have beneficial health implications if they are consumed conscientiously [38,39], cholesterol, in particular, is believed to be a crucial factor in the genesis of cardiovascular diseases [39]. Cholesterol is known to be an important risk factor for cardiovascular disease due to the induction of the elevation of LDL levels. It has also been found that HDL levels, which protect against cardiovascular diseases, increase after cholesterol consumption, so moderate consumption has been recommended in some studies [39].

However, to date the interactions between cholesterol intake and LDL and HDL blood levels have not been revealed completely [40]. With regard to caffeine, moderate consumption can increase physical and mental performance, while excessive intake can induce abuse or dependence [39]. Thus, it seems likely that both substances can be consumed moderately without any severe harm. However, being aware of potential detrimental side effects and therefore conscientious consumption is recommended.

Consumption of a high number of saturated fats is associated with cardiovascular diseases, such as stroke, myocardial infarction, and hypertension [8] (p. 414). Since vegan diets are characterized by a low percentage of saturated fats and a high percentage of omega-3 and omega-6 fatty acids [41], adhering to a plant-based diet can be a good way to improve cardiovascular health.

Health-effects of artificial sweeteners are controversial. While a couple of these products, such as aspartame, have previously received a generally recognized status as being safe from the United States Food and Drug Administration, there is also evidence for detrimental effects, such as the manifestation of glucose intolerance, weight gain and triggering of migraine in susceptible individuals [42]). Moreover, carcinogen effects could not be ruled out yet [43]. Overall, avoiding these agents appears to be advisable, so that the fact that the vegan endurance runners of our sample reported avoiding ingestion of sweeteners characterized them as being particularly health-conscious once again.

In addition to the avoidance of harmful substances, such as cholesterol and saturated fats, vegans reported choosing food in order to obtain phytochemicals. This finding supports the fact that vegan athletes are particularly health-conscious, since the consumption of phytochemical-rich foods is an important benefit of any plant-based diet in that it might help to mitigate the effects of excess inflammation and to promote recovery from training [41].

4.3. Allergies and Food Intolerances

There was a significant association between the prevalence of hypersensitivity reactions and diet, whereby vegan endurance runners reported least often that they had at least one allergy (20% in vegans vs. 32% in omnivores and 36% in vegetarians). Among those vegan endurance runners, 10-km runners had the lowest prevalence of allergies. At the same time, omnivores reported having a food intolerance least often (1% in omnivores vs. 10% in vegetarians and 12% in vegans).

Current evidence is sparse in this regard. One study has detected higher allergy rates among vegetarians [44], whereas others found a protective effect of a diet rich in fruits and vegetables on the occurrence of allergic asthma [45] and food allergies [46,47]. However, a relatively high incidence of allergies in a sample of endurance runners is not unexpected. It is well known that endurance athletes are more likely to have allergies (prevalence up to 13%) than people from the general population (prevalence 7% to 8%) [48]. This is usually attributed to the amount of time runners spend outdoors, which is supposed to be associated with a drying of the airways and an increased exposure to airborne allergens [49]. In the light of this, the finding that the vegan 10-km runners reported the lowest prevalence of allergies appears to be plausible because they usually have to cope with smaller training volumes (daily and weekly mileage) to successfully compete over shorter race distances. As a consequence, these runners do not spent as much time outdoors as long-distance runners, such as half-marathoners and (ultra-)marathoners.

Regarding food intolerances, the current literature does not provide clear data in this regard. One study indicated that a vegan diet might beneficially affect the intestinal flora, which seems to lower the risk of irritable bowel disease [47], whereas another study identified a long-term vegetarian diet as being the reason for the occurrence of irritable bowel disease [50]. However, endurance athletes, in general, are supposed to be more susceptible to symptoms of food sensitivities, which can be similar to those of irritable bowel disease. Constant training challenges the bowel to an extreme degree and endurance running, in particular, might cause gastrointestinal complaints. Thus, the ability to cope with additional gastrointestinal stress induced by food intolerances would be reduced [51].

4.4. Stress Perception

There was no significant difference found between vegetarians, vegans, and omnivores in reported stress and perceived pressure. *Mental health* scores were high, regardless of diet choice. However, vegan endurance runners had the highest scores for *mental health*. These findings were in line with previous studies, which showed that both endurance running [52,53] and adhering to a vegan dietary pattern caused good mood states [54]. Certain characteristics of vegans, such as a high degree of health-awareness [8] (p. 393), and the beneficial effects of endurance running, such as relaxation due to physical activity and an increase in stress resilience [52,53], appear to be the key factors in this regard.

In the light of this, finding the optimal dose of endurance running appears to be relevant, since too little exercise does not lead to a reduction in stress, whilst too much exercise might even increase stress levels [52]. According to the findings of the present study, half-marathon running appears to be a good way to cope with stress. These findings (unpublished data from our laboratory) are discussed in detail elsewhere [55]. Moreover, among the participants of the present study, there was a slight male predominance among those runners who reported as not suffering from stress. This was in line with previous research where it was reported that male endurance athletes possess a slightly higher degree of mental toughness than their female counterparts, allowing them to cope better with stress during exercise and in everyday life [56].

4.5. Chronic Diseases

There was no significant differences between the dietary subgroups when considering heart disease requiring treatment, state after heart attack, cancer, diabetes mellitus type 1 and 2, hypothyroidism and hyperthyroidism. In addition, there was a low overall incidence of these diseases among our participants. The only exceptions seemed to be apparently higher rates of cancer and hypothyroidism among vegetarians and vegans, which could be explained by a statistical bias.

There were five females who had suffered from breast cancer. Three of them had decided to change their dietary habits in favor of a vegetarian kind of diet after diagnosis of cancer, which skewed the results. The higher prevalence of hypothyroidism could be explained by the female predominance among the vegetarian and vegan subjects, as it is well known that eight times as many women suffer from thyroid diseases in general, and in particular from hypothyroidism, than men [57].

The fact that there was no association between diet and the prevalence rates of chronic diseases partially contradicts the body of evidence. Adhering to a vegetarian or vegan diet is usually associated with a lower incidence of diabetes mellitus type 2 [7,12,13], hypothyroidism (Tonstad et al. 2013 [58]), coronary artery disease [11,14], depression [54] and obesity [11] compared to an omnivorous diet. However, this effect might be compensated for by the fact that all our subjects were endurance athletes, who are usually supposed to be health-conscious, especially compared to non-active people of the general population [35]. Furthermore, the mean age of our participants was quite low (43.00 ± 18.00 in omnivores, 39.00 ± 16.00 in vegetarians, and 37.00 ± 15.00 in vegans), so that it can be assumed that the peak age for the manifestation of most diseases had not been reached yet. Furthermore, the fact that people who suffer from severe diseases usually do not become endurance runners might have led to a certain decrease in prevalence rates as well.

4.6. Medication Intake

There was no significant association found between the intake of medication with diet. All subgroups had similar *medication intake* scores. As there was a low prevalence of chronic diseases among our subjects, it was not surprising that there was also a low number of athletes who had to take any medication on a regular basis. The only exceptions were the intake of hormones and medication for the thyroid. The relatively high number of athletes who take hormones could be explained by the use of contraceptive pills or other interventions among the female runners. With regard to thyroid

medication, the relatively high incidence rates of hypothyroidism among the female subjects (8%) explains the number of subjects who were taking thyroid medication.

4.7. Smoking Habits

There was no significant association between diet and current or former smoking. Yet, a low rate of smokers in vegetarian, vegan and omnivorous runners was observed. Vegetarians had the best scores considering *smoking habits*. These findings were in line with previous research, which also showed low numbers of smokers among vegetarians and vegans [59,60]. Although the low rates among endurance runners could be explained by undesired performance limitations due to smoking [59], vegetarians and vegans are often particularly health-conscious and therefore the number of smokers would be quite low among them [8,60] (p. 393). In addition, we found that women were more likely to be non-smokers compared to men, which was in line with previous research [61]. Nonetheless, in the past years, the number of female smokers has increased, which is particularly displayed in the prevalence of smoking associated diseases, such as lung cancer [61].

4.8. Supplement Intake

The finding that percentages of supplement intake were similar in all diet groups is consistent with current evidence. At the same time, vegans had the highest *supplement intake* scores. These findings are in line with previous research which showed that vegetarian kinds of diet are not lacking in critical micronutrients and macronutrients, per se, but rather that nutrient deficits can occur in any kind of diet [62]. Plant-based diets, such as a vegan dietary pattern, are not worse in terms of daily nutrient intake than omnivorous kinds of diet [63]. A recent study showed that an omnivorous diet does not meet the required amount of intake of six nutrients on average (calcium, folate, magnesium, iron, copper, vitamin E), whereas in vegetarian diets the amount of the daily intake of three nutrients is too low on average (calcium, zinc, vitamin B12) [9]. Another study has even revealed higher diet quality scores in vegetarian runners than in non-vegetarian runners [17]. Thus, supplementation of certain nutrients can be recommended for omnivores, vegetarians, and vegans alike [63]. More than this, these findings underpinned the fact that vegans are particularly health-conscious, which has been confirmed in other studies as well [8,60] (p. 393).

The most frequently taken supplement mentioned by the participants was vitamin D. Vitamin D deficiency is usually not associated with a vegetarian or vegan diet [64], but is a common problem in the general population [65] and in particular among endurance runners. It was found that there is a very large difference between necessary and real intake in athletes, regardless of whether they adhere to a vegetarian, vegan, or omnivorous diet [66]. Thus, all endurance athletes have to be aware of vitamin D levels, irrespective of their dietary patterns.

4.9. Enhancement Substances

There was no significant association between dietary subgroups and the consumption of enhancement substances or anything to cope with stress. Vegans reported the lowest use of enhancement substances. As there was a low overall number of subjects who reported using such substances (n = 32 for the consumption of enhancement substances, n = 18 for the consumption of substances to cope with stress) it could be expected that the number among the dietary subgroups would be quite low as well. It is noteworthy that these findings contradicted a previous study by Wilson [18] who found that 40% of male marathon finishers reported the recent use of performance-enhancing supplements. However, since our subjects did (almost) not report using such substances, they are probably aware of the detrimental effects of substances to increase performance and would, therefore, have avoided intake. This applies especially to the vegan participants, who are known to be particularly health-conscious [8] (p. 393).

4.10. Healthcare Utilization

Vegans had the highest scores in *healthcare utilization*, although scores were similar for all dietary subgroups. Scientific data is sparse in this regard. In one study a higher need for healthcare has been found among vegetarians [44]. However, since our results showed a good state of health in vegan, vegetarian and omnivorous endurance runners, there seems to be no need for frequent doctor consultations. Furthermore, physical activity, such as endurance running, prevents diseases which could require consulting a doctor more frequently [52]. However, about half of the participants (54% in omnivores, 49% in vegetarians, and 61% in vegans) reported making use of routine health checks. Considering that the mean age of our participants was around 40 years, this was an encouraging result, as most health checks for the early recognition and treatment of severe diseases in Europe are recommended for people who are aged 40 years and older [67].

4.11. Limitations and Implications for Future Research

Some limitations of the study should be noted. The survey was based on self-reporting. Thus, the reliability of the data depended on the conscientiousness of the participants. However, this effect was controlled for diet, participation in running events and race distance by using control questions, each separated from the respective main question and included in different sections of the questionnaire. In addition, the small sample size and the pre-selection of the participants due to the fact that mainly highly motivated runners took part led to a lack of statistical representativeness which might have affected the results. Nonetheless, the high intrinsic motivation of the participants would also have led to an increase in the accuracy of their answers and thus to a high quality of the generated data. Moreover, it was striking that most subjects came from Germany (n = 177). This imbalance in the composition of the sample may have several causes. First, Germany has a population of 82 million, making it the largest German-speaking country [68]. As the core area of the present study were German-speaking countries, this predominance is displayed in the sample of the present study. Second, Germany has large vegetarian and vegan populations [69]. Since a couple of subjects were addressed via trade fairs on vegetarian and vegan nutrition and lifestyle, it was likely that the number of German participants would increase. Third, some of the largest running events, such as the Berlin Marathon [70], take place in Germany. Together, this might have led to an increase in German participants.

Nevertheless, the data contributes to the growing scientific interest in and research on vegetarianism and veganism as it relates to sports and exercise and can be taken as one step towards creating a broad body of evidence in this regard. Future studies should be performed on large randomized samples in order to improve statistical representativeness. Furthermore, measurement of the health status could be elaborated by including additional parameters, such as energy metabolism and fluid balance regulation. Thereby, the data generated from the participants' self-report could be specified.

5. Conclusions

In summary, the findings revealed that all endurance runners had a good health status, regardless of the diet choice. At the same time, vegan athletes appeared to be extraordinarily health-conscious, in particular due to their food choice habits. These findings support the notion that adhering to vegetarian kinds of diet, but in particular to a vegan dietary pattern, is compatible with ambitious endurance running and can be an appropriate, at least equal and healthy alternative to an omnivorous diet for athletes.

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