UNDERSTANDING THE INTERACTION BETWEEN PHYSICAL ACTIVITY AND DIET FOR THE PROMOTION OF HEALTH AND FITNESS

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PUBLISHED IN: Frontiers in Nutrition and Frontiers in Sports and Active Living

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-436-7
DOI 10.3389/978-2-88974-436-7

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UNDERSTANDING THE INTERACTION BETWEEN PHYSICAL ACTIVITY AND DIET FOR THE PROMOTION OF HEALTH AND FITNESS

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Citation: Koehler, K., Drenowatz, C., eds. (2022). Understanding the Interaction Between Physical Activity and Diet for the Promotion of Health and Fitness. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-436-7

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frontiers in Nutrition

EDITORIAL
published: 13 January 2022
doi: 10.3389/fnut.2021.835535

Editorial: Understanding the Interaction Between Physical Activity and Diet for the Promotion of Health and Fitness

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Keywords: exercise, eating behavior, weight loss, lifestyle, long-term health, energy balance

Editorial on the Research Topic

Understanding the Interaction Between Physical Activity and Diet for the Promotion of Health and Fitness

INTRODUCTION

The benefits of regular physical activity and a healthy diet are well-documented in the literature (1, 2). However, efforts to counter the global obesity epidemic and related metabolic diseases through interventions focusing on either physical activity or diet alone have been of limited success (3, 4), highlighting the need for new, more refined and integrated approaches. Although it appears intuitive that interventions combining both lifestyle components have the capacity to result in greater health benefits than singular approaches (5), it is also possible that changing behavior related to one component may result in compensatory changes in the other. For example, the obvious health benefits of increased physical activity may be overridden when the resulting increase in energy expenditure is compensated by an increase in dietary energy intake and unhealthier food choices (6). Likewise, dietary interventions such as caloric restriction may result in compensatory reductions in physical activity behavior (7), which could negatively affect physical fitness and performance, undermine weight loss success, and expose individuals to greater risk for future weight regain and other associated diseases (8–10).

The goal of this Research Topic was to strengthen our understanding of how physical activity and diet are related with each other in the context of health and fitness promotion. As such, this Research Topic includes research targeting physical activity, which refers to any bodily movement that results in energy expenditure, as well as exercise, a subset of physical activity with the goal of maintaining or improving physical fitness (11). The Research Topic combines a total of 9 original studies and systematic reviews, which cover three basic themes ranging from the interplay between nutrition and physical activity for weight loss (theme 1), the impact of exercise on food intake regulation (theme 2) and the potential for negative health consequences of excessive exercise (theme 3).

THEME 1: WEIGHT LOSS AND BODY COMPOSITION

Despite considerable efforts, global rates of overweight, and obesity continue to rise and excess body weight is considered a major threat to future public health (12). Accordingly, various strategies, including attempts to alter diet and physical activity, have been implemented to tackle the obesity

OPEN ACCESS

Edited and reviewed by:
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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *14 December 2021*
Accepted: *20 December 2021*
Published: *13 January 2022*

Citation:
Koehler K and Drenowatz C (2022) Editorial: Understanding the Interaction Between Physical Activity and Diet for the Promotion of Health and Fitness. Front. Nutr. 8:835535. doi: 10.3389/fnut.2021.835535

epidemic. In their systematic review Correia et al. highlight beneficial effects of intermittent fasting on body weight. Diet-induced changes in body weight, however, are generally short lived and even greater benefits can be accomplished with the inclusion of exercise training. Exercise also plays an important role for maintaining lean body mass during caloric restriction as indicated by Roth et al. who reported a decline in lean mass during caloric restriction despite assuring a high protein intake. In addition to structured programs, lifestyle adjustments also play a critical role in weight loss. Myers et al. show that particularly vigorous physical activity, along with a reduction in energy-dense foods, was associated with a more pronounced weight loss in women. Similarly, van Baak et al. report greater weight loss during caloric restriction in participants who increase their physical activity. Furthermore, increased physical activity was associated with beneficial changes in various cardio-metabolic risk factors and weight loss maintenance beyond the intervention period.

THEME 2: EXERCISE AND FOOD INTAKE REGULATION

While physical activity and exercise increase energy expenditure, most exercisers increase their dietary energy intake. This phenomenon, often referred to compensatory eating, was also described in a recent study by Horner et al. who showed that neither gastric emptying nor appetite-regulating hormones were significantly altered after a 4-week exercise intervention, suggesting that short-term changes in gastro-intestinal regulation play no major role in compensatory eating. Post-exercise food intake was also studied by Okada et al. who reported that administration of exogenous ketones impacted appetite-regulating hormones but failed to affect appetite perception and post-exercise energy intake. These two interventions are complemented by a systematic review and meta-analysis by Hubner et al. on the effects of exercise on appetite regulation in older adults. Despite limited research in this demographic, exercise, and physical activity appear to promote satiety sensitivity and appetite control, thereby providing an avenue for reducing disease burden later in life.

THEME 3: POSSIBLE NEGATIVE CONSEQUENCE

Besides the beneficial effects of physical activity and exercise there are also some possible harmful effects that need to be acknowledged. Ribeiro et al. discuss the potential detrimental effects of exacerbated exercise on the gastrointestinal environment that can, among others, impair gastric motility, and nutrient absorption. Moore et al. further address the increased risk for low energy availability in endurance athletes, and emphasize the need for adequate dietary energy intake during periods of high energy demands. The overall benefits of physical activity, however, should not be questioned by these results. Rather, these studies highlight the complex interaction between diet and physical activity and their effects on the human body and health.

SUMMARY

This overview of current research related to physical activity and diet highlights the importance of integrating both components regardless whether the goal is to maximize weight loss or to diminish the potential negative effects at the upper end of the activity spectrum. A deeper understanding of the interaction between these two critical lifestyle approaches is required for the development of combined interventions involving physical activity and diet that result in successful, long-term health improvements while avoiding unhealthy compensatory behavior in the other domain.

AUTHOR CONTRIBUTIONS

KK and CD wrote the introduction and the summary. CD summarized the publications pertaining to weight loss and excessive exercising. KK wrote the summaries of publications relating to the impact of exercise on food intake regulation. Both authors approved the submitted version.

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frontiers in Nutrition

ORIGINAL RESEARCH
published: 20 January 2021
doi: 10.3389/fnut.2020.626480

Exogenous Ketones Lower Post-exercise Acyl-Ghrelin and GLP-1 but Do Not Impact *Ad libitum* Energy Intake

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Ketosis and exercise are both associated with alterations in perceived appetite and modification of appetite-regulating hormones. This study utilized a ketone ester (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (KE) to examine the impact of elevated ketone body D-β-hydroxybutyrate (βHB) during and after a bout of exercise on appetite-related hormones, appetite perception, and *ad libitum* energy intake over a 2 h post-exercise period. In a randomized crossover trial, 13 healthy males and females (age: 23.6 ± 2.4 years; body mass index: 25.7 ± 3.2 kg·m^{-2}) completed an exercise session @ 70% VO_{2peak} for 60 min on a cycling ergometer and consumed either: (1) Ketone monoester (KET) (0.5 g·kg^{-1} pre-exercise + 0.25 g·kg^{-1} post-exercise); or (2) isocaloric dextrose control (DEX). Transient ketonaemia was achieved with βHB concentrations reaching 5.0 mM (range 4.1–6.1 mM) during the post-exercise period. Relative to the dextrose condition, acyl-ghrelin ($P = 0.002$) and glucagon-like peptide-1 ($P = 0.038$) were both reduced by acute ketosis immediately following exercise. AUC for acyl-ghrelin was lower in KET compared to DEX ($P = 0.001$), however there were no differences in AUC for GLP-1 ($P = 0.221$) or PYY ($P = 0.654$). Perceived appetite (hunger, $P = 0.388$; satisfaction, $P = 0.082$; prospective food consumption, $P = 0.254$; fullness, $P = 0.282$) and 2 h post-exercise *ad libitum* energy intake ($P = 0.488$) were not altered by exogenous ketosis. Although KE modifies homeostatic regulators of appetite, it does not appear that KE acutely alters energy intake during the post-exercise period in healthy adults.

Keywords: ketone monoester, energy intake, appetite, acyl-ghrelin, GLP-1, PYY

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *05 November 2020*
Accepted: *22 December 2020*
Published: *20 January 2021*

Citation:
Okada TE, Quan T and Bomhof MR (2021) Exogenous Ketones Lower Post-exercise Acyl-Ghrelin and GLP-1 but Do Not Impact Ad libitum Energy Intake. Front. Nutr. 7:626480. doi: 10.3389/fnut.2020.626480

INTRODUCTION

Recently there has been considerable interest in the utilization of ketogenic diets to help individuals control body weight. Although there is no clear evidence that low carbohydrate, ketogenic diets modify energy expenditure, evidence suggests that ketosis is associated with reduced hunger and improved appetite regulation (1). Weight reduction is generally accompanied by an orexigenic response, with prolonged elevations in the hunger hormone ghrelin and reduced satiety hormones leptin and peptide YY (PYY) (2). Elevated concentrations of ghrelin with weight loss are blunted with very low energy and low carbohydrate, ketogenic diets (3). Although the mechanisms by which ketosis elicits anorexigenic effects on regulators of appetite remain unclear, the ketone body D-β-hydroxybutyrate (βHB) is inversely associated with the hunger hormone ghrelin (3). To avoid

restricting carbohydrate or energy intake to induce endogenous ketosis, exogenous ketone esters have been utilized to safely and effectively induce a state of acute ketosis (4). Stubbs et al. (5) investigated the impact of a ketone monoester (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (KE), compared to an isocaloric dextrose control, on appetite and found that the acute elevation of blood βHB reduced hunger and acyl-ghrelin for up to 4 h. Research in rodents shows that a diet providing ∼30% of energy from ketone esters can suppress overall energy intake (6).

Exercise is another stimulus that transiently modifies homeostatic regulators of appetite. Both moderate and high intensity aerobic exercise sessions acutely reduce acyl-ghrelin and increase satiety hormones glucagon-like peptide-1 (GLP-1) and PYY for a post-exercise period of ∼30–90 min (7–10). The impact of ketosis during and after physical activity on homeostatic markers of appetite and energy intake remains to be fully elucidated. Similar to the impact of ketosis under sedentary conditions, ketone ester supplementation, on top of a background intake of 60 g carbohydrate·h^{-1}, during an intense and prolonged bout of exercise is associated with reductions in ghrelin and perceptions of hunger in elite athletes (11).

It remains to be determined whether ketosis, relative to an isocaloric control, impacts homeostatic regulators of appetite following a standardized, ecologically-valid exercise session in normal, healthy individuals. Furthermore, studies to date have not examined the influence of acute ketosis on *ad libitum* energy intake. The objective of our study was to examine the impact of ketosis during and after an exercise session, relative to an isocaloric control, on appetite-regulating hormones, *ad libitum* post-exercise energy intake, and perceived appetite in healthy adults.

MATERIALS AND METHODS

Participants and Ethical Approval

Thirteen participants (seven female; six male) were recruited to take part in the study from the University of Lethbridge and surrounding area using posters and word of mouth. Participants were self-reported, weight stable (had not gained or lost more than 2 kg within the previous 3 months) and had no history of serious physical injuries or metabolic disease (e.g., hypertension, cardiovascular disease, or diabetes). Participants provided written informed consent prior to taking part in the study. The study was approved by the University of Lethbridge Human Participant Research Committee and was conducted in accordance with the ethical principles of the 1964 *Declaration of Helsinki*.

Baseline Testing

Participants were asked to refrain from alcohol, caffeine, and moderate to vigorous exercise for 24 h prior to the baseline testing. Measures of height and weight were recorded to the nearest 0.1 cm and 0.1 kg, respectively, on a mechanical beam scale (Health-o-Meter Professional, McCook, IL, USA). Participants completed an incremental ramp test to exhaustion on an electromagnetically braked cycle ergometer (Velotron, QUARQ, Spearfish, SD, USA) to assess peak oxygen uptake (VO_{2peak}). After an initial 5 min, 50-watt warm-up, participants cycled at 50 W for 4-min at 80 rpm, followed by a constant increase in workload (30 W·min^{-1} for males; 25 W·min^{-1} for females), until volitional fatigue. Oxygen consumption was continuously measured using the Quark CPET (COSMED, Chicago, IL, USA) with breath-by-breath analysis. Heart rate was measured by a Garmin heart rate monitor (HRM-Dual, Garmin, Olathe, KS, USA). All participants achieved an RER > 1.1. VO_{2peak} was calculated by the maximum rolling 30-s VO_2 (mL O_2·min^{-1}·kg^{-1}) average.

Experimental Conditions

In a randomized crossover trial, participants completed a 1-h exercise session at 70% VO_{2peak} and consumed either: (1) ketone monoester drink (KET); or (2) isocaloric dextrose control drink (DEX) (**Figure 1A**). Six participants started with the DEX trial and seven started with the KET trial. Trials were scheduled at least 1 week apart for males and 4 weeks apart for females. To control for potential appetite fluctuations, all females completed both conditions within the first week of the follicular phase of the menstrual cycle (12, 13).

For each trial, participants reported to the lab in the morning (0800–0900 h) following an overnight fast (>10 h). Prior to each condition, participants were asked to refrain from consuming beverages containing caffeine and alcohol as well-moderate to vigorous exercise for 24 h prior to each trial. Participants were instructed to adhere to similar eating patterns for 24 h prior to the start of each experimental condition, but this was not confirmed using food records or self-reported compliance. Each condition consisted of a 5 min warm-up at 50 W, followed by 60 min at 70% VO_{2peak}. During exercise, each participant's oxygen consumption was measured until stable at 70% $VO_{2peak.}$ Subsequent spot checks of oxygen consumption were performed every 5 min to ensure consistent exercise intensity. Prior to the initiation of exercise, participants consumed 0.5 g·kg^{-1} body weight KE or an isocaloric amount of dextrose. Given that ketone bodies are oxidized at a greater rate during exercise (14), we provided an additional 0.25 g·kg^{-1} KE post-exercise or isocaloric amount of dextrose in the KET and DEX groups, respectively. The KET beverage was prepared using the commercially available ketone monoester (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (HVMN Inc., San Francisco, CA, USA). The isocaloric, dextrose placebo beverage was prepared by mixing dextrose powder in water. To mask the differences in taste, bitterness, and color between the KET and DEX beverages, zero-calorie stevia sweetener (SweetLeaf Sweetener; Wisdom Natural Brands, Gilbert, AZ, USA) and lemon- and cranberry raspberry-flavored Mio (Kraft Foods; H.J. Heinz Company Brands LLC., Glenview, IL, USA) were added to both experimental beverages. All drinks were diluted to 250 mL with water. Participants wore a nose clip and immediately consumed 20 mL of Powerade Zero (Coca-Cola Ltd., Toronto, ON, Canada) following the KET or DEX drink based on methods previously described (15). Using this protocol, pilot testing confirmed that research participants were not able to discern differences in the experimental beverages, however the success of the blinding was not directly verified amongst participants in this study.

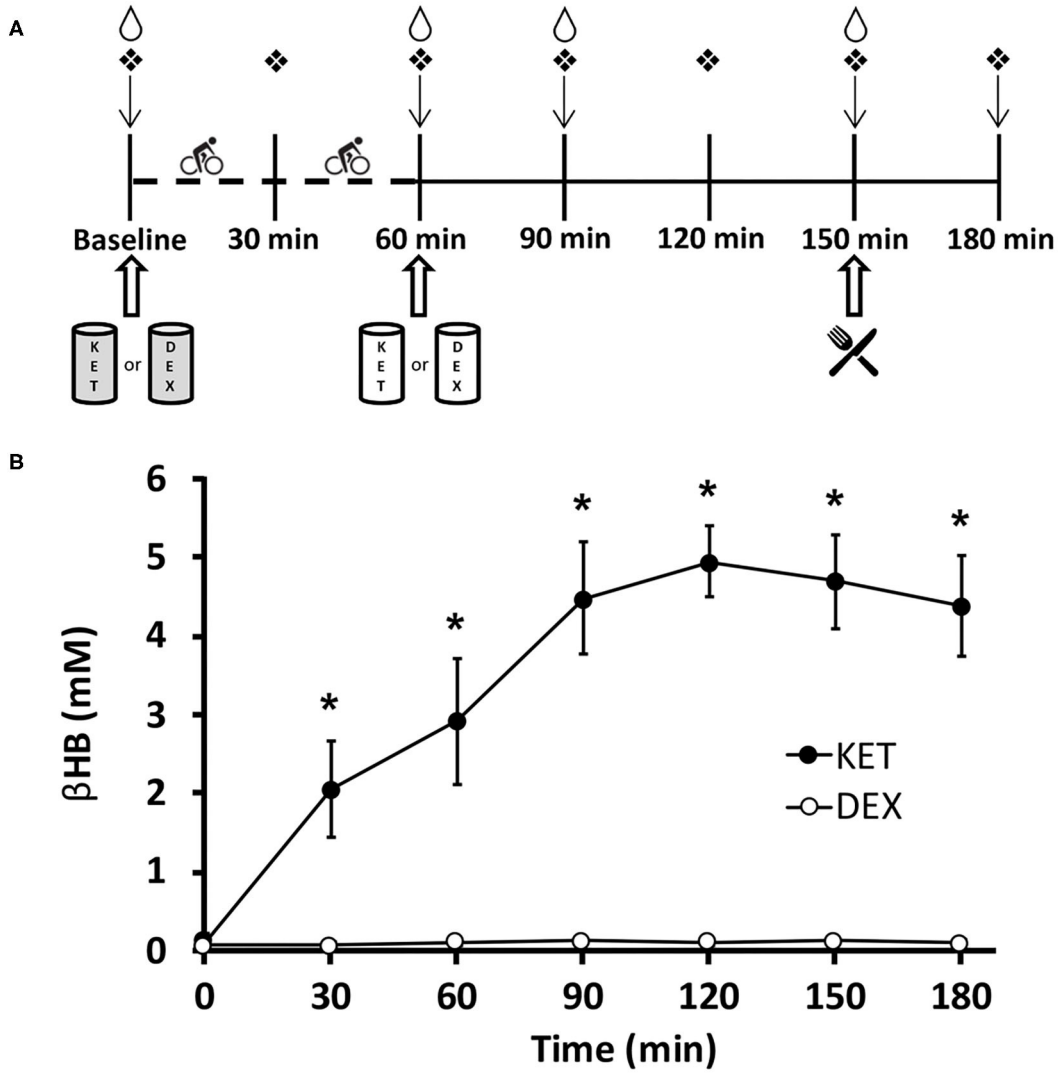

FIGURE 1 | (A) Timeline of experimental conditions. Participants arrived at the laboratory in the morning (0800–0900 h) in a fasted state (>10 h). ↓Appetite visual analog scale; ❖ finger capillary draw; venipuncture; *ad libitum* meal; 0.5 g·kg^{-1} KET or isocaloric DEX; 0.25 g·kg^{-1} KET or isocaloric DEX. Dashed timeline represents 1 h of exercise cycling at 70% VO_2peak. **(B)** Blood D-β-hydroxybutyrate levels in participants for both KET and DEX drink conditions. Values are means ± SD, $n = 13$. *$P < 0.05$ between KET and DEX conditions. βHB, D-β-hydroxybutyrate; DEX, dextrose; KET, ketone monoester.

Venipuncture was completed at baseline, 60, 90, and 150 min post-initiation of exercise. A validated, paper-based, 100 mm appetite visual analog scale (VAS) (16) was used to assess subjective measures of hunger, satisfaction, fullness, and prospective food consumption (PFC) at approximately the same time that each of the 4 venipuncture samples were collected, with an additional measure following the *ad libitum* meal. Further, finger capillary punctures were completed using a commercially available, contact-activated, 21G, 1.8 mm depth lancet (BD, Becton, Dickinson and Company, Mississauga, ON, Canada) at 7 time points throughout each condition (baseline, 30, 60, 90, 120, 150, and 180 min post-initiation of exercise) to measure blood βHB concentrations using β-ketone test strips (FreeStyle Precision Blood β-Ketone Test Strips; Abbott Laboratories, Saint-Laurent, QC, Canada) and a ketone monitoring system (FreeStyle Precision Neo; Abbott Laboratories).

Following the final blood draw (∼90 min post-exercise) and the 150 min appetite VAS, participants consumed a pre-weighed *ad libitum* meal. This approach for measuring energy intake has been previously validated and verified as reproducible in males and females (13, 17). The meal consisted of bottled water (Nestle, Toronto, ON, Canada), 2% milk (Saputo, Montreal, QC, Canada), and Michelina's Signature Chicken Teriyaki (Bellisio Foods, Inc., Duluth, MN, USA). Prior to consenting to participate in the study, all participants indicated that they had no allergies or aversion to the selected food items. All meals were provided in surplus and served in an isolated, distraction free room. Participants were asked to consume the meal until comfortably

full and were provided with 30 min to finish the meal. Post-meal, all remaining food was weighed using a digital scale to assess energy intake.

Blood Collection and Analysis

All blood samples were collected into pre-cooled 6 mL K_2EDTA spray-coated vacutainers (BD, Mississauga, ON, Canada). Immediately after collection, a protease inhibitor cocktail containing DPP IV inhibitor (10 $\mu L \cdot mL^{-1}$ blood; MilliporeSigma Corp., ON, Canada), Sigma protease inhibitor (1 $mg \cdot mL^{-1}$ blood; SigmaFast, MilliporeSigma Corp.), and Pefabloc (1 $mg \cdot mL^{-1}$ blood; MilliporeSigma Corp.) was added to the sample to prevent degradation of satiety hormones. Blood samples were centrifuged at 2,500 g for 10-min at 4°C. Plasma aliquots were stored at −80°C for later analysis.

The concentration of PYY was determined using the Human PYY (Total) ELISA kit (MilliporeSigma Corp.). GLP-1 concentration was assessed using the High Sensitivity GLP-1 Active Chemiluminescent ELISA kit (MilliporeSigma Corp.). Acyl-ghrelin concentration was assessed by the Human Ghrelin (Active) kit (MilliporeSigma Corp). All samples were assayed in duplicate. The intra- and inter-assay variation for these assays ranges between 2 and 7% in our laboratory, as previously described (18).

Statistical Analysis

SPSS software version 26 was used to analyze the data. Our primary outcome measures were appetite-regulating hormone changes following the exercise session. Sample size estimations were completed using G*Power ($\alpha = 5\%$, $\beta = 80\%$) using an effect size of 0.75 and the analysis of variance (ANOVA): repeated measures, between factors statistical test. The effect size was based on previously published research showing reductions in both GLP-1 and ghrelin with KE (5, 15) as well as previously determined standard deviation values from the measurement of these hormones in our lab (18). Data was assessed for normality using the Shapiro–Wilk test. Differences in perceived appetite, βHB, acyl-ghrelin, GLP-1, and PYY were assessed using two-way repeated measures ANOVA. When there was a significant condition × time interaction, *post-hoc* least significant difference pairwise comparisons were used to determine differences at specific time points. Within-condition differences were examined using a one-way repeated measures ANOVA. Effect size (d) was calculated using Cohen's d. Area under the curve (AUC) analysis was completed for hormonal analysis and appetite perceptions. AUC estimations were calculated using trapezoidal sums. Correlation analysis was calculated between energy intake and AUC for acyl-ghrelin, GLP-1, and PYY using a two-tailed Pearson test. Statistical significance was set at $P \leq 0.05$. All data in the manuscript are represented as mean ± SD.

RESULTS

Participants

Thirteen participants completed the study (7 female, 6 male). Participants were on average 23.6 ± 2.4 years of age, weighed 73.7 ± 10.9 kg and had a body mass index of 25.7 ± 3.2 $kg \cdot m^{-2}$ (**Table 1**). Average VO_{2peak} was 37.6 ± 4.3 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$.

TABLE 1 | Participant characteristics (n = 13; 7 female, 6 male).

	mean ± SD (range)
Age (years)	23.6 ± 2.4 (21–30)
Height (m)	1.69 ± 0.06 (1.59–1.82)
Weight (kg)	73.7 ± 10.9 (59.0–91.2)
BMI ($kg \cdot m^{-2}$)	25.7 ± 3.2 (21.2–30.0)
VO_2peak (mL $O_2 \cdot kg^{-1} \cdot min^{-1}$)	37.6 ± 4.3 (30.3–47.9)

Blood βHB Levels

Blood βHB was measured seven times (baseline, 30, 60, 90, 120, 150, and 180 min after the initiation of exercise) throughout each condition. A significant interaction effect of condition × time ($P < 0.001$), a main effect of condition ($P < 0.001$), and a main effect of time ($P < 0.001$) were observed for blood βHB levels. *Post-hoc* comparisons revealed higher concentrations of blood βHB in the KET condition compared to DEX at 30 ($P < 0.001$; $d = 3.23$), 60 ($P < 0.001$; $d = 3.59$), 90 ($P < 0.001$; $d = 6.23$), 120 ($P < 0.001$; $d = 10.47$), 150 ($P < 0.001$; $d = 7.85$), and 180 ($P < 0.001$; $d = 6.71$) min following the initiation of exercise. No differences in baseline measures of βHB ($P = 0.111$; $d = 0.48$) were observed between the KET and DEX conditions. Elevated ketonaemia was achieved with the pre-exercise 0.5 $g \cdot kg^{-1}$ KE dose, with βHB concentrations reaching ∼2.9 mM (range 1.2-4.5 mM) (**Figure 1B**). After the additional post-exercise 0.25 $g \cdot kg^{-1}$ KE, βHB concentrations were elevated to ∼5.0 mM (range 4.1–6.1 mM). The isocaloric dextrose drink had no impact on blood βHB levels (**Figure 1B**). With a total dose of 0.75 $g \cdot kg^{-1}$ KE, participants received an average of 265 kcal (∼4.8 $kcal \cdot g^{-1}$ KE). To create an isocaloric, dextrose-containing control beverage, participants received an average of 66 g total dextrose.

Appetite-Related Hormones

Acyl-Ghrelin

There was a significant condition × time interaction ($P < 0.001$), main effect of condition ($P = 0.001$), and main effect of time ($P < 0.001$) for acyl-ghrelin (**Figure 2A**). *Post-hoc* pairwise comparisons revealed that the KET condition had lower acyl-ghrelin compared to DEX at 60 min ($P = 0.002$; $d = 1.12$), 90 min ($P = 0.002$; $d = 1.06$), and 150 min ($P = 0.001$; $d = 1.30$) following the initiation of exercise. No differences in acyl-ghrelin concentrations were observed between conditions at baseline ($P = 0.622$; $d = 0.14$). Within-condition, acyl-ghrelin was reduced at 60 min ($P = 0.001$), 90 min ($P < 0.001$), and 150 min ($P < 0.001$) relative to baseline in the KET condition. In the DEX condition, acyl-ghrelin was reduced only at 60 min ($P = 0.003$) and 90 min ($P = 0.004$) relative to baseline. AUC comparisons revealed lower concentrations of acyl-ghrelin ($P = 0.001$; $d = 1.15$) during the KET condition compared to DEX.

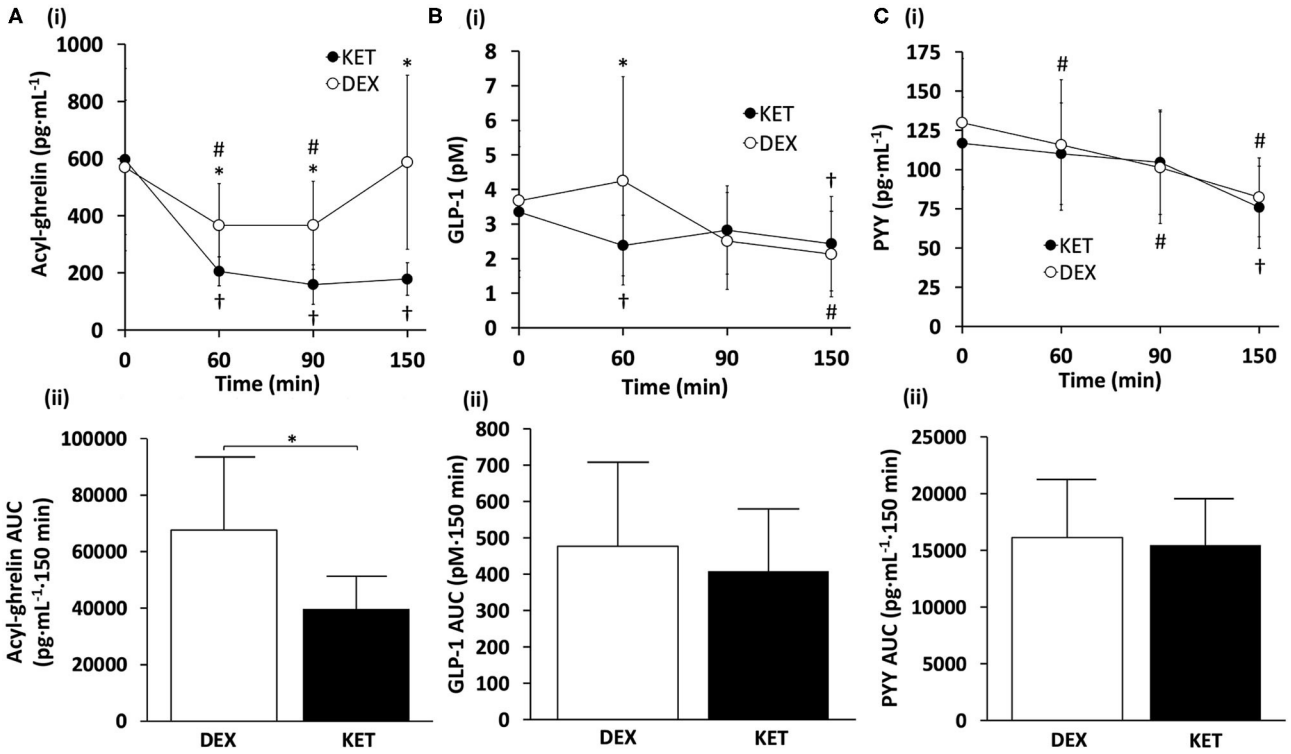

FIGURE 2 | (i) Hormone concentrations and (ii) AUC measures of **(A)** acyl-ghrelin, **(B)** GLP-1, and **(C)** PYY throughout the KET and DEX conditions. Values are means ± SD, $n = 13$. *$P < 0.05$ between KET and DEX; †$P < 0.05$ KET within-condition relative to baseline; #$P < 0.05$ DEX within-condition relative to baseline. DEX, dextrose condition; GLP-1, glucagon-like peptide-1; KET, ketone condition; PYY, peptide tyrosine tyrosine.

Glucagon-Like Peptide-1 (GLP-1)

For GLP-1, there was a significant condition × time interaction ($P = 0.026$) and a main effect of time ($P = 0.007$) (**Figure 2B**). No main effect of condition was observed ($P = 0.290$). *Post-hoc* comparisons for GLP-1 showed lower blood concentrations of GLP-1 in the KET condition at 60 min ($P = 0.038$; $d = 0.65$) relative to DEX. Between conditions, no differences were observed in GLP-1 at baseline ($P = 0.567$; $d = 0.16$), 90 min ($P = 0.366$; $d = 0.26$), or 150 min ($P = 0.286$; $d = 0.31$). Within-condition, GLP-1 in the KET condition was suppressed immediately after exercise, relative to baseline values ($P = 0.03$), and at 150 min for both DEX ($P = 0.014$) and KET ($P = 0.023$) conditions. No AUC differences were observed between conditions for GLP-1 ($P = 0.221$; $d = 0.36$).

Peptide Tyrosine Tyrosine (PYY)

No significant interaction of condition × time ($P = 0.228$) or main effect of condition ($P = 0.574$) were observed for PYY (**Figure 2C**). However, there was a significant main effect of time for PYY ($P < 0.001$). No differences were observed between conditions for baseline concentrations of PYY ($P = 0.166$; $d = 0.41$). Within-condition, PYY decreased at 60 min ($P = 0.030$) and 90 min ($P = 0.007$) with DEX, and 150 min for both DEX ($P < 0.001$) and KET ($P < 0.001$), in relation to baseline measures. There were no differences between conditions for AUC PYY ($P = 0.654$; $d = 0.13$).

Perceived Appetite

Levels of subjective hunger, satisfaction, fullness, and PFC were measured at 5 time points (baseline, 60, 90, 150, and 180 min following the initiation of exercise). No condition × time interactions (hunger, $P = 0.388$; satisfaction, $P = 0.082$; fullness, $P = 0.282$; PFC, $P = 0.254$) or main effects of condition (hunger, $P = 0.161$; satisfaction, $P = 0.65$; fullness, $P = 0.279$; PFC, $P = 0.083$; fullness, $P = 0.279$) were observed (**Figure 3**). There was a main effect of time for all appetite ratings ($P < 0.001$). There were no differences in perceived appetite measures at baseline (hunger, $P = 0.620$, $d = 0.14$; satisfaction, $P = 0.862$, $d = 0.04$; fullness, $P = 0.860$, $d = 0.05$; PFC, $P = 0.196$, $d = 0.52$). AUC comparisons between KET and DEX showed a trend toward increased satisfaction with KET ($P = 0.057$; $d = 0.59$). No differences in AUC were seen for feelings of hunger ($P = 0.154$; $d = 0.44$), fullness ($P = 0.239$; $d = 0.34$), or PFC ($P = 0.084$; $d = 0.52$).

Energy Intake

Ad libitum energy intake was measured 150 min following the initiation of exercise (90 min post-exercise). There were no differences in energy intake ($P = 0.488$; $d = 0.20$) between KET (964 ± 279 kcal; range 515–1,440 kcal) and DEX (997 ± 257 kcal; range 612–1,440 kcal) (**Figure 4**). No correlations were observed between energy intake and acyl-ghrelin ($r = 0.226$; $P = 0.266$), GLP-1 ($r = -0.015$; $P = 0.941$), and PYY ($r = -0.103$; $P = 0.617$).

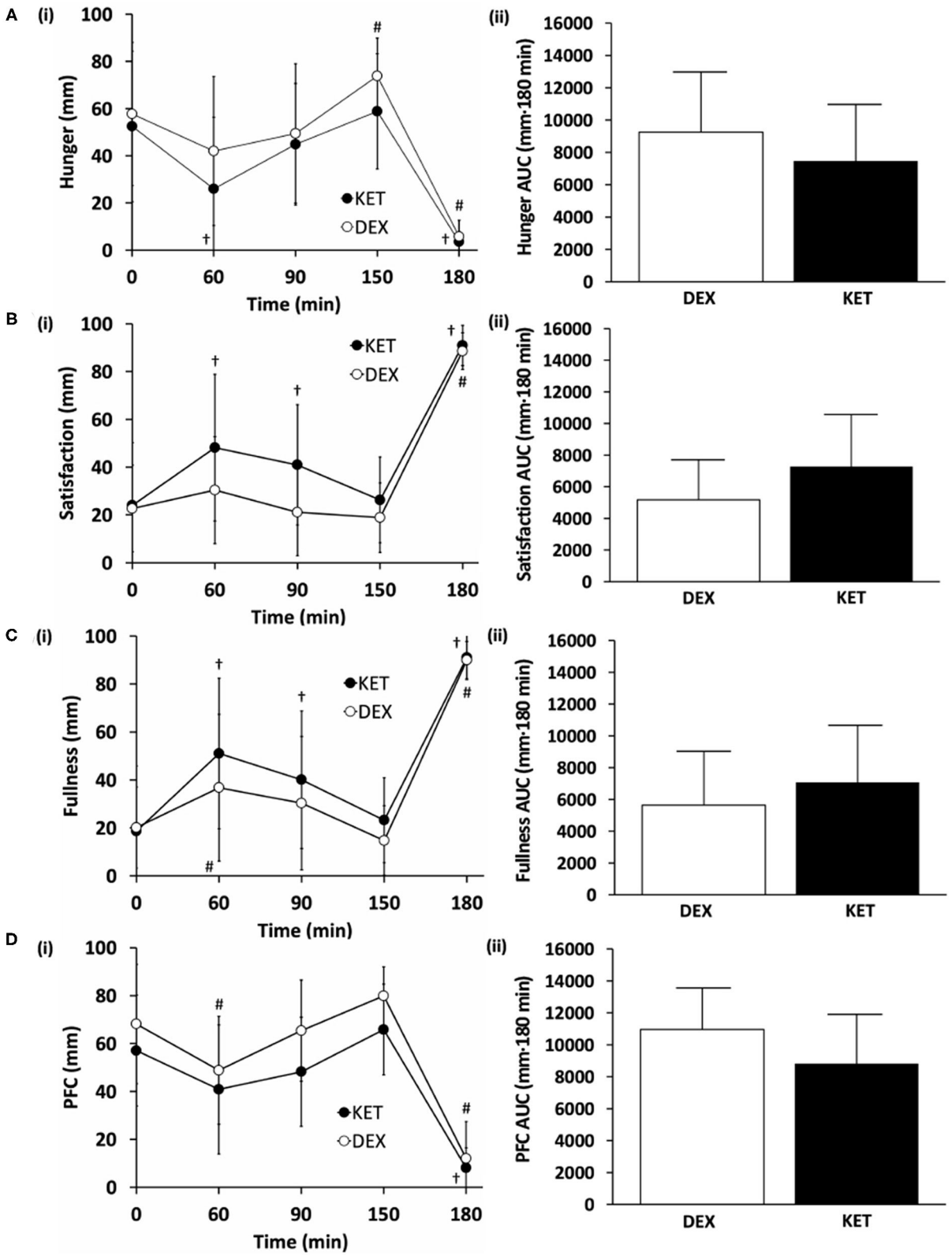

FIGURE 3 | Subjective measures of **(A)** hunger, **(B)** satisfaction, **(C)** fullness, and **(D)** prospective food consumption (PFC) on a (i) 100 mm visual analog scale (VAS) and (ii) total area under the curve (AUC) between the KET and DEX conditions. Values are means ± SD, $n = 13$. $^{\dagger}P < 0.05$ KET within-condition relative to baseline. $^{\#}P < 0.05$ DEX within-condition relative to baseline. DEX, dextrose condition; KET, ketone condition; PFC, prospective food consumption; VAS, visual analog scale.

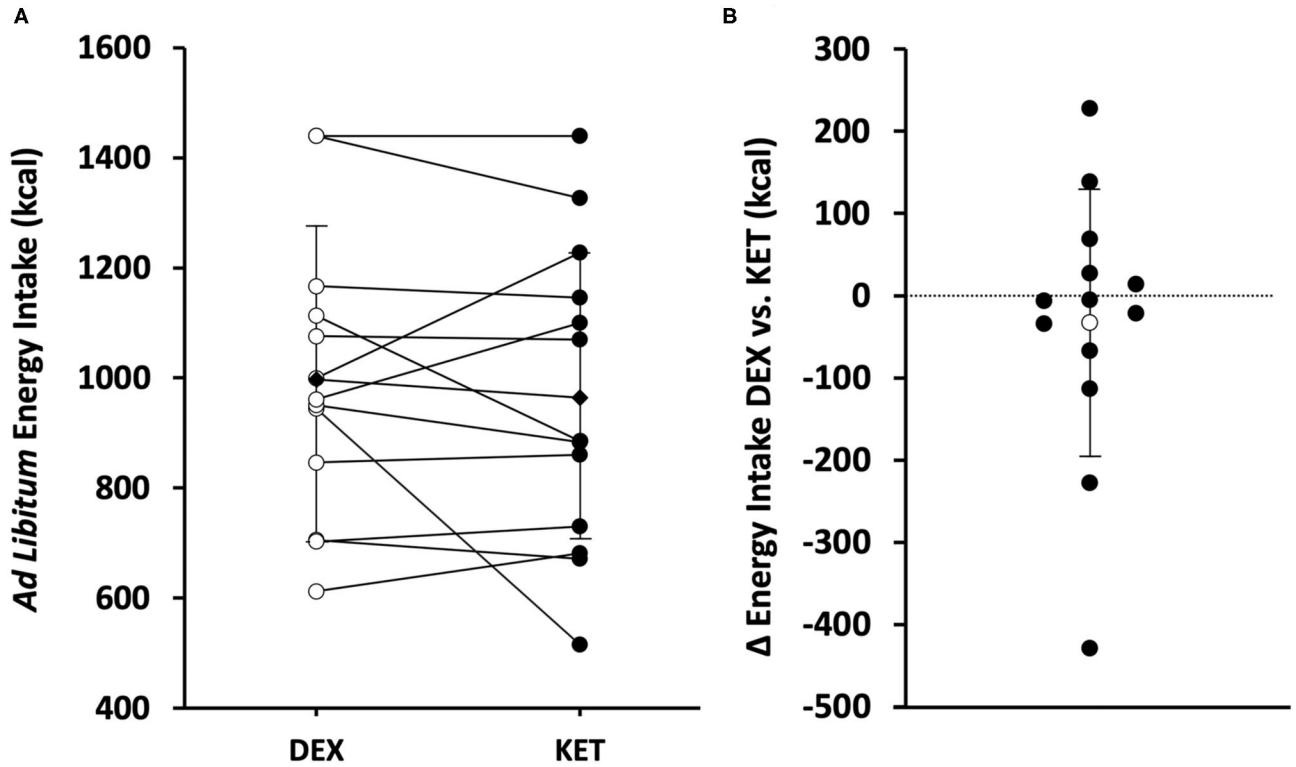

FIGURE 4 | (A) Individual (white circle = DEX; black circle = KET) and average (diamond) *ad libitum* energy intake (kcal) in the KET vs. DEX conditions. **(B)** Individual (black circle) and average (white circle) Δ in *ad libitum* energy intake from DEX to KET conditions. Values are means ± SD, $n = 13$. DEX, dextrose condition; KET, ketone condition.

DISCUSSION

This study examined the impact of exogenous ketosis during and after a bout of aerobic exercise on homeostatic regulators of appetite and *ad libitum* energy intake. Participants received a cumulative dose of 0.75 $g \cdot kg^{-1}$ KE and increased blood βHB concentrations to 4.1–6.1 mM during the post-exercise period. Ketosis, in association with exercise, reduced the hunger hormone acyl-ghrelin as well as the satiety hormone GLP-1. Post-exercise perceived appetite and *ad libitum* energy intake were not affected by exogenous ketosis. While βHB transiently modifies the secretion of appetite-regulating hormones following exercise, these changes do not appear to acutely affect subjective appetite and post-exercise energy intake/compensation.

Numerous studies have demonstrated that nutritionally-induced ketosis attenuates the increase in the hunger hormone ghrelin that occurs in association with weight loss (19–21). Similarly, acute exogenous ketosis under sedentary and exercise conditions has also been shown to induce transient reductions in acyl-ghrelin (5, 11). Consistent with these studies, we show a reduction in acyl-ghrelin with exogenous ketosis when compared to the consumption of an isocaloric dextrose beverage. Within-condition, both the ketone monoester and dextrose led to reductions in acyl-ghrelin at 60 min compared to baseline levels. The reduction in acyl-ghrelin in the DEX group is likely attributable to the combined impact of exercise and carbohydrate ingestion, both of which lead to transient reductions in acyl-ghrelin (18). Despite the absence of carbohydrate in the ketone ester group, the further reduction in acyl-ghrelin suggests that βHB elicits greater inhibitory action on acyl-ghrelin than carbohydrate alone. While many studies demonstrate an inverse association between βHB and acyl-ghrelin, the mechanism by which βHB reduces acyl-ghrelin is not completely understood. Acyl-ghrelin is produced in oxyntic cells in the stomach and the secretion of ghrelin is believed to be stimulated by the catecholamine-induced activation of β1-adrenergic receptors (22, 23). Interestingly, βHB has been demonstrated to suppress sympathetic tone by antagonizing GPR41 receptors expressed on sympathetic ganglion (24). It is not clear, however, whether sympathetic tone is reduced under exercise conditions with exogenous ketosis (11). Altogether, there is consistent evidence showing a reduction in acyl-ghrelin with KE ingestion, however the mechanisms mediating this reduction have yet to be fully elucidated.

In contrast to the anorexigenic effects of reduced acyl-ghrelin, the gut-derived satiety hormone GLP-1 was reduced post-exercise with ketone ester ingestion. Exercise, alone and in combination with nutrient intake, is known to lead to short-term increases in GLP-1 (8, 18). In two previous studies, ketone esters acutely suppressed GLP-1 under sedentary conditions (5, 15).

Given that dextrose is known to induce GLP-1 secretion (25), it is possible that the reduction in GLP-1 in the ketone ester group is due to a lack of dextrose-induced GLP-1 secretion. Evidence from Myette-Côté et al. (15), however, shows that ketone esters acutely suppress GLP-1 even when carbohydrate intake is the same between experimental groups, suggesting that βHB may have inhibitory action on GLP-1 secretion. βHB has antagonizing action on GPR41 receptors (24), the same receptors believed to be responsible for gut-stimulated GLP-1 secretion. In mice lacking the GPR41 receptor, short chain fatty acid-induced GLP-1 secretion is blunted (26). Based on this evidence, βHB may inhibit GLP-1 secretion by blocking GPR41 receptors on enteroendocrine cells within the gut. Overall, despite an unclear mechanism, the evidence suggests that acute ketosis elicits reductions in GLP-1, a finding that stands in contrast to the purported anorexigenic effects associated with ketosis.

Although numerous studies have examined changes in appetite signals with exogenous ketosis, there are no studies that have examined the effects of acute ketone ester supplementation on *ad libitum* energy intake in humans. Here we report that elevated ketone concentrations after an exercise session yield no changes in *ad libitum* energy intake. This finding is consistent with the observation that perceived appetite was not affected by ketone ingestion. Stubbs et al. (5) have previously reported that exogenous ketosis lowers subjective hunger and desire to eat under sedentary conditions. Exercise alone is known to reduce post-exercise appetite (27). The failure to see a reduction in perceived appetite in our study may be due to a greater anorexigenic effect of acute exercise in relation to exogenous ketosis. Despite this, under intense exercise conditions, ketosis has been found to lower post-exercise perceived appetite (11). After a 3 h intermittent cycling session, 15 min time trial, and an all-out sprint, Poffe et al. observed reductions in perceived levels of hunger and desire to eat when the exercise session was completed in a state of ketosis. Relative to our study findings, the reductions in perceived appetite reported by Poffe et al. may be due to factors including exercise duration/intensity, differential state of ketosis at the time of perceived appetite assessment, or additional energy consumption associated with exogenous ketone ingestion (∼277 kcal in the ketone condition relative to control) (11). In response to prolonged exogenous ketosis, animal studies show that ketones can reduce absolute energy intake (6, 28–30). Dietary R-3-hydroxybutyrate-R-1,3-butanediol monoester (∼30% energy) provided to C57BL/6J mice for a period of 12 weeks resulted in a 26% reduction in energy intake (28). Deemer et al. (6) showed that a diet with ∼30% of energy from ketone esters is necessary to induce this reduction in energy intake. It remains unknown, however, whether olfactory and taste aversion factors with ketone supplementation affects energy intake in animal studies (28). In contrast to prolonged animal studies showing reductions in energy intake with exogenous ketosis, it has been also been reported that ketone ester supplementation for a period of 3 weeks (50–57 g KE, 6×/week for 3 weeks) can increase energy intake in athletes performing high volumes of physical activity (31). It is speculated that the elevation in energy intake in a ketotic state is mediated by a reduction in the satiety signal growth differentiation factor 15. Potentially, given that ketone bodies cannot be converted into a storage form of energy (e.g., triglyceride) and are excreted in urine and breath if not oxidized (32), it is possible that ketone excretion elicits a greater energy deficit relative to isocaloric macronutrient ingestion, thus contributing to a greater compensatory response in energy intake. Taken together, the influence of exogenous ketosis on energy intake remains unclear. Under exercise conditions, our study suggests that acute ketosis has a neutral influence on energy intake.

There are several limitations with this present study. Our study did not include a non-exercise, sedentary control group. As such, it was not possible to determine whether there were any interaction effects between exercise and ketone ester intake on measures of appetite-regulating hormones, perceived appetite, or *ad libitum* energy intake. Additionally, our study may not have been adequately powered to detect differences in perceived appetite and energy intake. Although not statistically significant, there was a consistent pattern for exogenous ketosis to lower perceived appetite and energy intake, with effect sizes ranging from 0.2 to 0.6. Furthermore, given that this study was conducted in individuals with normal weight and average aerobic fitness, the findings from our study cannot be generalized to a population with obesity or high-performance aerobic athletes. Lastly, it was not possible to conduct the *ad libitum* meal tests using food items regularly consumed by all research participants. It is possible that the selected food items, unfamiliar eating environment, and restrictions on the timing of the *ad libitum* meal may have influenced energy intake measures.

In conclusion, our study provides evidence that acute exogenous ketosis during and after exercise elicits opposing action on homeostatic regulators of appetite by lowering the gut-derived, orexigenic hormone acyl-ghrelin as well as the anorexigenic hormone GLP-1. Associated with these changes, exogenous ketosis did not affect post-exercise appetite or alter *ad libitum* energy intake. In healthy adults, these data suggest that acute ketosis, in conjunction with a 1 h moderate to vigorous intensity aerobic exercise session, is not likely to have a large influence on short-term, post-exercise energy balance.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Lethbridge Human Participant Research Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TO recruited participants, collected and analyzed data, and wrote the manuscript. TQ recruited participants and collected data. MB designed study, obtained funding, and had final responsibility for

the study. All authors had access to the study data and reviewed and approved the final manuscript.

FUNDING

This study was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant (RGPIN-2018-05091). TO was supported through an NSERC-USRA.

ACKNOWLEDGMENTS

The authors would like to thank all participants who volunteered their time for this study.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

SYSTEMATIC REVIEW
published: 26 January 2021
doi: 10.3389/fnut.2020.625240

Effects of Ramadan and Non-ramadan Intermittent Fasting on Body Composition: A Systematic Review and Meta-Analysis

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Intermittent fasting (IF) has gained popularity for body-composition improvement purposes. The aim of this systematic review and meta-analysis was to summarize the effects of Ramadan vs. non-Ramadan IF on parameters of body composition. We conducted a comprehensive search of peer-reviewed articles in three electronic databases: PubMed, Scopus, and Web of Science (published until May 2020). Studies were selected if they included samples of adults (≥18 years), had an experimental or observational design, investigated any type of IF and included body composition outcomes. Meta-analytical procedures were conducted when feasible. Sixty-six articles met the eligibility criteria. We found that non-Ramadan IF is effective for decreasing body weight (−0.341 (95% CI [−0.584, −0.098], $p = 0.006$), body mass index (−0.699, 95% CI [−1.05, −0.347], $p < 0.001$), and absolute fat mass (−0.447, 95% CI [−0.673, −0.221], $p < 0.001$). When contrasting pre- post-intervention data on fat-free mass between treatments and controls, group-differences were non-significant ($p > 0.05$). Conversely, we observed a significant increase in fat-free mass when comparing pre- to post-intervention in a within design fashion (0.306, 95% CI [0.133, 0.48], $p = 0.001$). Finally, despite being accompanied by dehydration, Ramadan IF is effective in decreasing body weight (−0.353; 95% CI [−0.651, −0.054], $p = 0.02$) and relative fat mass (−0.533; 95% CI [−1.025, −0.04], $p = 0.034$). Ramadan IF seems to implicate some beneficial adaptations in weight management, although non-Ramadan IF appears to be more effective in improving overall body composition.

Keywords: Energy restriction, nutrition, diet, weight loss, calorie, exercise

OPEN ACCESS

Edited by:
Karsten Koehler, Technical University of Munich, Germany

Reviewed by:
Jennifer L. Scheid, Daemen College, United States
Giannis Arnaoutis, Harokopio University, Greece

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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *02 November 2020*
Accepted: *23 December 2020*
Published: *26 January 2021*

Citation:
Correia JM, Santos I, Pezarat-Correia P, Silva AM and Mendonca GV (2021) Effects of Ramadan and Non-ramadan Intermittent Fasting on Body Composition: A Systematic Review and Meta-Analysis. Front. Nutr. 7:625240. doi: 10.3389/fnut.2020.625240

KEY POINTS

- Both Ramadan and non-Ramadan intermittent fasting are effective on fat mass and body weight losses.
- Fat mass loss is more pronounced with non-Ramadan intermittent fasting and this type of intermittent fasting, combined with exercise training, leads to higher decreases in body mass index.

- Non-Ramadan intermittent fasting may be well-suited for eliciting small increases in fat-free mass, particularly under circumstances involving the simultaneous control of caloric intake.

INTRODUCTION

There are several forms of intermittent fasting (IF), all using fasting periods that extend well-beyond the duration of an overnight fast and implicating limited feeding time-windows, with or without caloric restriction (1, 2). For example, in time-restricted feeding/eating (TRF), eating is limited to a certain number of hours each day (3). Alternate-day fasting (ADF) consists of alternating between feasting and fasting days—feasting is compatible with *ad libitum* energy intake during 24 h and fasting implicates a caloric intake ≤ 25% of daily needs (∼ 500/600 kcal during 24 h) (4, 5). Recently, a focus on intermittent energy restriction (IER) has emerged in the scientific literature as an alternative approach to continuous energy restriction (CER) for improving body composition (6). It includes periods of energy restriction alternated with periods of habitual intake or minimally restricted dietary intake, allowing wider food choices (6, 7). Various forms of IER are currently being used for clinical purposes, including the "5:2 diet" and the "week-on-week-off" (7–9).

The ultimate goal of most IF regimens is to improve body composition. For this reason, they are suggested to maximize the loss of fat mass (FM), while attempting to preserve fat-free mass (FFM) (3). There is compelling evidence that IF elicits reductions in body weight and FM of ∼3–8% (4, 10–14) and ∼4–15%, respectively (1, 4, 10–13). Improvements in blood lipid profile, blood pressure and insulin sensitivity have also been consistently reported (5, 14). While the majority of studies explored the effectiveness of IF on improving body composition in adults with overweight/obesity (4, 5, 11, 13–15), there are only occasional reports that included normoponderal individuals (10, 13). Despite not being a universal finding (2, 16), IF has also been shown to induce slight increases in FFM [e.g., (17)]. In this specific study, the authors used a crossover design to compare TRF with an alternative diet involving three meals per day for a period of 8 weeks, with energy intake being individualized to each participant to ensure maintenance of body weight throughout both treatments (TRF and non-TRF). Under these conditions, TRF was shown to be effective in decreasing FM and increasing FFM (by ∼1.5 kg; $p = 0.06$) (17). In what concerns IER, and contrary to the initial belief, it seems to be unrelated to compensatory hyperphagia on *ad libitum* days; thus, similarly to that seen during CER, IER is accompanied by a negative caloric balance and this results in comparable changes in body composition between both interventions after 12 months of treatment (7). However, instead of being externally driven, caloric restriction during IER is purely spontaneous (18).

Apart from being used for clinical purposes, IF is also used in many religious practices. A notable example is Ramadan IF, which is the most widely-studied form of IF (19). During Ramadan, Muslims abstain from ingesting food and liquids between sunrise and sunset throughout a month-long period (20, 21). Thus, food and liquid intake becomes exclusively nocturnal. Even though Muslims eat *ad libitum* after sunset and before dawn, caloric restriction often accompanies Ramadan IF (22). Ramadan IF can last ∼12–18 h/day, depending on the geographic location and season of the year (23–25). Reductions in body weight, relative FM and resting metabolic rate are common consequences of Ramadan IF (20, 26). It has also been shown that Ramadan-related caloric restriction decreases total cholesterol, low-density lipoprotein (LDL) and fasting blood glucose levels. In parallel, Ramadan IF seems to improve body composition, possibly through enhancements in the ability to mobilize saturated fatty acids for metabolic processes (25). Changes in body composition may also reflect a balance between the unavoidable decrease of meal frequency (i.e., from 3–4 to 2) and sleep duration, together with a reduction of spontaneous daily physical activity and coercive dehydration (27–29). However, the effects of Ramadan IF on body composition are not conclusive, with some studies indicating significant reductions in body weight (19, 20, 23, 24, 26, 28, 30–34) and many other failing to show any relevant changes (21, 22, 27, 35–40). Such inconsistencies might be secondary to the specificities inherent to the participants included in each study. Alternatively, they may be dependent on differences between studies at the level of macro and micronutrient intake, or even arise as a consequence of cultural rituals or the number of fasting hours (23).

Taking all these points into consideration, this systematic review and meta-analysis aimed at summarizing the existing evidence on the effect of various IF regimens (i.e., TRF, ADF, IER, and Ramadan IF) on body composition. In addition, we sought to determine whether Ramadan and non-Ramadan IF exert a differential impact on human body composition. We hypothesized that various types of IF might contribute for improving body composition and that this effect might be further enhanced in circumstances compatible with a non-Ramadan dietary approach. To our knowledge, this is the first study providing such a comprehensive perspective.

METHODS

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (41) and is registered on PROSPERO (registration number CRD42020191161).

Eligibility Criteria

Articles focusing on the interaction between IF and body composition outcomes were retrieved. Only original research published in peer-reviewed scientific journals were considered. Studies were selected for this review if they respected the

Abbreviations: IF, intermittent fasting; Ramadan IF, Ramadan intermittent fasting; TRF, time-restricted feeding; ADF, alternate-day fasting; IER, intermittent energy restriction; CER, continuous energy restriction; BMI, body mass index; FM, fat mass; FFM, fat-free mass; ATP, adenosine triphosphate; SMD, standardized mean difference; PRISMA, preferred reporting items for systematic reviews and meta-analysis; EPHPP, effective public health practice project; CMA, comprehensive meta-analysis; CI, confidence interval.

following criteria: (i) included samples of adults (≥18 years), regardless of sex, (ii) had an experimental or observational design, (iii) investigated any type of IF (i.e., TRF, ADF, IER, or Ramadan IF), and (iv) included body composition outcomes (i.e., body weight, FFM, FM, muscle mass/volume, bone density, waist circumference, or body mass index—BMI). Studies including persons with specific health conditions, taking medication or having diseases/conditions known to affect metabolism/body composition (e.g., cancer, thyroid disease, diabetes, bariatric surgery, total parenteral nutrition, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), organ transplant, Prader–Willi Syndrome, polycystic ovary syndrome, chronic obstructive pulmonary disease, or acute illnesses, such as infections or traumatic injury), or pregnant women (or breastfeeding) were not included. Review articles, case studies, protocols, as well as abstracts/conference papers were also excluded.

Search Strategy

A comprehensive search of peer-reviewed articles was conducted in May 2020 in the following electronic databases: PubMed, Scopus and Web of Science. Searches included combinations of two sets of terms: (i) terms concerning the dietary intervention (i.e., IF) and (ii) terms representing the outcome of interest (i.e., body composition). Free-text terms used for the literature search were as follows (based on keywords): ("intermittent fasting" OR "alternate-day-fasting" OR "time-restricted feeding" OR "time-restricted eating" OR "periodic fasting" OR "intermittent calorie restriction" OR "intermittent energy restriction" OR "fasted state" OR "Ramadan") AND ("body composition" OR "body mass" OR "body mass index" OR "Quetelet index" OR "fat-free mass (FFM)" OR "fat-free mass" OR "fat mass" OR "fat percentage" OR "body weight" OR "waist circumference" OR "muscle mass" OR "muscle volume" OR "bone density"). There were no restrictions regarding the language of publication. Additionally, manual cross-referencing of literature cited in previous key reviews were performed.

Screening and Data Extraction

All titles and abstracts identified from the literature searches were screened for potential inclusion eligibility by three authors (JC, IS and GVM), based on the eligibility criteria mentioned above. Duplicate entries were removed. Relevant studies were fully screened by the same authors. A data extraction form was developed to compile information about the article (i.e., authors and year of publication), participants (i.e., sample size and demographics), study design, intervention characteristics (i.e., methods, protocols and length of intervention), and outcomes of interest. Endnote® X7 for Windows 10 was used to manage the references. The first author (JC) extracted the data and uncertainties were resolved by consensus.

Quality Assessment

Study quality was assessed with the Quality Assessment Tool for Quantitative Studies developed by the Effective Public Health Practice Project (EPHPP) (42), evaluating five key methodological domains: selection bias, study design, confounders, data collection method and withdrawals/dropouts. Since it is not possible to blind participants regarding a fasting condition, the sixth item of the original scale (blinding) was not assessed. Each domain was classified as strong, moderate or weak methodological quality. A global rating was determined based on the scores of each component. The five domains and overall quality were rated independently by two authors (JC and IS), and discrepancies were discussed until a consensus was reached. Inter-rater agreement across categories varied from moderate (Cohen's $k = 0.6$) to strong ($k = 1$).

Data Synthesis

For each study, results were summarized by: (i) intervention characteristics (intervention protocols, methods and duration), (ii) changes in body weight, (iii) changes in BMI, (iv) changes in absolute and relative FM, and (v) changes in FFM.

Data Analysis

Analyses were conducted using the Comprehensive Meta-Analysis (CMA) software version 3.0 (43). Separate meta-analyses were performed for each outcome (BMI, FM, FFM, and body weight) considering all studies, only Ramadan IF and only non-Ramadan IF studies. Meta-analyses were conducted using both random-effects and fixed-effect models, in which the summary effect size (ES) is the standardized mean difference (SMD) of a distribution (44, 45). Random-effects models were chosen for most analyses due to the high number of available studies ($k \geq 6$), assuming that the true effect size varies from study to study and that the studies in our analysis represent a random sample of effect sizes. When the number of studies per outcome was limited ($k < 6$), we used fixed-effect models, assuming that the true effect size is the same in all studies and the only reason for the effect size to vary between studies is sampling error (44). SMDs were calculated based on sample size, standard differences in means (between pre- and post-intervention time points and between pre- and post-intervention data of both treatment and control groups) and effect direction, and were interpreted according to Cohen's guidelines, with values of 0.2, 0.5 and 0.8 for small, medium and large SMD, respectively (46). The 95% confidence interval (CI) and corresponding p-values were considered as indicators of statistical significance. To evaluate the amount of variation in the effects of included studies, we tested for heterogeneity using the following approach: (i) the Cochran's Q statistic (47), for which a significant p-value (<0.05) demonstrates that studies do not share a common SMD (i.e., there is heterogeneity in the effect sizes between studies); and (ii) the I^2 statistic (48) that assesses the proportion of observed dispersion that is due to real differences in the actual SMD and is not affected by low statistical power. The I^2 ranges from 0 to 100%, where a value of 0% indicates no observed heterogeneity and values of 25, 50, and 75% reflect low, moderate and high heterogeneity, respectively (48). Some studies did not provide sufficient data to estimate the SMD and therefore the number of studies per outcome reported in the qualitative synthesis may differ from the ones included in the meta-analyses.

Subgroup analyses were conducted to examine whether the effect of IF on body composition outcomes varied according

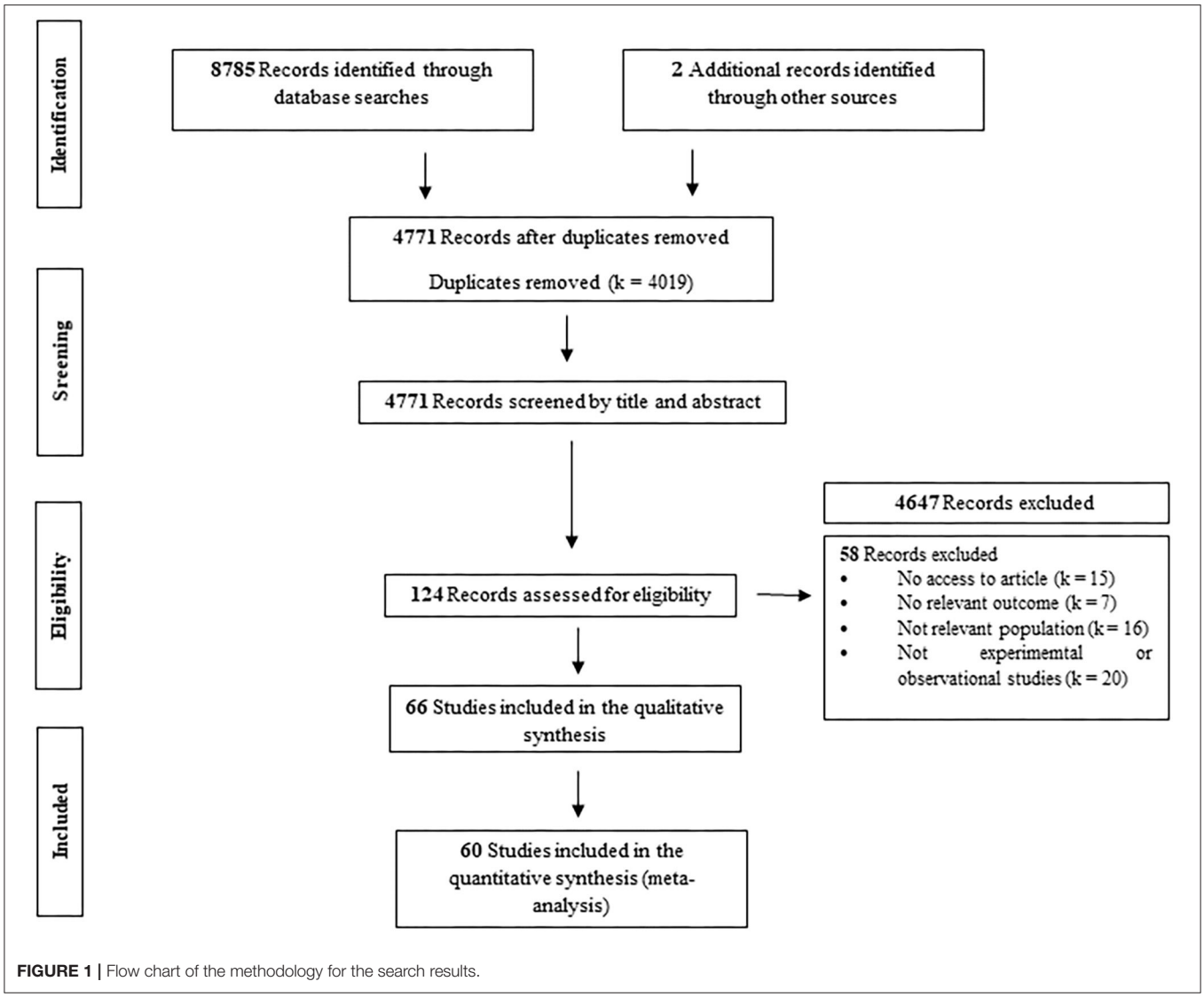

FIGURE 1 | Flow chart of the methodology for the search results.

to the pre-intervention nutritional status (overweight/obesity vs. normal body weight), exercise (protocols with vs. without exercise), and caloric intake (controlling for vs. not controlling for caloric intake). These subgroup analyses were conducted using mixed-effect models (i.e., random-effects model was conducted within subgroups and a fixed-effect model was used across subgroups) (49). Between-groups Q statistic and corresponding *p*-values were used to compare the mean effect across subgroups. Some studies included samples with both individuals showing normal body weight and excess weight/obesity, and therefore could not be included in moderation analyses.

Sensitivity Analyses

Sensitivity analyses were carried out to explore if the results were affected by methodological quality. Therefore, primary analyses were repeated excluding studies with poor methodological quality. Publication bias was also examined by visual inspection of funnel plots for asymmetry. To quantitatively confirm the visual impression, Egger's test (50) and Duval and Tweedie's trim-and-fill method (51) were used, but only for meta-analyses with 10 or more studies per outcome and no substantial heterogeneity, since the power is too low to distinguish chance from real asymmetry.

RESULTS

Study Selection

PubMed, Scopus and Web of Science searches generated 8,785 publications. Two publications were manually added from previous key reviews. Of 4,771 articles (after duplicates were removed), 124 were considered potentially relevant. Sixty-six articles met all inclusion criteria and were included in the present review (**Figure 1**). Of these, 60 provided sufficient data to be included in the meta-analyses.

TABLE 1 | Meta-analytic results for the effects of intermittent fasting on body composition.

Outcomes	*k*	Point estimate	CI lower	CI upper	*P*	Heterogeneity		
						Q-value	*P*	I^2
Body Weight (kg)*								
All studies	25	−0.347	−0.488	−0.205	**<0.001**	153.762	**<0.001**	84.391
Only Ramadan IF studies	23	−0.234	−0.341	−0.127	**<0.001**	77.564	**<0.001**	71.636
Only non-Ramadan IF studies	2	−1.845	−2.213	−1.477	**<0.001**	0.016	0.899	0.000
Body Weight (kg)**								
All studies	28	−0.334	−0.537	−0.13	**0.001**	85.219	**<0.001**	68.317
Only Ramadan IF studies	6	−0.353	−0.651	−0.054	**0.02**	0.839	0.974	0.000
Only non-Ramadan IF studies	22	−0.341	−0.584	−0.098	**0.006**	83.593	**<0.001**	74.878
BMI (kg/m^2)*								
All studies								
Only Ramadan IF studies[a]	16	−0.212	−0.353	−0.072	**0.003**	69.694	**<0.001**	78.477
Only non-Ramadan IF studies								
BMI (kg/m^2)**								
All studies	17	−0.655	−0.97	−0.341	**<0.001**	70.116	**<0.001**	77.181
Only Ramadan IF studies	3	−0.439	−1.017	0.138	0.136	1.229	0.541	0.000
Only non-Ramadan IF studies	14	−0.699	−1.05	−0.347	**<0.001**	68.735	**<0.001**	81.087
Fat Mass (kg)*								
All studies	13	−0.996	−1.351	−0.641	**<0.001**	196.692	**<0.001**	93.899
Only Ramadan IF studies	5	−0.376	−0.618	−0.135	**0.002**	47.803	**<0.001**	85.357
Only non-Ramadan IF studies	5	−1.963	−2.243	−1.682	**<0.001**	6.682	0.154	40.138
Fat Mass (kg)**								
All studies	24	−0.435	−0.65	−0.22	**<0.001**	78.722	**<0.001**	70.783
Only Ramadan IF studies	2	−0.286	−0.985	0.413	0.423	0.002	0.966	0.000
Only non-Ramadan IF studies	22	−0.447	−0.673	−0.221	**<0.001**	78.717	**<0.001**	73.322
Fat Mass (%)*								
All studies	15	−0.324	−0.477	−0.171	**<0.001**	58.248	**<0.001**	75.965
Only Ramadan IF studies[a]	12	−0.197	−0.312	−0.082	**0.001**	25.459	**0.008**	56.793
Only non-Ramadan IF studies	3	−1.073	−1.4	−0.746	**<0.001**	3.621	0.164	44.763
Fat Mass (%)**								
All studies	12	−0.237	−0.449	−0.025	**0.029**	6.04	0.871	0.000
Only Ramadan IF studies	4	−0.533	−1.025	−0.04	**0.034**	2.408	0.492	0.000
Only non-Ramadan IF studies	8	−0.169	−0.404	0.066	0.158	1.93	0.964	0.000
Fat Free Mass (kg)*								
All studies	15	−0.023	−0.193	0.146	0.788	66.465	**<0.001**	78.936
Only Ramadan IF studies	10	−0.142	−0.324	0.041	0.129	39.933	**<0.001**	77.462
Only non-Ramadan IF studies	5	0.306	0.133	0.48	**0.001**	5.869	0.209	31.843
Fat Free Mass (kg)**								
All studies	22	0.032	−0.122	0.186	0.684	33.663	**0.039**	37.617
Only Ramadan IF studies	5	−0.203	−0.599	0.192	0.314	0.214	0.995	0.000
Only non-Ramadan IF studies	17	0.073	−0.106	0.251	0.425	32.287	**0.009**	50.445

**Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control; *BMI, all studies (k = 16); *Body Weight (kg), all studies (k = 25); *Body Weight (kg), Ramadan IF studies (k = 23); *Body Weight (kg), non-Ramadan IF studies (k = 2); **Body Weight (kg), all studies (k = 28); **Body Weight (kg), Ramadan IF studies (k = 6); **Body Weight (kg), non-Ramadan IF studies (k = 22); *BMI, Ramadan IF studies (k = 16); **BMI, all studies (k = 17); **BMI, Ramadan IF studies (k = 3); **BMI, non-Ramadan IF studies (k = 14); *Fat Mass (kg), all studies (k = 13); *Fat Mass (kg), Ramadan IF studies (k = 8); *Fat Mass (kg), non-Ramadan IF studies (k = 5); **Fat Mass (kg), all studies (k = 24); **Fat Mass (kg), Ramadan IF studies (k = 2); **Fat Mass (kg), non-Ramadan IF studies (k = 22); *Fat Mass (%), all studies (k = 15); *Fat Mass (%), Ramadan IF studies (k = 12); *Fat Mass (%), non-Ramadan IF studies (k = 3); **Fat Mass (%), all studies (k = 12); **Fat Mass (%), Ramadan IF studies (k = 4); **Fat Mass (%), non-Ramadan IF studies (k = 8); *Fat Free Mass, all studies (k = 15), *Fat Free Mass, Ramadan IF studies (k = 10); *Fat Free Mass, non-Ramadan IF studies (k = 5); **Fat Free Mass, all studies (k = 22), **Fat Free Mass, Ramadan IF studies (k = 5); **Fat Free Mass, non-Ramadan IF studies (k = 17).*

CI, confidence interval; BMI, body mass index; IF, intermittent fasting.

[a]*Sensitivity analysis: Results are no longer significant when excluding studies with weak methodological quality. The bold values are statistically significant values.*

Study Characteristics

Characteristics of included studies are summarized in **Supplementary Table 1**. Twenty-seven studies were randomized controlled trials, 13 studies were non-randomized controlled trials, four studies were quasi-experimental (i.e., used a single-group pre- post-test design, where participants were their own controls) and 22 were observational studies. All IF protocols were conducted in laboratories ($k = 66$). More than half were undertaken during the month of Ramadan ($k = 34$) and the others explored the effects of TRF vs. normal diet on body composition ($k = 12$), during a period of 12 weeks ($k = 3$), 8 weeks ($k = 5$), 6 weeks ($k = 2$), 4 weeks ($k = 1$), and 10 days ($k = 1$); ADF vs. CER ($k = 4$) during 24 weeks ($k = 2$), 16 weeks ($k = 1$), and 8 weeks ($k = 1$), or ADF vs. no diet ($k = 8$) during 24 weeks ($k = 1$), 12 weeks ($k = 4$), and 8 weeks ($k = 3$); and IER vs. CER ($k = 7$) during 24 weeks ($k = 1$), 12 weeks ($k = 3$), and 8 weeks ($k = 3$), or IER vs. no diet ($k = 1$) during 3 weeks. Body composition was assessed with dual X-ray absorptiometry ($k = 17$), air displacement plethysmography ($k = 3$), bioimpedance ($k = 24$) or anthropometry ($k = 22$). Eighteen studies included only women and twenty-one studies tested individuals with overweight and/or obesity. Approximately 48% of the studies controlled only for dietary intake ($k = 32$) and 29 studies assessed dietary intake plus exercise. The sample sizes varied from 9 to 332 experimental participants and their mean ages ranged between 18 and 72 years.

Quality Assessment

Supplementary Table 2 shows the detailed classification of each quality domain and the overall methodological quality of each study. Of the 66 studies identified as relevant for this systematic review, the overall methodological quality of 40 studies was rated as "moderate" and 26 were classified as "weak." Twenty-seven studies (randomized controlled trials) were rated as strong and 13 (non-randomized controlled trials) were rated as moderate regarding the quality of study design; the remaining studies were rated as weak. All studies ($k = 66$) were classified as weak regarding selection bias (representativeness) because the samples were self-selected or composed by volunteers. With regard to the adjustment of analyses for confounders, all studies ($k = 66$) were classified as strong because there was no bias arising from lack of controlled elements. In what concerns data collection methods, all studies were rated as strong because they all used valid and reliable tools. Finally, regarding the reporting of withdrawals and dropouts, 20 studies were rated as strong, 19 studies were rated as moderate, and one study was rated as weak.

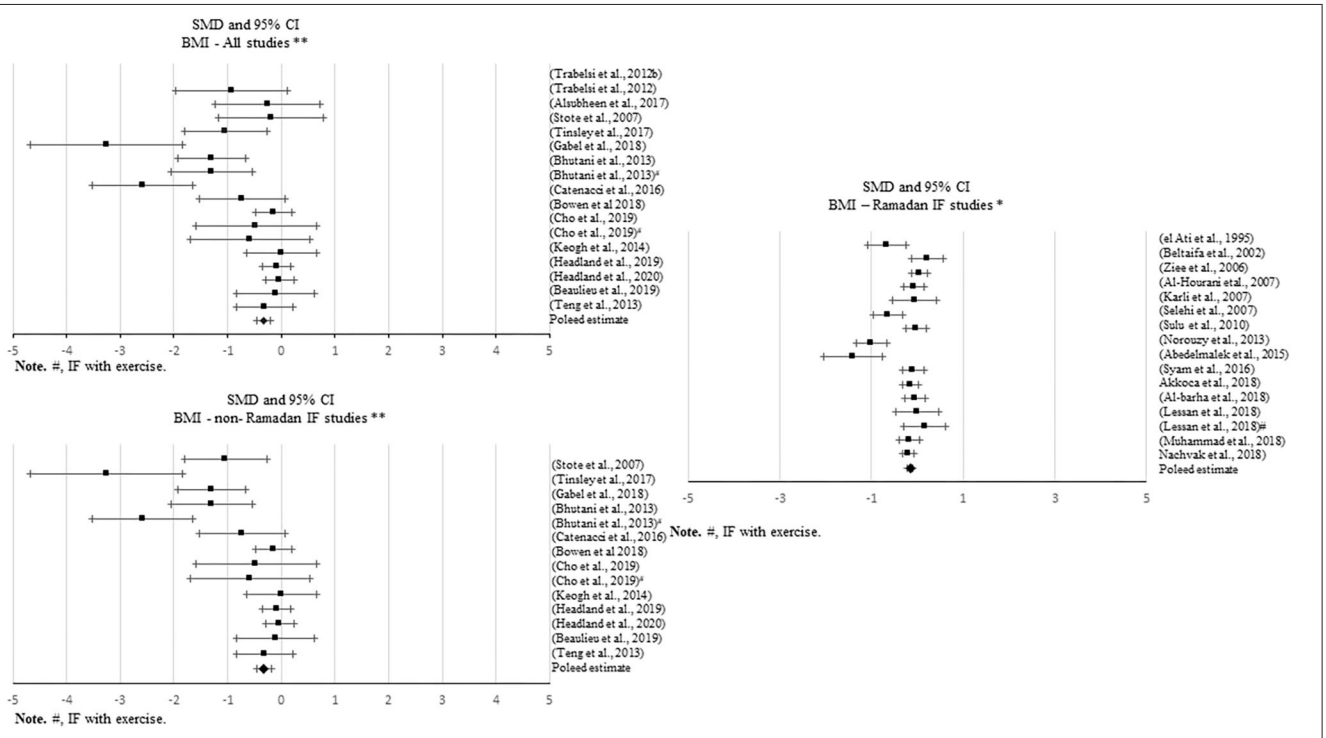

FIGURE 2 | Forest plot of the effects from a random effects and fixed-effects meta-analysis shown as standardized mean difference with 95% confidence intervals on absolute body weight (BW) for all studies, only Ramadan IF and only non-Ramadan IF studies. For each study, the square represents the standardized mean difference between pre-post data intervention and control groups, with the horizontal line intersecting it as the lower and upper limits of the 95% confidence interval. Forest plot of the effects from a random effects and fixed-effects meta-analysis shown as standardized mean difference with 95% confidence intervals on absolute body weight (BW) for all studies, only Ramadan IF and only non-Ramadan IF studies. For each study, the square represents the standardized mean difference between pre- and post-intervention time points. The rhombi represents the pooled estimated standardized mean difference. *Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control.

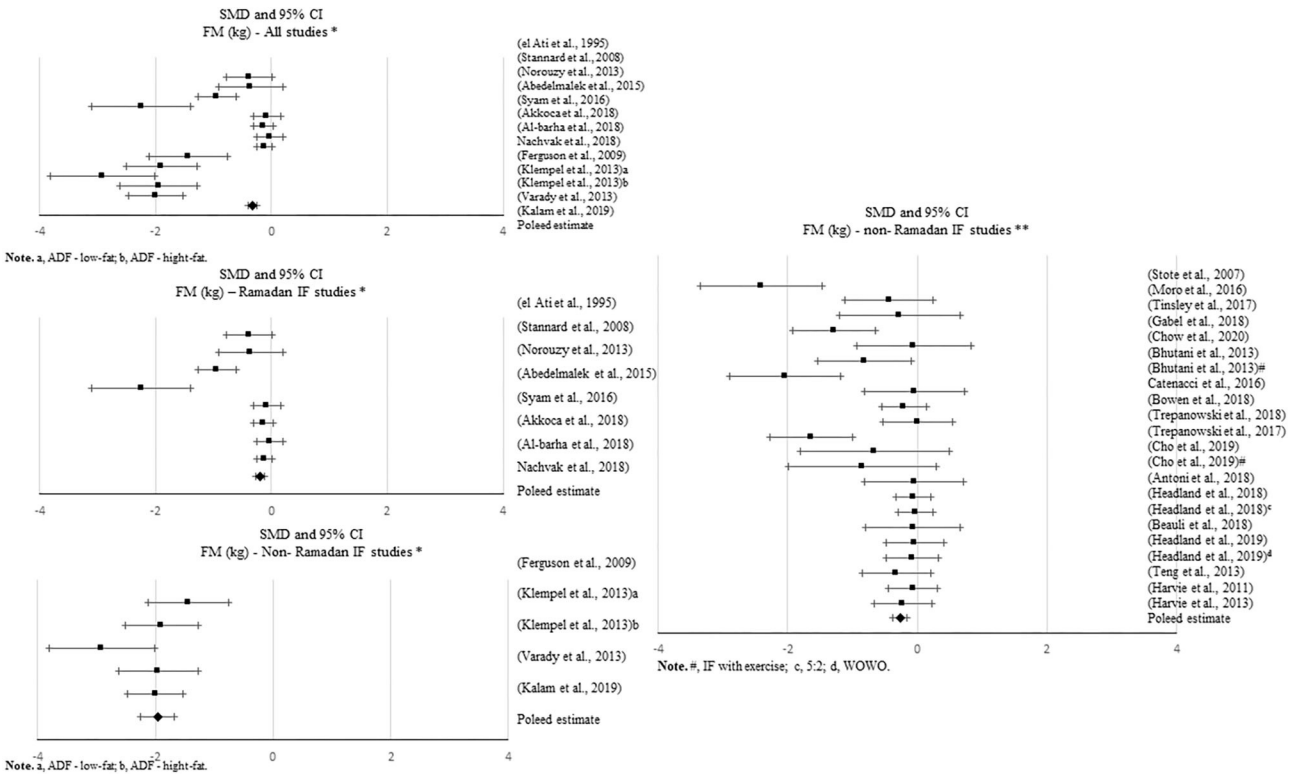

FIGURE 3 | Forest plot of the effects from a random-effects meta-analysis shown as standardized mean difference with 95% confidence intervals on body mass index (BMI) for all studies and only non-Ramadan IF studies. For each study, the square represents the standardized mean difference between pre-post data intervention and control groups, with the horizontal line intersecting it as the lower and upper limits of the 95% confidence interval. Forest plot of the effects from a random-effects meta-analysis shown as standardized mean difference with 95% confidence intervals on body mass index (BMI) for only Ramadan IF studies. For each study, the square represents the standardized mean difference between pre- and post-intervention time points. The rhombi represents the pooled estimated standardized mean difference. *Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control.

Effects of Intermittent Fasting on Specific Outcomes

A data analytic synthesis of the effects of IF on the four tested body composition outcomes (i.e., body weight, BMI, absolute and relative FM, and FFM) is shown in **Supplementary Table 1**. **Table 1** shows the meta-analytic results for the pooled estimates of these effects, and **Figures 2–6** the respective forest plots. **Tables 2–4** show the moderation effect of nutritional status, exercise and caloric control, respectively, on these estimates.

Changes in Body Weight

Sixty-four studies reported effects of IF on body weight, with ∼66% of them attaining significant changes in this specific outcome. Some studies obtained significant decreases in body weight with Ramadan IF ($k = 20$) and non-Ramadan IF [TRF ($k = 8$), ADF ($k = 8$) and IER ($k = 5$)]. Approximately 23% of the studies that observed a significant decrease in body weight had an exercise intervention ($k = 15$).

When assessing changes in body weight, significant negative pooled estimates emerged, when comparing pre-post-intervention data between treatments and the control groups, including all IF studies ($k = 28$, −0.334 (95% CI [−0.537, −0.13], $p = 0.001$), only Ramadan IF studies ($k = 6$, −0.353 (95% CI [−0.651, −0.054], $p = 0.02$), and only non-Ramadan IF studies ($k = 22$, −0.341 (95% CI [−0.584, −0.098], $p = 0.006$). There was evidence of high heterogeneity between non-Ramadan IF studies ($Q = 83.593$, $p < 0.001$, $I^2 = 75\%$) and moderate heterogeneity between all studies ($Q = 85.219$, $p < 0.001$, $I^2 = 68\%$). No heterogeneity was observed between Ramadan IF studies ($Q = 0.839$, $p = 0.974$, $I^2 = 0\%$). Similar results were found when considering pre- post-intervention data only (all: $k = 25$; SMD = −0.347, 95% CI [−0.488, −0.205], $p < 0.001$ ($Q = 153.762$, $p < 0.001$, $I^2 = 84\%$); only Ramadan IF interventions: $k = 23$; SMD = −0.234, 95% CI [−0.341, −0.127], $p < 0.001$ (Q =77.564, $p < 0.001$, $I^2 = 71\%$); and only non-Ramadan IF interventions: $k = 2$; SMD = −1.845, 95% CI [−2.213, −1.477], $p < 0.001$ ($Q = 0.016$, $p = 0.899$, $I^2 = 0\%$). Similar results were observed when excluding studies with weak methodological quality.

There was evidence of publication bias based on the visual inspections of the funnel plots for body weight, when comparing pre-post data between treatments and controls including all studies and non-Ramadan IF studies only, and when considering pre-post intervention data only for all studies, but due to the moderate-to-high heterogeneity observed, there was not enough power to differentiate chance from real asymmetry.

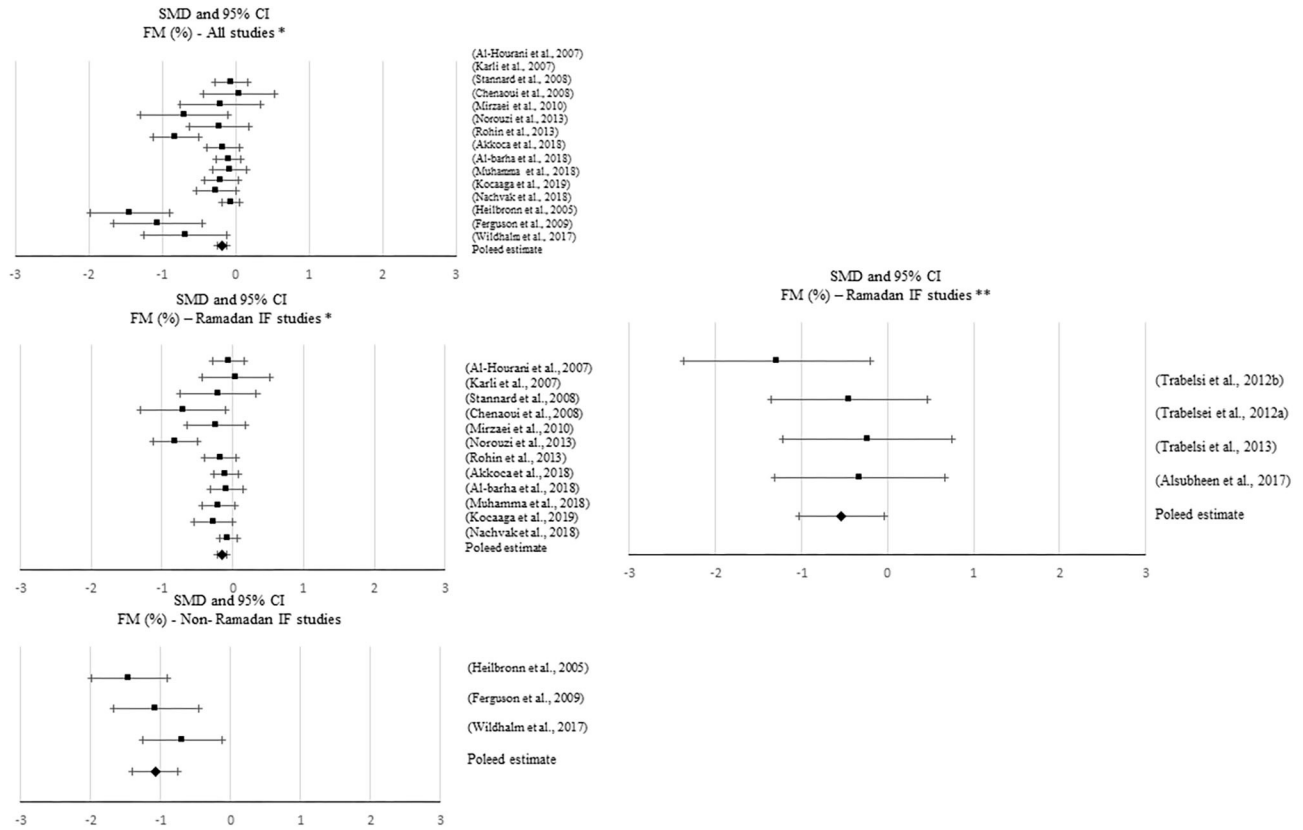

FIGURE 4 | Forest plot of the effects from a random-effects meta-analysis shown as standardized mean difference with 95% confidence intervals on absolute fat mass (FM) for all studies and only non-Ramadan IF studies. For each study, the square represents the standardized mean difference between pre-post data intervention and control groups, with the horizontal line intersecting it as the lower and upper limits of the 95% confidence interval. Forest plot of the effects from a random-effects and fixed-effects, respectively, meta-analysis shown as standardized mean difference with 95% confidence intervals on absolute FM for all studies and only non-Ramadan IF studies. For each study, the square represents the standardized mean difference between between pre- and post-intervention time points. The rhombi represents the pooled estimated standardized mean difference. *Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control.

Subgroup analyses showed a moderation effect of exercise on the impact of IF on body weight when considering pre-post-intervention data for all studies ($Q = 5.330$, $p = 0.021$): a small negative effect was found when exercise was not included ($k = 17$; SMD $= -0.430$, 95% CI $[-0.609, -0.251]$, $p < 0.001$). Additionally, significant differences were found between interventions with and without caloric control when considering pre- post-intervention data for all studies ($Q = 67.857$, $p < 0.001$): significantly higher negative effects emerged when calories were taken into account: $k = 2$; SMD $= -1.845$, 95% CI $[-2.213, -1.477]$, $p < 0.001$ vs. $k = 23$; SMD $= -0.234$, 95% CI $[-0.341, -0.127]$, $p < 0.001$).

Changes in Body Mass Index

Thirty-six studies presented data for the effect of IF on BMI, with ∼69% of the studies showing significant changes. Most of these studies reported positive results, i.e., a significant decrease in BMI with Ramadan IF ($k = 12$) and non-Ramadan IF [TRF ($k = 4$), ADF ($k = 5$), and IER ($k = 3$)]. Only one study reported an increase in BMI with Ramadan IF. Approximately 16% of the studies showing a significant decrease in BMI had an exercise intervention ($k = 6$).

Significant decreases in BMI were found when quantitatively comparing pre- post-intervention data between IF treatments and the control groups, including all studies ($k = 17$; SMD $= -0.655$, 95% CI $[-0.97, -0.341]$, $p < 0.001$) and non-Ramadan IF studies only ($k = 14$; SMD $= -0.699$, 95% CI $[-1.05, -0.347]$, $p < 0.001$). There was evidence of high heterogeneity between all studies ($Q = 70.116$, $p < 0.001$; $I^2 = 77\%$) and non-Ramadan studies only ($Q = 68.735$, $p < 0.001$; $I^2 = 81\%$). Non-significant results emerged when considering Ramadan IF studies only ($p > 0.05$). Although this pooled estimate is significant when meta-analyzing pre-post-intervention data only ($k = 16$; SMD $= -0.212$, 95% CI $[-0.353, -0.072]$, $p = 0.003$; $Q = 69.694$, $p < 0.001$; $I^2 = 78\%$), sensitivity analysis showed that when excluding the studies with weak methodological quality, it became non-significant ($p > 0.05$).

There was evidence of publication bias based on the visual inspection of the funnel plots, but due to the large heterogeneity observed including all studies and non-Ramadan IF studies

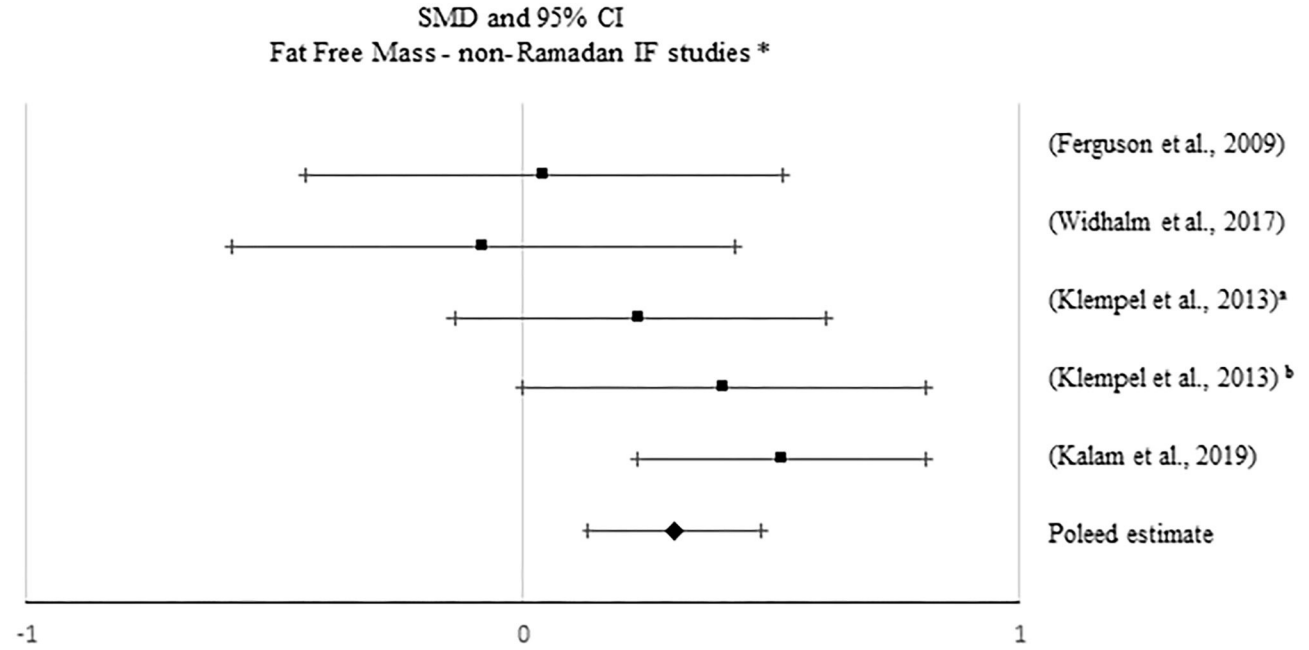

FIGURE 5 | Forest plot of the effects from a random-effects and fixed-effects, respectively, meta-analysis shown as standardized mean difference with 95% confidence intervals on relative fat mass (FM) for all studies and only Ramadan IF studies. For each study, the square represents the standardized mean difference between pre-post data intervention and control groups, with the horizontal line intersecting it as the lower and upper limits of the 95% confidence interval. Forest plot of the effects from a random-effects and fixed-effects, meta-analysis shown as standardized mean difference with 95% confidence intervals on relative FM for all studies, only Ramadan IF studies and only non-Ramadan IF studies. For each study, the square represents the standardized mean difference between pre- and post-intervention time points. The rhombi represents the pooled estimated standardized mean difference. *Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control.

only, there was not enough power to differentiate chance from real asymmetry.

Subgroup analyses showed that exercise moderated the effect of IF on BMI when comparing pre- post-intervention data between non-Ramadan IF treatments and the control groups ($Q = 4.660$, $p = 0.031$): a higher negative effect was found when individuals exercised ($k = 3$; SMD $= -2.112$, 95% CI [-3.634, -0.59], $p = 0.007$ vs. $k = 11$; SMD $= -0.411$, 95% CI [-0.675, -0.147], $p = 0.002$).

Changes in Fat Mass

A total of 49 studies reported on the effect of an IF dietary intervention on FM. Six studies reported satisfactory results as a significant decrease in absolute FM with Ramadan IF and 18 studies with non-Ramadan IF [five studies with TRF, five studies with IER, and eight studies with ADF]. Approximately 13% of the studies that observed a significant decrease in FM had an exercise intervention ($k = 6$).

Meta-analytic results showed a negative, small but significant effect of IF on absolute FM when comparing pre- post-intervention data between IF treatments and the control groups, including all studies ($k = 24$; SMD $= -0.435$, 95% CI [-0.65, -0.22], $p < 0.001$; Q $= 78.722$, $p < 0.001$; $I^2 = 70\%$) and non-Ramadan IF studies only ($k = 22$; SMD $= -0.447$, 95% CI [-0.673, -0.221], $p < 0.001$; Q $= 78.717$, $p < 0.001$; $I^2 = 73\%$). When considering pre- post-intervention data, significant pooled effects were observed with all IF interventions ($k = 13$; SMD $= -0.996$, 95% CI [-1.351, -0.641], $p < 0.001$; Q $= 196.692$, $p < 0.001$; $I^2 = 94\%$), Ramadan IF interventions only ($k = 8$; SMD $= -0.376$, 95% CI [-0.618, -0.135], $p = 0.002$; Q $= 47.803$, $p < 0.001$; $I^2 = 85\%$) and non-Ramadan IF interventions only ($k = 5$; SMD $= -1.963$, 95% CI [-2.243, -1.682], $p < 0.001$; Q $= 6.682$, $p = 0.154$; $I^2 = 40\%$). Similar results were found after conducting sensitivity analyses (i.e., removing studies with weak methodological quality).

Results of the meta-analyses for relative FM showed a negative, moderate effect when comparing pre- post-intervention data between Ramadan IF treatments and the control groups ($k = 4$; SMD $= -0.533$, 95% CI [-1.025, -0.04], $p = 0.034$; Q $= 2.408$, $p = 0.492$; $I^2 = 0\%$) and a negative, but small effect when comparing pre- post-intervention data between all IF treatments and the control groups ($k = 12$; SMD $= -0.237$, 95% CI [-0.449, -0.025], $p = 0.029$; Q $= 6.04$, $p = 0.871$; $I^2 = 0\%$). Again, when considering pre- post-intervention data only, significant pooled estimates were observed with all IF interventions ($p \leq 0.001$).

Sensitivity analyses showed that, when excluding the studies with poor quality, the effect of Ramadan IF interventions on relative FM became non-significant ($p > 0.05$).

Regarding publication bias, visual inspections of the funnel plots for absolute FM showed the presence of asymmetry between all studies and between non-Ramadan IF studies only, when comparing pre- post-intervention data between treatments and

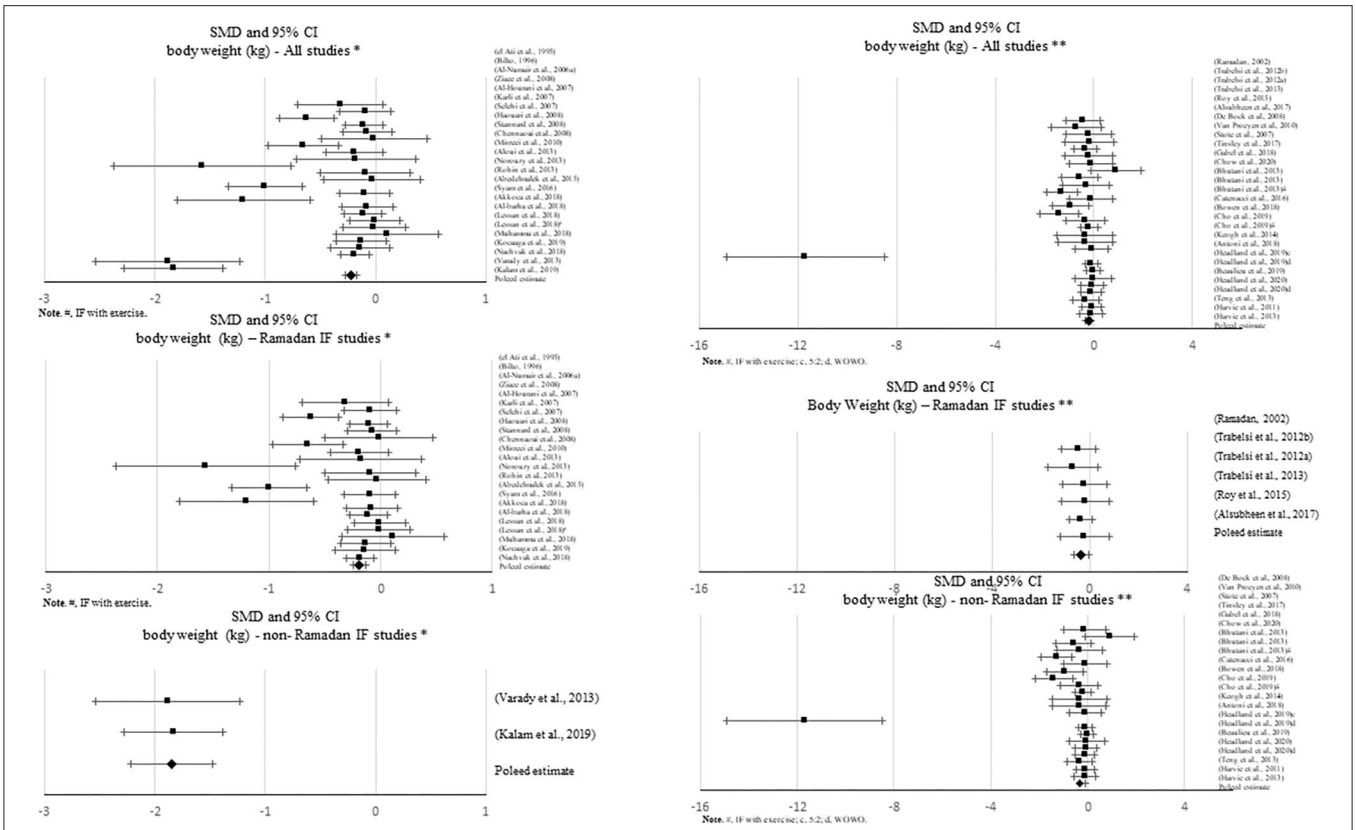

FIGURE 6 | Forest plot of the effects from a fixed-effects meta-analysis shown as standardized mean difference with 95% confidence intervals on fat-free mass (FFM) for only non-Ramadan IF studies. For each study, the square represents the standardized mean difference between pre- and post-intervention time points, with the horizontal line intersecting it as the lower and upper limits of the 95% confidence interval. The rhombi represents the pooled estimated standardized mean difference. *Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control.

controls, and between all studies when considering pre- post-intervention data only. Visual inspections of the funnel plots for relative FM also demonstrated asymmetry between all studies, when considering pre- post-intervention data only. However, due to the moderate-to-high heterogeneity observed, there was not enough power to differentiate chance from real asymmetry.

Subgroup analyses showed that, when considering pre- post-intervention data for all studies, there were significant differences in the effect of IF on absolute and relative FM between studies with and without caloric control (absolute FM: $Q = 49.961$, $p < 0.001$; FM (%): $Q = 6.002$, $p = 0.014$): significantly higher negative effects were observed when calories were taken into account (absolute FM: $k = 5$; SMD $= -1.979$, 95% CI [-2.351, -1.606], $p < 0.001$ vs. $k = 8$; SMD $= -0.376$, 95% CI [-0.618, -0.135], $p = 0.002$; relative FM: $k = 1$; SMD $= -1.059$, 95% CI [-1.659, -0.459], $p = 0.001$ vs. $k = 14$; SMD $= -0.287$, 95% CI [-0.435, -0.14], $p < 0.001$).

Changes in Fat-Free Mass

Approximately 62% of the studies reported effects of IF on FFM ($k = 41$). Only one study (TRF protocol) showed a significant increase in FFM. Fourteen studies reported significant decreases in this specific outcome with Ramadan IF ($k = 3$) and non-Ramadan IF [TRF ($k = 2$), ADF ($k = 5$), and IER ($k = 4$)]. Only 2 and 7% of the studies showing, respectively, significant increases and decreases in FFM, included exercise.

No significant pooled estimates emerged when comparing pre- post-intervention data between the IF treatments and the control groups ($p > 0.05$). However, when meta-analyzing pre-post-intervention data, a significant increase in FFM was found with non-Ramadan IF interventions ($k = 5$; SMD $= 0.306$, 95% CI [0.133, 0.48], $p = 0.001$; $Q = 5.869$, $p = 0.209$; $I^2 = 32\%$). Sensitivity analysis revealed no significant changes.

Regarding publication bias, visual inspections of the funnel plots did not show the presence of asymmetry, which was confirmed with Egger's tests ($p > 0.05$).

Subgroups analyses revealed significant differences in the effect of IF on FFM between individuals with normal weight and overweight, when considering pre- post-intervention data for all studies ($Q = 4.142$, $p = 0.042$), although changes in each subgroup were not significant ($p > 0.05$). Significant differences in the effect of IF on FFM between studies with and without caloric control were also found, when considering pre- post-intervention data for all studies ($Q = 13.365$, $p < 0.001$): a small positive effect was found when caloric

TABLE 2 | Subgroup analysis assessing the effect of nutritional status as a moderator of the impact of intermittent fasting on body composition.

Moderators	*k*	Point estimate	CI lower	CI upper	*P*	Heterogeneity		
						Q-value	*P*	I^2
Body Weight (kg)*								
All studies								
Normal weight	21	−0.325	−0.488	−0.162	**<0.001**	0.535	0.465	83.885
Overweight/Obesity	4	−0.469	−0.818	−0.120	**0.008**			88.869
Only Ramadan IF studies								
Normal weight	19	−0.175	−0.281	−0.069	**0.001**	2.497	0.114	59.739
Overweight/Obesity	4	−0.469	−0.818	−0.12	**0.008**			88.869
Body Weight (kg)**								
All studies								
Normal weight	6	−0.384	−0.683	−0.085	**0.012**	0.083	0.773	0.000
Overweight/Obesity	17	−0.446	−0.734	−0.157	**0.002**			79.950
Only non-Ramadan IF studies								
Normal weight	2	−0.477	−1.053	0.099	0.104	0.009	0.924	0.000
Overweight/Obesity	17	−0.446	−0.734	−0.157	**0.002**			79.950
BMI (kg/m^2)*								
Only Ramadan IF studies								
Normal weight	11	−0.118	−0.272	0.035	0.130	1.516	0.218	66.978
Overweight/Obesity	6	−0.336	−0.646	−0.025	**0.034**			86.499
BMI (kg/m^2)**								
All studies								
Normal weight	4	−1.261	−2.275	−0.247	**0.015**	1.563	0.211	74.830
Overweight/Obesity	11	−0.574	−0.935	−0.214	**0.002**			79.285
Only non-Ramadan IF studies								
Normal weight	2	−2.061	−4.233	0.111	0.063	1.752	0.186	86.428
Overweight/Obesity	11	−0.574	−0.935	−0.214	**0.002**			79.285
Fat Mass (kg)*								
All studies								
Normal weight	11	−1.134	−1.605	−0.663	**<0.001**	1.696	0.193	94.131
Overweight/Obesity	2	−0.516	−1.318	0.287	0.208			95.255
Only Ramadan IF studies								
Normal weight	6	−0.334	−0.624	−0.045	**0.024**	0.173	0.678	80.912
Overweight/Obesity	2	−0.516	−1.318	0.287	0.208			95.255
Fat Mass (kg)**								
All studies								
Normal weight	4	−0.838	−1.807	0.130	0.090	0.826	0.364	79.531
Overweight/Obesity	18	−0.377	−0.604	−0.149	**0.001**			70.702
Only non-Ramadan IF studies								
Normal weight	3	−1.021	−2.286	0.245	0.114	0.964	0.326	85.085
Overweight/Obesity	18	−0.377	−0.604	−0.149	**0.001**			70.702
Fat Mass (%)*								
All studies								
Normal weight	12	−0.328	−0.511	−0.146	**<0.001**	0.001	0.973	71.809
Overweight/Obesity	3	−0.336	−0.716	0.045	0.084			89.480
Only Ramadan IF studies								
Normal weight	9	−0.138	−0.228	−0.048	**0.003**	0.982	0.322	0.000
Overweight/Obesity	3	−0.336	−0.716	0.045	0.084			89.480
Fat Mass (%)**								
All studies								
Normal weight	4	−0.508	−0.994	−0.022	**0.040**	0.633	0.426	0.000
Overweight/Obesity	6	−1.354	−3.379	0.672	0.190			96.642

(Continued)

TABLE 2 | Continued

Moderators	*k*	Point estimate	CI lower	CI upper	*P*	Heterogeneity		
						Q-value	*P*	I^2
Only non-Ramadan IF studies								
Normal weight	1	−0.253	−1.186	0.680	0.595	0.936	0.333	0.000
Overweight/Obesity	6	−1.354	−3.379	0.672	0.190			96.642
Fat Free Mass (kg)*								
All studies								
Normal weight	12	0.097	−0.022	0.216	0.109	4.142	**0.042**	28.246
Overweight/Obesity	3	−0.423	−0.91	0.064	0.088			93.218
Only Ramadan IF studies								
Normal weight	7	0.000	−0.116	0.116	0.999	2.745	0.098	0.000
Overweight/Obesity	3	−0.423	−0.91	0.064	0.088			93.218
Fat Free Mass (kg)**								
All studies								
Normal weight	5	0.038	−0.331	0.407	0.841	2.238	0.135	0.000
Overweight/Obesity	14	−0.767	−1.754	0.221	0.128			97.762
Only non-Ramadan IF studies								
Normal weight	2	0.290	−0.214	0.795	0.259	3.491	0.062	3.942
Overweight/Obesity	14	−0.767	−1.754	0.221	0.128			97.762

**Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control; *BMI, Ramadan IF studies (k = 15); *Body Weight (kg), all studies (k = 25); **Body Weight (kg), all studies (k = 23); *Body Weight (kg), Ramadan IF studies (k = 23); **Body Weight (kg), non-Ramadan IF studies (k = 19); **BMI, all studies (k = 15); **BMI, non-Ramadan IF studies (k = 13); *Fat Mass (kg), all studies (k = 13); **Fat Mass (kg), all studies (k = 22); *Fat Mass (kg), Ramadan IF studies (k = 8); **Fat Mass (kg), non-Ramadan IF studies (k = 21); *Fat Mass (%), all studies (k = 15); **Fat Mass (%), all studies (k = 10); *Fat Mass (%), Ramadan IF studies (k = 12); **Fat Mass (%), non-Ramadan IF studies (k = 7); *Fat Free Mass, all studies (k = 15), **Fat Free Mass, all studies (k = 19); *Fat Free Mass, Ramadan IF studies (k = 10); **Fat Free Mass, non-Ramadan IF studies (k = 16).*
CI, confidence interval; BMI, body mass index; IF, intermittent fasting. The bold values are statistically significant values.

intake was taken into account ($k = 4$; SMD = 0.351, 95% CI [0.154, 0.548], $p < 0.001$).

DISCUSSION

This study sought to summarize the effects of different IF approaches on body composition-related outcomes. To our knowledge, this is the first systematic review and meta-analysis presenting comprehensive results on changes in specific parameters of body composition resulting from Ramadan vs. non-Ramadan IF, which is of considerable relevance given the recent popularity of these approaches among researchers and clinical nutritionists/sports physiologists.

Our findings showed that, when comparing pre- with post-intervention data between treatment and control groups, Ramadan IF elicits significant reductions in some body composition parameters, namely in relative FM and body weight. Similar results were also found when considering pre- to post-intervention (within-subjects design); specifically, data indicated that Ramadan IF leads to significant reductions in BMI, FM (relative and absolute), and body weight. Our findings also suggest that, with Ramadan IF, weight loss seems to be more pronounced in persons exhibiting higher BMI values, although there were no significant differences between groups (normal weight vs. overweight/obesity). These findings are in line with those of past research (30). A greater loss of body fluids in individuals with overweight/obesity during Ramadan IF offers a likely explanation for such relationships (52).

In the general population, weight loss subsequent to Ramadan IF is largely caused by the unconscious restriction of food intake. Under these circumstances, the reduction in meal frequency is causally linked with the prolonged daily periods of standing and praying (19, 24, 26, 31, 32, 36). In addition, there is a metabolic shift toward the predominant use of fatty acids as fuel for adenosine triphosphate (ATP) synthesis during Ramadan IF and this lowers body fat (25). The optimization of energy reserves during this type of IF may also serve as a possible explanation for this phenomenon. According to this concept, while being effective in preserving protein pools via a reduction in basal metabolism, Ramadan IF also leads to a decreased secretion of anabolic hormones (e.g., insulin) and an increased secretion of catabolic hormones (i.e., adrenaline and glucagon) (53). Interestingly, it has been contended that weight loss resulting from Ramadan IF is also accompanied by reductions in FFM (>35% in normoponderal people and ∼20–30% in those with overweight/obesity) (54). Our meta-analytic findings do not support this contention, revealing no significant effects of Ramadan IF on FFM. From a physiological standpoint, this is a relevant finding because FFM is well-known to play an important role on functional capacity, resting energy expenditure and blood glucose homeostasis (55). However, despite representing an important opportunity for some people to lose body weight and FM, the adaptations inherent to Ramadan IF are typically transient and largely reversible within a small amount of

TABLE 3 | Subgroup analysis assessing the effect of exercise as a moderator of the impact of intermittent fasting on body composition.

Moderators	*K*	Point estimate	CI lower	CI upper	*P*-value	Heterogeneity		
						Q-value	*P*-value	I-squared
Body Weight (kg)*								
All studies								
With exercise	8	−0.127	−0.312	0.059	0.182	5.330	**0.021**	52.046
Without exercise	17	−0.430	−0.609	−0.251	**<0.001**			87.986
Only Ramadan IF studies								
With exercise	8	−0.127	−0.312	0.059	0.182	1.681	0.195	52.046
Without exercise	15	−0.276	−0.407	−0.146	**<0.001**			76.520
Body Weight (kg)**								
All studies								
With exercise	8	−0.386	−0.788	0.016	**0.06**	0.078	0.780	48.868
Without exercise	20	−0.320	−0.557	−0.082	**0.008**			72.681
Only Ramadan IF studies								
With exercise	3	−0.419	−0.775	−0.064	**0.021**	0.457	0.499	0.000
Without exercise	3	−0.193	−0.744	0.357	0.491			0.000
Only non–Ramadan IF studies								
With exercise	5	−0.290	−1.048	0.469	0.454	0.016	0.900	69.912
Without exercise	17	−0.342	−0.603	−0.081	**0.010**			76.994
BMI (kg/m^2)*								
Only Ramadan IF studies								
With exercise	4	−0.037	−0.181	0.106	0.612	4.417	**0.036**	0.000
Without exercise	12	−0.280	−0.456	−0.105	**0.002**			83.232
BMI (kg/m^2)**								
All studies								
With exercise	5	−1.481	−2.581	−0.381	**0.008**	3.550	0.060	80.758
Without exercise	12	−0.396	−0.648	−0.144	**0.002**			61.208
Only Ramadan IF studies								
With exercise	2	−0.572	−1.286	0.142	0.117	0.381	0.537	0.000
Without exercise	1	−0.189	−1.171	0.793	0.706			0.000
Only non-Ramadan IF studies								
With exercise	3	−2.112	−3.634	−0.59	**0.007**	4.660	**0.031**	81.242
Without exercise	11	−0.411	−0.675	−0.147	**0.002**			64.721
FM (kg)*								
All studies								
With exercise	3	−0.552	−1.305	0.200	0.150	1.793	0.181	86.618
Without exercise	10	−1.146	−1.58	−0.712	**<0.001**			94.999
Only Ramadan IF studies								
With exercise	2	−0.093	−0.346	0.161	0.473	3.404	0.065	13.04
Without exercise	6	−0.466	−0.772	−0.161	**0.003**			88.977
Only non-Ramadan IF studies								
With exercise	1	−1.434	−2.117	−0.75	**<0.001**	2.764	0.096	0.000
Without exercise	4	−2.069	−2.376	−1.762	**<0.001**			23.425
FM (kg)**								
All studies								
With exercise	5	−0.779	−1.462	−0.095	**0.026**	1.279	0.258	65.227
Without exercise	19	−0.364	−0.584	−0.145	**0.001**			70.205
Only Ramadan IF studies								
With exercise	1	−0.271	−1.263	0.721	0.593	0.002	0.966	0.000
Without exercise	1	−0.301	−1.286	0.685	0.549			0.000

(Continued)

TABLE 3 | Continued

Moderators	*K*	Point estimate	CI lower	CI upper	*P*-value	Heterogeneity		
						Q-value	*P*-value	I-squared
Only non-Ramadan IF studies								
With exercise	4	−0.897	−1.718	−0.077	**0.032**	1.487	0.223	71.000
Without exercise	18	−0.368	−0.594	−0.142	**0.001**			71.850
FM (%)*								
All studies								
With exercise	9	−0.386	−0.646	−0.125	**0.004**	0.448	0.503	76.645
Without exercise	6	−0.274	−0.470	−0.079	**0.006**			78.206
Only Ramadan IF studies								
With exercise	7	−0.148	−0.267	−0.028	**0.016**	0.573	0.449	0.000
Without exercise	5	−0.236	−0.432	−0.04	**0.018**			79.574
Only non-Ramadan IF studies								
With exercise	2	−1.271	−1.674	−0.869	**<0.001**	2.749	0.097	0.000
Without exercise	1	−0.686	−1.249	−0.124	**0.017**			0.000
FM (%)**								
All studies								
With exercise	5	−0.523	−0.968	−0.078	**0.021**	2.054	0.152	0.000
Without exercise	7	−0.153	−0.394	0.088	0.215			0.000
Only Ramadan IF studies								
With exercise	3	−0.603	−1.172	−0.034	**0.038**	0.235	0.627	7.948
Without exercise	1	−0.321	−1.307	0.665	0.524			0.000
Only non-Ramadan IF studies								
With exercise	2	−0.396	−1.112	0.32	0.278	0.432	0.511	0.000
Without exercise	6	−0.142	−0.391	0.107	0.263			0.000
Fat Free Mass (kg)*								
All studies								
With exercise	7	0.017	−0.101	0.135	0.779	0.178	0.673	0.000
Without exercise	8	−0.054	−0.358	0.251	0.731			88.948
Only Ramadan IF studies								
With exercise	6	0.015	−0.106	0.137	0.806	3.282	0.070	0.000
Without exercise	4	−0.357	−0.742	0.027	0.068			89.896
Only non-Ramadan IF studies								
With exercise	1	0.042	−0.438	0.523	0.862	1.333	0.248	0.000
Without exercise	4	0.346	0.160	0.532	**<0.001**			33.853
Fat Free Mass (kg)**								
All studies								
With exercise	6	0.015	−0.309	0.339	0.928	0.021	0.884	0.000
Without exercise	16	0.043	−0.138	0.223	0.643			48.726
Only Ramadan IF studies								
With exercise	4	−0.234	−0.667	0.198	0.289	0.119	0.731	0.000
Without exercise	1	−0.046	−1.026	0.934	0.927			0.000
Only non-Ramadan IF studies								
With exercise	2	0.337	−0.243	0.918	0.255	0.872	0.350	29.138
Without exercise	15	0.047	−0.140	0.234	0.624			52.128

**Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control; *Body Weight (kg), all studies (k = 25); **Body Weight (kg), all studies (k = 28); *Body Weight (kg), Ramadan IF studies (k = 23); **Body Weight (kg), Ramadan IF studies (k = 6); **Body Weight (kg), non-Ramadan IF studies (k = 22); **BMI, all studies (k = 17); *BMI, Ramadan IF studies (k = 16); **BMI, Ramadan IF studies (k = 3); **BMI, non-Ramadan IF studies (k = 14); *Fat Mass (kg), all studies (k = 13); **Fat Mass (kg), all studies (k = 24); *Fat Mass (kg), Ramadan IF studies (k = 8); **Fat Mass (kg), Ramadan IF studies (k = 2); *Fat Mass (kg), non-Ramadan IF studies (k = 5); **Fat Mass (kg), non-Ramadan IF studies (k = 22); *Fat Mass (%), all studies (k = 15); **Fat Mass (%), all studies (k = 12); *Fat Mass (%), Ramadan IF studies (k = 12); **Fat Mass (%), Ramadan IF studies (k = 4); *Fat Mass (%), non-Ramadan IF studies (k = 3); **Fat Mass (%), non-Ramadan IF studies (k = 2); *Fat Free Mass, all studies (k = 15), **Fat Free Mass, all studies (k = 22); *Fat Free Mass, Ramadan IF studies (k = 10); **Fat Free Mass, Ramadan IF studies (k = 5); *Fat Free Mass, non-Ramadan IF studies (k = 5); **Fat Free Mass, non-Ramadan IF studies (k = 17).*
CI, confidence interval; BMI, body mass index; IF, intermittent fasting. The bold values are statistically significant values.

TABLE 4 | Subgroup analysis assessing the effect of caloric control as a moderator of the impact of intermittent fasting on body composition.

Moderators	*K*	Point estimate	CI lower	CI upper	*P*-value	Heterogeneity		
						Q-value	*P*-value	I-squared
Body Weight (kg)*								
All studies								
With caloric control	2	−1.845	−2.213	−1.477	**<0.001**	67.857	**<0.001**	0.000
Without caloric control	23	−0.234	−0.341	−0.127	**<0.001**			71.636
Body Weight (kg)**								
All studies								
With caloric control	19	−0.295	−0.547	−0.043	**0.022**	0.925	0.336	75.001
Without caloric control	9	−0.474	−0.736	−0.212	**<0.001**			6.854
Only non-Ramadan IF studies								
With caloric control	19	−0.295	−0.547	−0.043	**0.022**	0.630	0.427	75.001
Without caloric control	3	−0.629	−1.415	0.156	0.116			64.495
BMI (kg/m^2)**								
All studies								
With caloric control	12	−0.502	−0.814	−0.190	**0.002**	1.646	0.199	73.887
Without caloric control	5	−1.095	−1.944	−0.245	**0.012**			74.074
Only non-Ramadan IF studies								
With caloric control	12	−0.502	−0.814	−0.190	**0.002**	2.834	0.092	73.887
Without caloric control	5	−2.168	−4.082	−0.254	**0.026**			83.819
FM (kg)*								
All studies								
With caloric control	5	−1.979	−2.351	−1.606	**<0.001**	49.961	**<0.001**	40.138
Without caloric control	8	−0.376	−0.618	−0.135	**0.002**			85.357
FM (kg)**								
All studies								
With caloric control	19	−0.421	−0.655	−0.187	**<0.001**	0.092	0.762	73.632
Without caloric control	5	−0.510	−1.034	0.014	0.057			44.933
Only non-Ramadan IF studies								
With caloric control	10	−0.421	−0.655	−0.187	**<0.001**	0.155	0.694	73.632
Without caloric control	3	−0.593	−1.415	0.229	0.158			67.603
FM (%)*								
All studies								
With caloric control	1	−1.059	−1.659	−0.459	**0.001**	6.002	**0.014**	0.000
Without caloric control	14	−0.287	−0.435	−0.14	**<0.001**			74.042
Only non-Ramadan IF studies								
With caloric control	1	−1.059	−1.659	−0.459	**0.001**	0.003	0.957	0.000
Without caloric control	2	−1.079	−1.47	−0.689	**<0.001**			72.359
FM (%)**								
All studies								
With caloric control	6	−0.176	−0.428	0.077	0.172	0.752	0.386	0.000
Without caloric control	6	−0.382	−0.772	0.009	0.055			0.000
Only non-Ramadan IF studies								
With caloric control	6	−0.176	−0.428	0.077	0.172	0.020	0.888	0.000
Without caloric control	2	−0.127	−0.767	0.514	0.699			0.000
Fat Free Mass (kg)*								
All studies								
With caloric control	4	0.351	0.154	0.548	**<0.001**	13.365	**<0.001**	10.401
Without caloric control	11	−0.137	−0.31	0.036	0.119			74.985
Only non-Ramadan IF studies								
With caloric control	4	0.358	0.173	0.542	**<0.001**	2.521	0.112	10.401

(Continued)

TABLE 4 | Continued

Moderators	*K*	Point estimate	CI lower	CI upper	*P*-value	Heterogeneity		
						Q-value	*P*-value	I-squared
Without caloric control	1	−0.079	−0.586	0.427	0.759			0.000
Fat Free Mass (kg)**								
All studies								
With caloric control	15	0.090	−0.105	0.286	0.365	1.594	0.207	56.342
Without caloric control	7	−0.144	−0.45	0.162	0.357			0.000
Only non-Ramadan IF studies								
With caloric control	15	0.090	−0.105	0.286	0.365	0.297	0.586	56.342
Without caloric control	2	−0.055	−0.538	0.429	0.824			0.000

**Pre-post tests, IF intervention; **Pre-post tests, IF intervention vs. control; *Body Weight (kg), all studies (k = 25); **Body Weight (kg), all studies (k = 28); **Body Weight (kg), non-Ramadan IF studies (k = 22); **BMI, all studies (k = 17); **BMI, non-Ramadan IF studies (k = 14); *Fat Mass (kg), all studies (k = 13); **Fat Mass (kg), all studies (k = 24); **Fat Mass (kg), non-Ramadan IF studies (k = 22); *Fat Mass (%), all studies (k = 15); **Fat Mass (%), all studies (k = 12); *Fat Mass (%), non-Ramadan IF studies (k = 3); **Fat Mass (%), non-Ramadan IF studies (k = 8); *Fat Free Mass, all studies (k = 15), **Fat Free Mass, all studies (k = 22); *Fat Free Mass, non-Ramadan IF studies (k = 5); **Fat Free Mass, non-Ramadan IF studies (k = 17).*

CI, confidence interval; BMI, body mass index; IF, intermittent fasting. The bold values are statistically significant values.

time (26). Thus, the prescription of maintenance strategies at termination of Ramadan is of extreme value for the long-term preservation of healthy body composition.

Caloric restriction and different forms of fasting are well-known to exert a powerful physiological impact on humans, namely on health status and body composition (5). According to our analyses, more than half of the studies (pre- post-intervention within-subjects design) examining the effects of non-Ramadan IF on FFM revealed small, but significant improvements in FFM. TRF was shown to be particularly effective in inducing slight increases in FFM [e.g., (17, 56)]. In fact, it has been argued that, when combined with resistance training, TRF has an adjunctive role in preserving or delaying possible FFM losses (3, 57). Moreover, fasting triggers numerous endocrine responses that may well contribute for heightened metabolic rate and preserved FFM (e.g., increased serum levels of noradrenaline and growth hormone, respectively) (58). In addition, exercising while fasting increases the expression of sirtuin 1 (SIRT1) as well as the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), both of which have numerous effects on gene expression (i.e., regulation of mitochondrial biogenesis) (59). Yet, it has been disputed whether the interaction between TRF and gains in FFM might be mediated by the amount of dietary protein intake, together with the final energy balance achieved on a daily basis (3, 60). This is partially supported by our moderation analyses because, when calorie consumption was taken into consideration, a significant effect of IF on FFM emerged. In addition, when comparing pre- post-intervention data between treatment and control groups, the beneficial effects of IF on decreasing BMI were more pronounced after accounting for exercise training. Non-Ramadan IF also led to significant reductions in body weight and absolute FM when comparing pre-post-intervention data between treatment and control groups, as well as when comparing pre- post-intervention on within-subjects designs. In fact, studies specifically designed to test the effects of IF on body composition have consistently shown that different approaches of IF (i.e., ADF, IER, and TRF) are well-suited for managing successful losses in body weight and FM (2, 10, 13, 61). One possible explanation is that 12 h of fasting generate a lipolytic-prone state that is compatible with decreased fat storage and enhanced fatty acid mobilization, together with ketogenesis (62). Moreover, in the short term, non-Ramadan IF (i.e., ADF protocol 5:2) appears to be superior (due to higher compliance) than CER for reducing FM (61).

According to the present beliefs, Ramadan IF is more effective for weight loss than non-Ramadan IF due to a greater loss of body water resulting from fluid deprivation (53). Although the opposite was found when considering our meta-analytical results involving pre- post-intervention analyses (within-subjects designs) (i.e., higher effects derived from non-Ramadan IF), similar results were observed for Ramadan vs. non-Ramadan IF interventions when comparing treatments and controls. However, there are clear differences in what concerns to absolute FM, with non-Ramadan IF showing greater improvements in this specific parameter, therefore being unequivocally linked to improved body composition.

This study has some limitations. First, our findings should be interpreted with caution because analyses were carried out on different populations. Second, since we did not include individuals from all ages, ethnicities, religions, geographical areas and educational backgrounds, our findings cannot be extrapolated to the general population. Third, the degree of calorie restriction varied between studies and the non-fasting control groups also ranged from "normal diet" to CER. Fourth, the nutritional profile of foods consumed during the *ad libitum* feeding period should be further monitored. Fifth, the analyses of body composition were made with different techniques and, due to the limited number of studies per technique, it was not possible to conduct moderation analyses. Bioimpedance, a technique that assumes individual euhydration, was used in many studies. Since Ramadan IF is typically accompanied by dehydration, in this specific context, the assessments of body composition using

this method may be affected. The preferable use of gold standard techniques, such as the four-component model or even the single use of dual x-ray absorptiometry (only 16 studies here included used this method) in future research is fundamental. This approach would greatly reduce the possibility of measurement inaccuracies. Finally, it would have been important to discriminate the moderating effects of different types of exercise (e.g., endurance vs. resistance training). However, the low number of studies on each specific type of exercise precluded us from conducting separate moderation analyses. Instead, all exercise studies were compiled and used as a single moderator.

In summary, our meta-analytic findings revealed a negative significant pooled estimate effect of both Ramadan and non-Ramadan IF on FM and body weight. However, we found that decreases in FM are more pronounced with non-Ramadan IF and this type of IF, combined with exercise training, leads to higher decreases in BMI. Our data also provide preliminary evidence that non-Ramadan IF may be well-suited for eliciting small increases in FFM, particularly under circumstances involving the simultaneous control of caloric intake. Thus, although Ramadan IF certainly implicates some beneficial adaptations in weight management, non-Ramadan IF appears to be more effective in improving overall body composition (particularly, if combined with exercise training and controlled calorie intake), despite the fact it does not imply dehydration.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JC, IS, GM, PP-C, and AS designed the research and wrote the paper. JC, IS, and GM conducted the research, analyzed the data, and performed the statistical analysis. All authors had equal responsibility for the final content of the paper, read, and agreed to the published version of the manuscript.

FUNDING

This work was partly supported by the Fundação para a Ciência e Tecnologia, under Grant UIDB/00447/2020 to CIPER—Centro Interdisciplinar para o Estudo da Performance Humana (unit 447) and by Universidade de Lisboa (grant attributed to JC-n C00246e).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.625240/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

REVIEW
published: 08 March 2021
doi: 10.3389/fnut.2021.627289

Is There an Exercise-Intensity Threshold Capable of Avoiding the Leaky Gut?

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Endurance-sport athletes have a high incidence of gastrointestinal disorders, compromising performance and impacting overall health status. An increase in several proinflammatory cytokines and proteins (LPS, I-FABP, IL-6, IL-1β, TNF-α, IFN-γ, C-reactive protein) has been observed in ultramarathoners and triathlon athletes. One of the most common effects of this type of physical activity is the increase in intestinal permeability, known as leaky gut. The intestinal mucosa's degradation can be identified and analyzed by a series of molecular biomarkers, including the lactulose/rhamnose ratio, occludin and claudin (tight junctions), lipopolysaccharides, and I-FABP. Identifying the molecular mechanisms involved in the induction of leaky gut by physical exercise can assist in the determination of safe exercise thresholds for the preservation of the gastrointestinal tract. It was recently shown that 60 min of vigorous endurance training at 70% of the maximum work capacity led to the characteristic responses of leaky gut. It is believed that other factors may contribute to this effect, such as altitude, environmental temperature, fluid restriction, age and trainability. On the other hand, moderate physical training and dietary interventions such as probiotics and prebiotics can improve intestinal health and gut microbiota composition. This review seeks to discuss the molecular mechanisms involved in the intestinal mucosa's adaptation and response to exercise and discuss the role of the intestinal microbiota in mitigating these effects.

Keywords: leaky gut, exercise threshold, gastrointestinal disorder, gut microbiota, gut injury

OPEN ACCESS

Edited by:
Karsten Koehler, Technical University of Munich, Germany

Reviewed by:
Kaatje Lenaerts, Maastricht University, Netherlands
Jonathan Peake, Queensland University of Technology, Australia

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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *08 November 2020*
Accepted: *10 February 2021*
Published: *08 March 2021*

Citation:
Ribeiro FM, Petriz B, Marques G, Kamilla LH and Franco OL (2021) Is There an Exercise-Intensity Threshold Capable of Avoiding the Leaky Gut? Front. Nutr. 8:627289. doi: 10.3389/fnut.2021.627289

INTRODUCTION

Physical exercise is a non-pharmacologic agent in preventing and managing non-communicable chronic diseases, where its beneficial effect is well-documented in the musculoskeletal and cardiovascular systems. In addition to these systems, physical exercise also promotes positive adaptations in the gastrointestinal tract, such as a decrease in colon cancer risk (1). However, exacerbated exposure to exercise stress and even moderate-intensity training (depending on volume, environment and age) may negatively impact the gastrointestinal environment, contributing to the worsening of other clinical conditions (2–4). In this context, the array of

normal physiological responses to exercise that disturb and affect gastrointestinal integrity and function was dubbed "exercise-induced gastrointestinal syndrome," estimated to present a 70% of the maximum work capacity prevalence among endurance athletes (3).

Exercise-induced gastrointestinal syndrome results from two distinct and communicable pathways: The circulatory-gastrointestinal pathway and the neuroendocrine-gastrointestinal pathway. The first pathway redistributes blood flow to working muscles and peripheral circulation, reducing total splanchnic perfusion, while the neuroendocrine-gastrointestinal pathway is related to the increase in sympathetic activation and the consequent reduction in the gastrointestinal functional capacity (5, 6). Thus, it is believed that intestinal ischemia is considered the leading cause of abdominal pain, nausea, vomiting, and diarrhea (and bloody diarrhea), occurring 2-fold more in running athletes compared to other endurance sports (e.g., cycling or swimming), and 1.5–3 times more in elite athletes compared to amateurs (7). Nevertheless, both pathways lead to gastrointestinal symptoms with acute or chronic health complications (8).

Strenuous exercise's negative effects ($\geq$60–70% VO_{2max}) may not be limited to the gastrointestinal system and the intestinal microbiota, affecting its structure and functionality. Deterioration of the gastrointestinal mucosal barrier may also occur, increasing its permeability to bacterial endotoxins, and low-grade systemic inflammation may not only affect gastrointestinal homeostasis but also overall health (9, 10). However, not every type of physical exercise negatively affects the gut microbiota; on the contrary, there is compelling evidence that exercise has positive effects on the colon, increasing the microbiota's diversity and increasing butyrate-producing bacteria as well as butyrate concentration (9).

Despite that, exercise varieties and their dynamics of intensity and volume have not yet been widely studied to establish the ideal dose-response ratio of exercise to its protective or restorative effect on the gastrointestinal tract (11). To this end, the present bibliographic review aimed to (1) report the molecular and physiological changes in intestinal permeability caused by exercise (2) describe whether it is currently possible to determine an exercise "threshold" to avoid the leaky gut phenomenon and the factors involved in this process and (3) mention the main factors that contribute to minimizing the occurrence of intestinal injury. For this, a search strategy was used focusing on exercise and intestinal permeability, as well as the factors that influence this process.

SEARCH STRATEGY

The following search strategy was carried out by searching for full-text articles indexed in Pubmed. The terms used for the search were: "exercise AND intestinal permeability"; "exercise AND intestinal injury"; "exercise AND leaky gut"; "exercise AND gut microbiota." All individual terms were used to assess related topics on exercise and intestinal permeability and the other factors that boost this relationship.

Gastrointestinal Physiological and Molecular Adaptations to Exercise

The intestinal environment is a complex of different cells, acting together to generate motility, digestion, absorption, and secretion, as shown in **Figure 1**. Above the intestinal epithelial cells (IECs) and in contact with the intestinal lumen, a mucus layer contains the intestinal microbiota, composed of trillions of microorganisms with metabolic, immunological, and physiological roles in symbiosis with the host. Different IECs exist in the intestine's innermost layer, such as enterocytes, Paneth cells, goblet cells, enterocytes, and microfold cells, each with a distinct function. In general, these cells protect the IECs by creating a barrier with narrow spaces between them and secreting mucus and various antimicrobial agents to defend the epithelial layer. In addition, a covering layer of connective tissue known as the *lamina propria* is responsible for establishing molecular communication between the microbiota and the immune cells. The last layer comprises smooth muscle, regulated by interstitial cells; this layer is responsible for intestinal motility (12). The myenteric and submucosal plexuses form the enteric nervous system and are responsible for regulating the local bloodstream and intestinal secretions (13). Thus, physiological responses to exercise are changes in a large group of cells (14), in addition to modulations in the intestinal microbiota (15).

It is well-known that physical exercise leads to an increase in the skeletal muscle's energy demand and the organism's adaptation to supply this demand. Through this stimulus, the sympathetic nervous system's activity alters hemodynamics, reducing and redistributing the blood flow from vital organs to the exercising muscles. It has been shown that the decrease in splanchnic blood flow occurs at around 70–80% of the maximum oxygen consumption (VO_{2max}) during exercise (5, 16). Thus, the type of exercise and its intensity can promote changes in the gastrointestinal system through its hypoxic effect.

Local intestinal ischemia is one of the main characteristics of vigorous endurance (17). This is one of the main physiological factors that cause cell damage and disorders, due to a reduction in adenosine triphosphate (ATP) synthesis in mitochondrial respiration (18, 19). Splanchnic hypoperfusion and subsequent ischemia can damage the specialized antimicrobial protein-secreting cells (Paneth cells), the mucus-producing cells (such as goblet cells), and the tight junction proteins (claudin and occludin) that prevent the infiltration of pathogenic organisms into the systemic circulation (8). Thus, endotoxins such as lipopolysaccharide (LPS) and proinflammatory cytokines may pass through epithelial cells due to their permeability, an effect known as "leaky gut" (20, 21). This phenomenon may explain, in part, the impaired absorption of intestinal nutrients observed after strenuous exercise (22).

An increase in sympathetic system stimuli can also lead to subsequent alterations in intestinal motility and absorption capacity (8, 23). This malabsorption is observed in endurance running, and it is not yet known whether it is due to local ischemia or down-regulated intestinal transporter activity, or a combination of both (22, 24). Together, the above exercise-related responses are associated with lower-gastrointestinal symptoms such as flatulence, lower-abdominal bloating, urge to

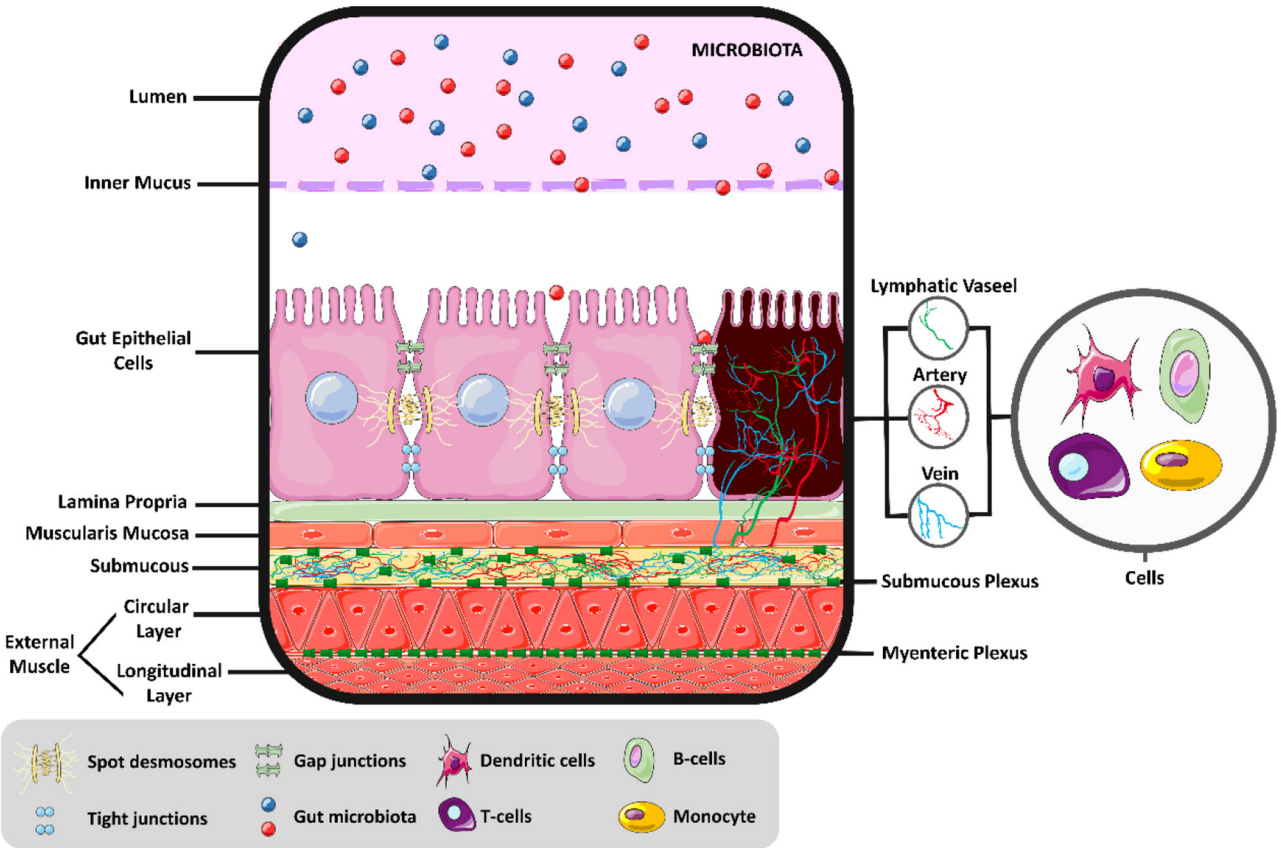

FIGURE 1 | Intestinal molecular environment. Intestinal health and the permeability balance depend on the homeostasis of the intestinal environment.

defecate, abdominal pain, abnormal defecation, such as diarrhea, and bloody stools (8, 14, 17, 22).

From a molecular perspective, the Caco-2 TJ permeability induced by the increase of IL-1β is regulated by synthesis and increased transcription of MLCK mRNA (25, 26). The IL-1β causes a rapid increase in mitogen-activated protein kinase kinase kinase 1 (MEKK1), and this plays an important role in the regulation of a variety of biological activities in intestinal epithelial cells (27). Further, the MLCK activation pathway appears to be an essential molecular issue in TJ regulation and intestinal permeability (26, 28, 29). Similarly, the increase in permeability occurs with the increase of tumor necrosis-alpha (TNF-α) (30). Thus, physical exercise can increase intestinal permeability due to the increased expression of these molecules caused by physiological changes in exercise.

Strenuous exercise may affect the intestinal epithelial cells (31), tight junction (TJs) proteins (32), smooth muscle cells (33), and the composition and function of the gut microbiota (GM) (34), compromising gastrointestinal homeostasis. This phenomenon has been observed in ultramarathon athletes, where the profile of proinflammatory proteins and cytokines such as C-reactive protein, interleukin-6 (IL-6), IL-1β, TNF-α, and interferon-gamma (IFN-γ) increased (20). Similarly, LPS, IL-6, and C-reactive protein levels also increase in other types of ultra-endurance exercise (e.g., ∼8 h of triathlon) (35). Apparently, the increase in intestinal permeability caused by strenuous exercise seems to coincide with the gut microbiota changes (36). The molecular and tissue changes in the intestine caused by exercise are shown in **Figure 2**.

Strenuous exercise is also known to induce the synthesis of enterocyte-derived intestinal fatty-acid binding protein (I-FABP), an intestinal biomarker of enterocyte damage and ischemia (8). The increased release of I-FABP into circulation indicates damage to mature enterocytes, and is observed after prolonged exercises (≥1 h) and after shorter periods of resistance training (30 min) (8). Besides these factors, hyperthermia (>40°) and acute local ischemia are exercise-related factors that are known to disturb the tight junctions, increasing intestinal permeability (31, 32).

The increase in intestinal permeability also allows LPS to pass into the bloodstream. This increase in the concentration of LPS in the blood occurs in exercise with short duration (<20 min) (37), long (>1 h) duration (38, 39), and performed in a hot environment (40, 41). However, there is evidence that moderate exercise can decrease circulating LPS concentrations (42). These data show a similarity between the increases in circulating LPS and I-FABP, as well as the increase in proinflammatory cytokines.

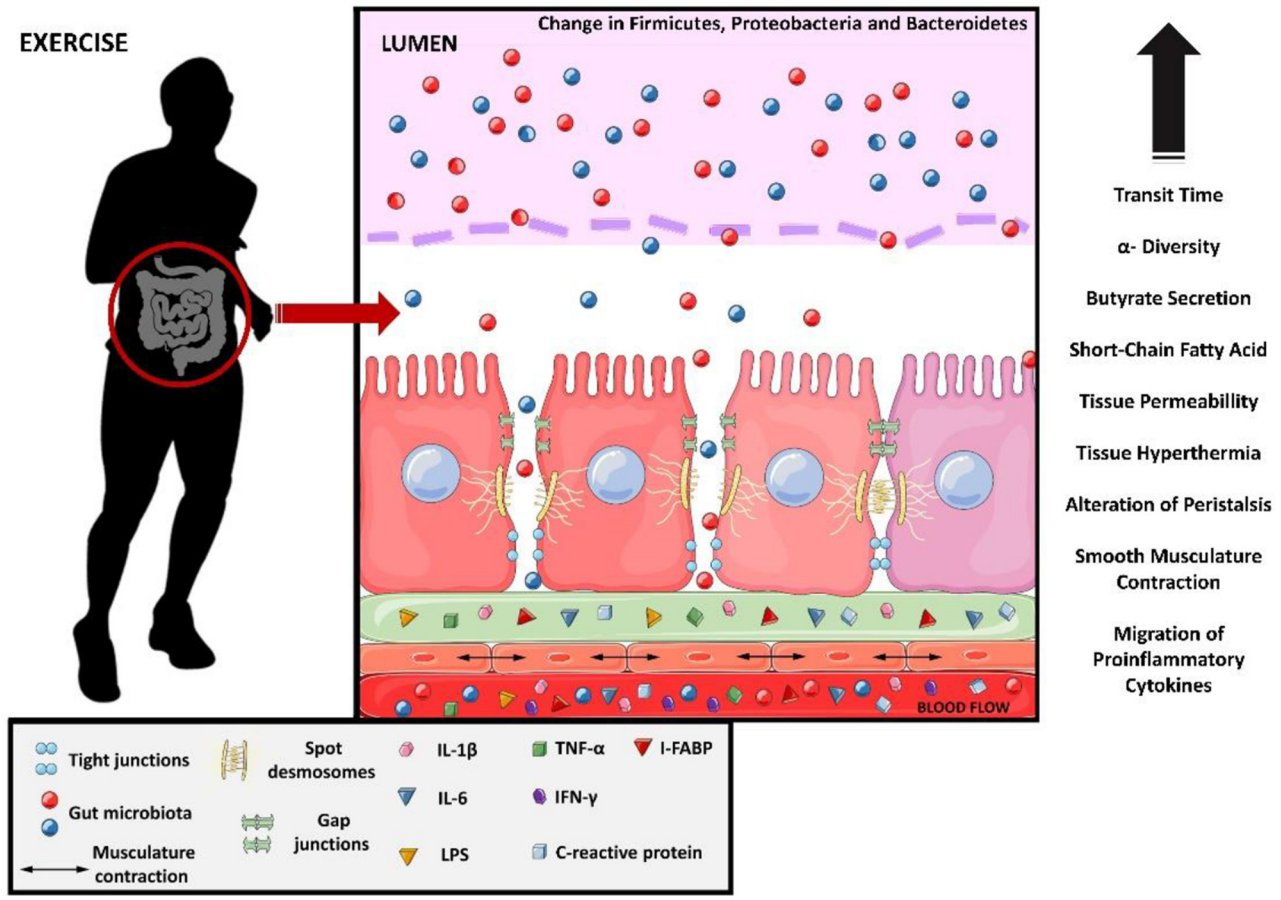

FIGURE 2 | Molecular changes from exercise causing leaky gut. Interleukin 1 beta, IL-1B; interleukin 6, IL-6; lipopolysaccharides, LPS; tumor necrosis factor-alpha, TNF-alfa; interferon gamma, IFN-y and intestinal fatty-acid binding protein, I-FABP.

After exploring the main molecular changes caused by exercise, the next topic aims to highlight whether it is possible to determine an exercise "threshold" that leads to the "leaky gut" phenomenon.

A Possible Exercise "Treshold" to Avoid Leaky Gut

While low-to-moderate intensity is associated with positive effects on the gastrointestinal tract, including mucosa preservation and improved intestinal motility, ischemia and hypoperfusion associated with strenuous exercise are commonly associated with reduced gastric motility, epithelial injury, disturbed mucosa integrity, enhanced permeability, impaired nutrient absorption, and endotoxemia with local and systemic low-grade inflammation (8) (**Figure 2**). It is therefore essential to identify the appropriate exercise dose-response or safe thresholds that do not generate these adverse effects or even act as a recovery agent for the intestinal mucosa.

Naturally, it should be noted that different exercise stimuli may lead to adverse impacts on the intestine, also considering their intensity and duration, and the environmental conditions in which they take place. It is known that high altitudes can have adverse effects on the small intestine (43, 44) and that high temperatures (hyperthermia) induced by intense exercise may lead to gut ischemia (45). Also, variations in physical training such as intensity, volume, continuity (alternation between increasing stresses and the proportional recovery period), training time (46) and fluid restriction during exercise are determinant factors that may contribute to leaky gut (8, 47, 48). Finally, the impact of exercise on the intestinal microbiota (IM) composition must be considered, as the IM is a crucial component for maintaining the gastrointestinal mucosa's integrity.

The increase in intestinal permeability has already been identified in several types of exercise: cycling (49), swimming (50), and running (51, 52). Although there is still no comparison between the types of exercise and the leaky gut, apparently the determining factors for the increase in permeability are the intensity and volume of training. The assessment of mucosal-injury induced by exercise is often done by a dual-sugar test with lactulose and rhamnose (L/R ratio's) or claudin-3 concentrations for analysis of the small intestine and the analysis of I-FABP

concentration as an intestinal biomarker of epithelial injury (5), as shown in **Table 1**. These studies show that ≥70% of maximum working capacity and with a volume >1 h can lead to an increase in intestinal permeability. However, as shown in **Table 1**, several factors can increase or minimize the permeability: temperature, food during the training process, fluid restriction and training at different times of the day.

It has been evidenced that 60 min of running exercise at an 80% VO_{2Peak} leads to an enhanced lactulose/rhamnose ratio, compared to lower intensities of 40 and 60% of the VO_{2Peak} (48). Furthermore, trained individuals submitted to a fluid restriction protocol (glucose or sweetened water) and 60 min of exercise at 70% of VO_{2max} presented an enhanced lactulose/rhamnose ratio, indicating that dehydration may increase intestinal permeability (47). On the other hand, exercise-induced hyperthermia has been one of the leading hypotheses for increasing intestinal permeability and exercise-induced endotoxemia (65). Healthy people who trained for 60 min at 70% of the VO_{2max} in hot environments [33°C, 50% relative humidity (rH)] and cold (22°C, 62% rH), led to the same alteration in intestinal permeability compared to control (same claudin-3 alterations). The hot environment group had a significant increase in blood LPS, indicating the effect of exercise-induced endotoxemia (60).

Similarly, 60 min of running and cycling at a moderate intensity led to an increased concentration of I-FABP (6, 55, 56), with the highest concentration seen in hot environments (30°C) (56). It was recently identified that 45 min of cycling at an intensity of 70% of VO_{2max} at different temperatures (30° or 20°) raised I-FABP levels in a similar way (59). Thus, the effect of temperature and endurance training on I-FABP is still unclear, due to methodological differences in their analysis (53). Besides, several dietary interventions can influence I-FABP concentrations in the context of physical exercise (58, 62, 66). For example, sucrose supplementation may alleviate the concentration of circulating I-FABP elevated by exercise (49). Thus, great caution is needed when analyzing the relationship between physical exercise and serum levels of I-FABP to presume an intestinal injury.

Although the above studies have shown that 60 min at an intensity at 70% of VO_{2max} are related to an increase in intestinal permeability, the athlete's training level must be considered. It has been previously reported that local ischemia and hyperthermia are the main factors for leaky gut. The progressive increase in catecholamines by vigorous endurance exercise is one of the main signs of this gastrointestinal ischemia (67). In this sense, catecholamine levels tend to rise above the lactate threshold, on average, in a range of 60–80% of VO_{2max}, where lactate is accumulated. Endurance-trained, sprint-trained, and weightlifter-trained athletes tend to have higher catecholamine concentrations at rest than inactive subjects (68). Endurance athletes also tend to have a rise in post-exercise adrenaline concentrations comparable to untrained subjects, even working at the same relative training level (69). This suggests that local intestinal ischemia should still be investigated in groups with different levels of training.

After 30 min of local intestinal ischemia, the circulating concentration of the L/R ratio is increased, but after 120 min of reperfusion, there are no changes (70). I-FABP concentrations are observed to be similar at the same times. There is evidence that only 60 min of reperfusion is capable of resealing the epithelial barrier and that remnants of removed apoptotic epithelial cells have been observed in the lumen (71). An acute bout of high-intensity interval training (HIIT) (eighteen 400-m runs at 120% maximal oxygen uptake) can increase permeability (increase in L/R ratio's and I-FABP) despite not experiencing symptoms (52). However, although acute exercise generates an increase in permeability, it has been hypothesized that chronic training may enhance gut barrier integrity overall through several mechanisms (72). Thus, it is not known how much physical training can damage the intestine, and the comparison between the acute and chronic effects of training on the intestinal injury still needs to be explored.

Low-to-moderate exercise (30–60% of maximum oxygen consumption, VO_{2max}) accelerates gastric emptying and may decrease the risk for Gastroesophageal Reflux Disease (GERD) (73). It was shown that moderate aerobic training improved gastrointestinal motility after 12 weeks of training (74), reducing transient stool time, which benefits the host by decreasing pathogens' contact with the gastrointestinal mucus layer (75). A similar effect on gut transit was observed after 1 week of running or cycling at a moderate intensity (50% of VO_{2max}) (76). Even an acute bout of swimming exercise increased the ileum's contractile reactivity in an animal model (77). These observations demonstrate the intestinal mucosa's sensitivity to physical exercise and its most diverse manifestations; however, exercise-induced gastrointestinal syndrome has been more associated with strenuous exercise.

The studies revealed that variations in the intensity, volume, and/or training time of exercise training make it difficult to unify the relationship between physical training and leaky gut. There is some evidence that vigorous endurance training (≥60 min and ≥70% of maximum work capacity) may lead to injury and increased intestinal permeability. Depending on variables such as temperature, moderate to prolonged exercise (>60 min) can also lead to intestinal injury, based on elevations in the circulating I-FABP (56). It is still uncertain what the acute and chronic effects of exercise are on intestinal injury. Moreover, high altitude and dehydration also increase intestinal damage and intestinal permeability. It is worth mentioning that exercise performed above 70% of the maximum work capacity can generate benefits in other organs, such as a greater and faster increase in VO_{2max} or a greater decrease in total fat mass (78, 79). Thus, it is difficult to determine a "threshold" of exercise to avoid leaky gut. Although intensities over ≥70% of maximum work capacity and a duration of ≥60 min is an approximate parameter, several variables can act in the intestinal environment, and this possible "threshold" becomes variable. Therefore, the emergence of new studies with a focus on determining the "threshold" is extremely important for active people to have a safe training parameter aimed at intestinal health.

TABLE 1 | Changes in intestinal permeability caused by exercise and the influencing factors.

Subjects	Exercise type	Exercise intensity	Exercise volume	Contribution influence factor	Minimization influence factor	Change in permeability	References
Endurance trained M and W ($n = 7$)	Acute running	70% of VO_{2max}	60 min	30°C T_{amb} (12 to 20% RH)	At 20 min of exercise: 27 g of Cho	Increase in I-FABP by exercise and decreased hours after exercise in the Cho group	(53)
Recreationally trained M ($n = 12$)	Resistance-type exercise (combined cycling with a leg press)	Load progression of 40–55–70% between sets	30 min	–	–	Increase in I-FABP by exercise	(54)
Competitive cyclists M and W ($n = 13$)	Acute cycling	70% W_{max} + Time trial	45 min of 70% W_{max} + 15 min of time trial	7 days of gluten-containing diet	7 days of gluten-free diet	Increase in I-FABP after 15 min time trial (no difference by diet)	(55)
Recreationally trained M ($n = 8$)	Acute running and cycling	Cycling at 50% HRR + running at 80% HRR + maximal-distance trial) + cycling at 50% HRR, respectively	15 (cycling)-30 (running)-30 (maximal running)-15 min (cycling), respectively	30°C T_{amb} (50% RH)	1.7 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ of bovine colostrum (COL) supplementation	Increase in I-FABP by exercise (no difference by diet). This increase was greater with 6 training sessions per wk than 3 sessions	(56)
Active runners ($n = 20$)	Running	70% of VO_{2max}	60 min	–	–	Increase in I-FABP by exercise	(6)
cyclists and triathletes M ($n = 9$)	Acute cycling	70% W_{max}	60 min	400 mg ibuprofen intake before cycling	–	Increase in I-FABP by exercise and ibuprofen	(57)
Endurance trained M ($n = 8$)	5 consecutive days of Running	78% of VO_{2max} (4 mMol/L blood lactate) until T_c increases 2.0°C or volitional exhaustion	Volitional exhaustion = 24 min	T_{amb} 40°C (40% RH)	–	Increase in I-FABP by exercise in the heat. This increase was decreased from the 1° to the 5° day of exercise	(41)
Well-trained athletes M ($n = 16$)	Acute cycling	70% W_{max}	60 min	–	Acute ingestion of sodium nitrate (NIT; 800 mg NO_3), sucrose (SUC; 40 g) or water (Placebo)	Increase in I-FABP by during exercise and post-exercise. I-FABP was attenuated in SUC vs. PLA	(49)
Endurance runners M and W ($n = 25$)	Running	60% of VO_{2max}	2 h	–	Gel-disks containing 30 g carbohydrates (2:1 glucose-fructose, 10% w/v) every 20 min	Increase in I-FABP by exercise (no difference by supplementation)	(22)
Healthy M ($n = 12$)	Acute running	70% of VO_{2peak}	60 min	–	14 days of 20 g/day supplementation with *bovine colostrum* (Col)	Increase in I-FABP by exercise. I-FABP attenuated by Col supplementation post-exercise	(58)
Health M ($n = 12$)	Acute cycling	70% of VO_{2max}	45 min	T_{amb} 30°C (40% RH)	T_{amb} 20°C (40% RH)	Increase in I-FABP by exercise (no difference by temperatures groups)	(59)
Endurance runners ($n = 16$)	Running	60% of VO_{2max}	3 h	Training at night (21:00 h)	Training in the morning (09:00 h)	Increase in I-FABP by exercise (both trials). Night resulted in greater total-gastrointestinal symptoms	(46)
Active M and W ($n = 15$)	Running	70% of VO_{2max}	60 min	T_{amb} 33°C (50% RH)	–	Increase in plasma claudin by exercise	(60)
Triathletes ($n = 15$)	Swimming, cycling, and mountain running	1,500-m swimming, 36-km cycling, and 10-km mountain running	–	–	0.7 ± 0.3 L of water and 1.5 ± 0.5 L of isotonic drinks	Increase in plasma zonulin by exercise	(50)

(Continued)

TABLE 1 | Continued

Subjects	Exercise type	Exercise intensity	Exercise volume	Contribution influence factor	Minimization influence factor	Change in permeability	References
Active runners ($n = 17$)	Acute running	80% of the speed of their best 10 km race time.	90 min	Runners with history of experiencing GI symptoms during running (symptomatic group)	–	Increase of L/R ratios, I-FABP and zonulin after exercise. No difference between asymptomatic and symptomatic group	(51)
Endurance runners M and W ($n = 7$)	Running	60% of VO_{2max}	3 x of 2 h	T_{amb} 35°C (50% RH) - Exertional heat stress (EHS)	15 g glucose (GLUC) or energy-matched whey protein hydrolysate (WPH)	GLUC and WPH minimized I-FABP and L/R ratios	(61)
Trained runners M ($n = 7$)	High-intensity interval running	120% of VO_{2max} with 18 × 400 m interval efforts	Separated by 3 min of complete rest	–	–	Increase of L/R ratios and I-FABP after exercise	(52)
Healthy M ($n = 12$)	Running	80% of VO_{2max}	20 min	–	20 g/day bovine colostrum (14 days)	Increase of L/R ratios by exercise and attenuated by colostrum supplementation	(62)
M and W endurance runners ($n = 20$)	Running	70% of VO_{2max}	60 min	Fluid restriction	4% glucose solution	Increase of L/R ratios by exercise + fluid restriction	(47)
Active M and W ($n = 6$)	Running	40–60–80% VO_{2peak}	60 min	–	–	Increase of L/R ratios by 80% VO_{2peak} compared to other intensities	(48)
marathon runners M and W ($n = 15$)	Acute running	Road marathon competition	2 h 43 min to 5 h 28 min	–	Vitamin E (1,000 IU daily)	Increase of L/R ratios by exercise (no difference by supplementation)	(63)
Soldiers M ($n = 73$)	4-day cross-country ski march	51 km cross-country ski-march while 139 carrying a ∼45 kg pack	50:10 min work-to-rest ratios	–	–	Increase of L/R ratios by exercise	(36)
Endurance trained M and W ($n = 7$)	Acute running	65–70% of VO_{2max}	60 min	T_{amb} 30°C (12–20% RH)	Oral glutamine supplementation (0.9 g/kg) for 7 days	Increase of L/R ratios by exercise and decreased with glutamine supplementation	(64)

I-FABP, intestinal fatty-acid binding protein; HRR, heart rate reserve; L/R ratios, Men, M; dual-sugar test with lactulose and rhamnose; Post-exercise (or peak) core temperature (T_c), RH, relative humidity; T_{amb}, ambient temperature; VO_{2max}, maximum oxygen consumption, W, women; W_{max}, watt maximum; wk, week.

Exercise as a Restorative Agent of the Gastrointestinal Environment

The gut microbiota's responsiveness to external factors has received much attention in recent years due to these changes' clinical potential effects on the host's health. Among these factors, dietary intervention and physical exercise are recurrent elements in studies involving the GM's composition and its systemic impacts across different tissues and physiologic systems (80). Naturally, adequate eating habits and physical activity are two external factors that receive much attention from the scientific community due to their role in preventing diseases and maintaining health (81).

As previously described, prolonged and excessive exercise stimuli may affect the gastrointestinal environment, impacting the mucosa's integrity and increasing its permeability to external agents such as endotoxins. This process is associated with the onset of proinflammatory signaling, affecting gastrointestinal health. Dehydration, bloody diarrhea episodes, and abdominal discomfort are typical responses in endurance athletes (17). These effects are also expected to compromise sports performance and affect overall health (39, 82). As a result, several strategies have been considered to restore the gastrointestinal mucosa by modulating the gut microbiota. To date, the mutual interaction among exercise, dietary supplementation, and gut microbiota is speculated to be a key strategy to reduce the effects of gastrointestinal distress caused by strenuous exercise and even a game-changer concerning sports performance.

Unlike what is observed in response to strenuous exercise stimuli, certain intensities positively modify the GM's quality and function, favoring the host's health. In this way, a body of evidence has shown that exercise is a potent modulator of intestinal microbiota composition and function, leading to enrichment and bacterial proliferation, improvement of intestinal barrier integrity, and the synthesis of immunomodulatory and antimicrobial agents (83). Moderate endurance exercise has been associated with preserving the intestinal mucosa and the upregulation of β-defensin 1, α-defensin 5, regenerating gene Type IIIb (Reg IIIb), and Reg IIIc (84). The defensins and the Reg 3 family are proteins with antimicrobial actions that act as barriers, protecting body surfaces against microorganisms (85, 86). This exercise intensity was also shown to reduce irritable bowel syndrome (80) effectively, which is a condition often observed and underdiagnosed in endurance athletes (87).

Recent research on the GM's response to exercise, especially endurance, has shed light on the cross-talk between skeletal muscle and the GM, and its influence on muscle bioenergetics. In the gastrointestinal tract, some of these effects include the proliferation and stimuli of intestinal microbes and the synthesis of microbe-metabolites (88). Among these metabolites, the short-chain fatty acids (formate, acetate, propionate, and butyrate) significantly impact human metabolism and protect the gut mucosa (89). In this matter, an injection of gastric and intestinal SCFAs can lead to increased mRNA abundance of Occludin and Claudin-1 (TJs), decreasing the mRNA and protein abundances of IL-1β in the colon, and diminishing infiltration of neutrophils to the gut *lamina propria* (90, 91). Thus, the hypothesis arises that exercise changes may increase SCFAs, similarly to the direct injection of these metabolites.

Studies with humans have shown that cardiovascular capacity is positively correlated with increased bacterial diversity and SCFAs producing bacteria (92). However, some of these effects might depend on body composition (93). In this study, endurance exercise altered the gut microbiota in lean and obese subjects; however, the production of microbe-SCFAs (acetate, propionate, and butyrate) was enhanced only in the lean group. Together, these studies establish new clinical perspectives for manipulating the GM and novel insights on the cross-talk between gut microbes and their metabolites and the skeletal muscle, especially concerning the host metabolism and exercise capacity regulation.

The GM interacts with the intestinal immune function by activating G protein-coupled receptor (GPR41 and GPR43) and histone activation deacetylases (HDAC) in leucocyte endothelial cells. SCFAs can bind to Gpr43 (SCFA-Gpr43 signaling) and reduce inflammatory responses of neutrophils and eosinophils and be capable of inhibiting HDAC, preventing colorectal cancer (94, 95). In this context, moderate-to-vigorous physical training for only 6 weeks can increase fecal SCFAs and possibly activate the molecular pathways mentioned above, although these pathways have not yet been clinically explored in the context of exercise (93). This is one explanation for why exercise can prevent and treat colorectal cancer (1, 96).

The transplantation of fecal microbiota containing *Veillonella atypica* isolated from a marathon runner was shown to increase the submaximal running time to exhaustion on mice. Considering that *Veillonella atypica* metabolizes lactate into propionate and acetate through the methyl malonyl-CoA pathway, it is speculated that the lactate produced during exercise is converted into SCFAs, improving exercise capacity (88). Moreover, several probiotic supplements can decrease intestinal damage caused by strenuous training (97–99), as shown in **Table 1**. The probiotics *Escherichia coli* strain Nissle 1917 (100), UCC118 (99) and bovine colostrum (98), in addition to different dietary applications (61, 101, 102) seem to exert this softening effect on the permeability caused by strenuous exercise.

Intestinal epithelial barrier properties are also maintained by cellular junctions called desmosomes, shown in **Figure 1**. The only desmosome expressed in enterocytes (Desmoglein 2, Dsg2) is activated under the same conditions as p38 mitogen-activated protein kinases (p38 MAPK) (103, 104). Although there is still no study showing the effects of exercise on Dsg2 of enterocytes, it is known that physical training can activate p38 MAPK in different muscles (105, 106).

If, on the one hand, intestinal dysbiosis is associated with a quantitative and qualitative reduction of the intestinal microbes, on the other hand, exercise at specific doses may be a key strategy to restore the composition and function of the gut microbiota, improving gastrointestinal mucosa and reducing inflammatory signaling. It may also operate an intricate process of bidirectional communication with the skeletal muscle metabolism (83).

CONCLUSION

Physical exercise acts as a modulator of the intestinal environment due to the demands of skeletal muscle. Strenuous exercise leads to higher gastrointestinal ischemia and hyperthermia. So far, it is believed that vigorous endurance training with ≥60 min at ≥70% of the maximum work capacity increases the intestinal permeability, with an enhanced effect observed in hot environments, at high altitude, and under dehydration. In response to strenuous exercise, leaky gut is associated with increased I-FABP and infiltration of bacterial endotoxins within the blood circulation. On the other hand, non-prolonged moderate exercise may preserve the intestinal mucosa by accelerating gastric emptying, improving intestinal motility, increasing the abundance and diversity of the gut microbiota, also increasing butyrate-producing bacteria and the synthesis of short-chain fatty acids. However, to date, an exercise "threshold" that may lead to increased gut permeability is still uncertain.

The determination of a "threshold" is essential for the intestinal health of individuals who are athletes or who seek to be active. It is necessary to standardize the analyses that indicate the leaky gut. After that, it is advisable to carry out research that analyzes these factors (I-FABP, sugar test, LPS, among others) with a progression of intensities and volumes of exercise. Obviously, confounding factors such as temperature, altitude, dehydration and degree of trainability need to be controlled for. Thus, more studies are needed in order to emphasize the role of exercise in intestinal permeability and to pinpoint other variables that may influence this phenomenon at the time of activity.

AUTHOR CONTRIBUTIONS

FR, BP, and GM: writing of the manuscript and elaboration of the figures. LHK: writing of the manuscript. OF: writing of the manuscript and general review. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by CNPq (437308/2018-9), CAPES, FUNDECT e FAPDF.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 28 May 2021
doi: 10.3389/fnut.2021.654902

Effect of Combined Interval and Continuous Exercise Training on Gastric Emptying, Appetite, and Adaptive Responses in Men With Overweight and Obesity

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Background/Objectives: Characterizing compensatory and adaptive responses to exercise assists in understanding changes in energy balance and health outcomes with exercise interventions. This study investigated the effects of a short-term exercise intervention (combining high intensity interval (HII) and continuous exercise) on (1) gastric emptying, appetite and energy intake; and (2) other adaptive responses including cardiorespiratory fitness, in inactive men with overweight/obesity.

Methods: Fifteen men (BMI: 29.7 ± 3.3 kg/m^{-2}) completed a 4-wk supervised exercise intervention, consisting of 5 exercise sessions per week alternating between HII (30 s at 100% VO_2max followed by 30 s recovery) and continuous (at 50% VO_2max) training on a cycle ergometer, progressing from 30 to 45 min session duration. Gastric emptying (^{13}C-octanoic acid breath test), appetite (visual analog scale), energy intake (*ad libitum* lunch meal), body composition (air displacement plethysmography), non-exercise activity (accelerometery) VO_2max, blood pressure, and fasting concentrations of glucose, insulin, and ghrelin were measured before and after (≥48 h) the intervention.

Results: Gastric emptying, glucose, insulin and ghrelin were unchanged, but energy intake at the *ad libitum* lunch test meal significantly increased at post-intervention (+171 ± 116 kcal, $p < 0.01$). Body weight (−0.9 ± 1.1 kg), waist circumference (−2.3 ± 3.5 cm) and percent body fat (−0.9 ± 1.1%) were modestly reduced ($P < 0.05$). VO_2max increased (+4.4 ± 2.1 ml.kg.min^{-1}) by 13% and systolic (−6.2 ± 8.4 mmHg) and diastolic (−5.8 ± 2.2 mmHg) blood pressure were significantly reduced ($P \leq 0.01$ for all).

Conclusions: Four weeks of exercise training did not alter gastric emptying, indicating gastric emptying may only adapt to a higher volume/longer duration of exercise or changes in other characteristics associated with regular exercise. The combination of HII and continuous exercise training had beneficial effects on body composition, cardiorespiratory fitness, and blood pressure and warrants further investigation in larger randomized controlled trials.

Keywords: appetite, energy intake, energy balance, cardiorespiratory fitness, compensatory responses, high intensity interval

OPEN ACCESS

Edited by:
Karsten Koehler, Technical University of Munich, Germany

Reviewed by:
Kristine Beaulieu, University of Leeds, United Kingdom
Eric Doucet, University of Ottawa, Canada

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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *17 January 2021*
Accepted: *26 April 2021*
Published: *28 May 2021*

Citation:
Horner KM, Byrne NM and King NA (2021) Effect of Combined Interval and Continuous Exercise Training on Gastric Emptying, Appetite, and Adaptive Responses in Men With Overweight and Obesity. Front. Nutr. 8:654902. doi: 10.3389/fnut.2021.654902

INTRODUCTION

Exercise has many health benefits, including weight maintenance, and should be an effective weight loss strategy by increasing energy expenditure. However, the efficacy of exercise for weight loss is modest (1) and will depend on changes in other components of energy balance including energy intake and non-exercise activity (2). Although it is intuitive that exercise drives an increase in appetite and energy intake, the relationship between exercise and appetite is more complex. Evidence indicates that exercise improves the sensitivity of appetite control (3–8) and that exercise influences at least two processes of appetite control: both the drive to eat and the satiating efficiency of a meal (5). As the strength of these processes may determine whether individuals lose weight with exercise, understanding the effects of exercise on energy intake and the underlying mechanisms is vital.

Gastrointestinal peptides and gastric emptying (the rate at which food empties from the stomach) have an important integrative role in the short-term control of food intake. For example, a slower gastric emptying is associated with increased gastric distension, postprandial fullness and reduced energy intake at a subsequent test meal (9, 10). However, a slower gastric emptying also delays the interaction of nutrients with the intestine, blunting the release of satiety related gut peptides in individuals with obesity (11). The relative influence of intestinal and gastric signals on appetite may be influenced by factors such as the time interval between meals, characteristics of the individual or of the meal. Cross-sectional studies have shown gastric emptying is faster in active compared to inactive individuals (12–14), and is associated with activity energy expenditure (14). Faster gastric emptying has been proposed as a mechanism which may increase desire for food intake with chronic physical activity (12). We have also previously hypothesized that faster gastric emptying with chronic exercise could be one mechanism contributing to an overall increase in meal frequency and energy intake by reducing gastric distension and fullness, but improved ability to match daily energy intake to expenditure in active individuals through enhanced intestinal satiety signaling (15). However, such relationships have yet to be investigated. In addition, while cross-sectional studies can provide important information, they do not allow for a causal relationship between changes in gastric emptying with repeated exercise training to be determined.

Potential mechanisms contributing to changes in gastric emptying and energy intake include alterations in fasting ghrelin (16), blood glucose (17) and insulin sensitivity (18), which have been shown to change in response to exercise training (3, 19–23). Therefore, examining changes in these blood markers may provide further mechanistic insight into changes in appetite with exercise.

Compensatory responses in other components of energy balance, including activity outside of the prescribed exercise program are also important factors when considering exercise prescription for individuals with overweight and obesity (24–26). In addition to changes in energy intake, non-exercise activity may be influenced by exercise intensity and reduced to a greater extent as a compensatory response to high intensity exercise (27, 28). Thus, changes in non-exercise activity could potentially undermine beneficial effects of higher intensity exercise on total daily activity levels.

Combining high intensity interval (HII) exercise sessions with continuous lower intensity exercise sessions may serve to provide benefits for increasing both cardiorespiratory fitness, along with increasing the total amount of exercise–an important factor contributing to body weight and fat loss (29). Exercise programs (aimed at improving total daily activity, cardiorespiratory fitness, body composition, and adherence) should include a combination of low- and high-intensity exercise (30), and have been shown to result in substantial improvements in VO_2max in trained and untrained individuals (31). However, to the best of our knowledge the effects of combining HII and moderate intensity continuous exercise on compensatory responses and other health-related outcomes have not been widely examined in individuals with overweight and obesity.

The present study was undertaken to investigate the effects of a 4-week exercise intervention (combining HII and continuous exercise) on (1) gastric emptying, appetite and energy intake; and (2) body composition, non-exercise activity, cardiorespiratory fitness and related health markers in inactive men with overweight and obesity.

MATERIALS AND METHODS

Participants

Based on our previous work examining the reproducibility of gastric emptying in individuals with overweight and obesity without any intervention (32), a minimum of 15 participants was required to detect a mean difference of at least 10% for all gastric emptying parameters, with a power of 80% and $\alpha = 0.05$. Participants were recruited in the university and local area. Inclusion criteria were: male, aged 18–60 years, BMI 25–40 $kg.m^{-2}$, weight stable ($\pm$ 4 kg over last 6 months), non-diabetic, no history of GI surgery or disorder, no medical conditions, and not taking any medication known to influence the outcome measures, willing to consume study test meals, not a heavy smoker (<10 per day) and inactive (participating in one structured exercise session or less per week and not engaged in strenuous work). All participants completed the Sports Medicine Australia pre-exercise screening questionnaire and those with any risk factors were required to present approval by their medical doctor prior to participation. Ethical approval was granted by Queensland University of Technology Research Ethics Committee, the study was conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent prior to taking part. No incentive was provided.

Design

Participants attended the laboratory on 2 separate test days (at least 48 h apart) in the week prior to the 4-week exercise intervention (baseline) and on 2 separate test days in the week following the exercise intervention (post-intervention) (at least 48 h after the last exercise session to avoid any acute effects of exercise). At one testing session, fasting blood samples, body

composition and VO_2max were measured. At the second test session, gastric emptying, subjective appetite sensations and *ad libitum* lunch energy intake were assessed. The order of testing sessions was the same for all participants. On both occasions, participants attended the laboratory after a 12 h overnight fast, and having avoided alcohol and strenuous exercise for 24 h. One glass of water was allowed upon waking. Participants were instructed to repeat these procedures prior to the post-test. There was no dietary intervention, similar to others assessing the impact of exercise without dietary intervention (23, 33–35).

Exercise Intervention

The exercise intervention consisted of five exercise sessions per week for 4 weeks. All sessions were supervised and involved indoor cycling on a cycle ergometer (Monark 884E Ergomedic Sprint Bike, Monark Exercise AB, Vansbro, Sweden). Exercise sessions alternated between continuous cycling and HII exercise, with participants prescribed ten of each type over the course of the 4 weeks. The continuous exercise sessions involved cycling at a constant workload equivalent to 50% VO_2max for the duration of the session. HII sessions consisted of 30 s cycling at 100% VO_2max followed by 30 s recovery (unloaded cycling or static recovery) each minute for the duration of the session. Thus, an identical relative workload and time duration was prescribed.

Exercise duration progressed by 5 min/week from 30 min in week 1 to 45 min in week 4. Each session started with a 5 min warm up of unloaded cycling and finished with a cool down. Participants wore a heart rate monitor (Polar Electro Oy, Kempele, Finland) during each exercise session. HR and RPE using the Borg Scale (36) were recorded every 5 min. In HII sessions, recordings were taken immediately at the end of a HII bout. Workloads were prescribed based on each participant's baseline VO_2max test using individual regression equations for each subject. Percent VO_2max data calculated during the last 30 s of each stage of the test was plotted against stage workload and 50% and 100% VO_2max were used to calculate the corresponding prescribed workloads.

Anthropometry and Body Composition

Height was measured without shoes to the nearest 0.5 cm and weight to the nearest 0.01 kg. Waist and hip circumferences were taken and body composition was measured using air displacement plethysmography (Bodpod, Concord, CA).

Maximal Oxygen Consumption (VO_2 Max)

VO_2 max was assessed using a TrueMax 2400 Metabolic Cart (ParvoMedics Inc, USA). All tests were conducted on the same cycle ergometer (Monark Bike 839E, Monark Exercise AB, Sweden) and consisted of 2 phases [similar to Wood et al. (37)]. Phase 1 consisted of a graded exercise test performed to volitional exhaustion and phase 2 consisted of a verification test. Participants were instructed to maintain cycling cadence at 70 rpm. Participants performed a 2-min warm up at the start of the graded test. Subsequently, workload was increased each minute by either 21 or 28 W (determined prior to the test based on the participant's predicted VO_2max). Following phase 1, the participant was given a 5-min rest and a small glass of water. Participants then resumed cycling at the workload of the third last 1-min stage of the preceding maximal continuous incremental test for phase 2 (the verification test) (37). The workload was increased each minute until volitional exhaustion. This two-phase test was used as it has been suggested that a verification or "booster" test may provide a time-efficient means of verifying whether a VO_2peak is indicative of a true maximal VO_2 (38).

The continuous incremental exercise test (phase 1) was deemed to be a valid maximal test on the basis of achievement of at least three of the following criteria during the final 30 s of the last completed stage (37): Increase in $VO_2 < 50\%$ of that expected for the change in mechanical work, heart rate (HR) within +/– 11 bpm of age-predicted maximum, calculated as 220 –age, respiratory exchange ratio (RER) $\geq$ 1.15, RPE $\geq$ 18. Ventilatory threshold was calculated using the combined approach (39).

Blood Pressure

Systolic and diastolic blood pressure were assessed using an Omron IA1B blood pressure monitor (Omron Healthcare Singapore PTE Ltd, Singapore) in a seated position. Measurements were taken in duplicate following 10 min of sitting to ensure the participant was rested and relaxed.

Blood Samples

Fasting samples were collected by venepuncture into potassium oxylate, serum and EDTA tubes containing aprotinin and DPP-IV inhibitors. Potassium oxylate and EDTA tubes were immediately centrifuged (refrigerated at 2,000 g for 10 min) and the serum tube was allowed to stand for 30 min before centrifugation. Samples were immediately aliquoted, placed in liquid nitrogen and stored at −80 degrees until analysis. Plasma glucose was measured colourimetrically using standard laboratory techniques, insulin by chemiluminescent immunoassay and total ghrelin using an established RIA. All analyses were conducted in duplicate, and mean values are reported. Intra-sample CV's were 0.7 ± 1.1% for glucose, 2.1 ± 1.3% for insulin and 6.5 ± 5.2% for ghrelin. Insulin resistance by homeostasis model (HOMA-IR) was calculated according to Matthews et al. (40): HOMA-IR = fasting glucose × fasting insulin/22.5.

Non-exercise Activity

Non-exercise activity (i.e., activity outside of the prescribed exercise) was monitored using a tri-axial GT3X accelerometer (Actigraph, Fort Walton Beach, FL, USA). Participants were provided with the accelerometer to wear for 7 days prior to the intervention and again in week 4 of the intervention, a duration estimated to result in 90% reliability (41). The accelerometer was attached to an elastic belt and worn on the waist, in line with the right hip. Data were processed using ActiLife software (version 6.4.5). VM3 counts were summed over 60 s epochs and levels of activity were defined as counts per minute using cut point values according to validated recommendations (42). Data were checked for spurious values (counts per minute of >15,000). A non-wear period was defined as at least 90 min of consecutive zero counts without interruption (43). Wear time exceeding 600 min was considered a valid day (44) and

a valid dataset was considered a combination of at least 3 week days and 1 weekend day (45). Data during prescribed exercise times were excluded from analysis. Mean minutes per day of time spent in moderate and vigorous (combining vigorous and very vigorous) activity were calculated. Activity count data were converted to activity energy expenditure (AEE) using the "Freedson VM3 combination (11)" option in Actilife software (version 6.4.5). Accelerometery data were compared between pre- and post- exercise intervention. Data were also compared in participants who had a complete 24 h dataset following a single continuous and HII exercise session in week 4 to examine whether subsequent 24 h AEE was impacted by the type of training session.

Energy Compensation

To estimate energy expenditure from the prescribed exercise, individual energy expenditure regression equations were developed for each participant using the heart rate and energy expenditure values recorded during the last 30 s of each stage of the VO_2max test, similar to previous work (46–48). Heart rates recorded during the prescribed exercise sessions were then inserted into the individual regression equations to predict energy expenditure. Net energy cost of exercise was calculated by subtracting resting energy expenditure from energy expenditure during prescribed exercise. Resting energy expenditure was measured over 30 min at baseline by indirect calorimetry using an identical procedure to previous work (14).

Energy compensation was calculated following Riou et al. (46, 49) based on the total estimated energy expended during prescribed exercise (EE), and changes in fat (FM) and fat free mass (FFM) observed using energy equivalents for fat mass and fat free mass previously described (50) as follows:

$$\text{Energy Compensation } (\%) = \frac{100}{\text{EE (kcal)}} \times \left[\left(\text{FM } (\text{kg}) \times 9,500 \text{ kcal}\right) + \left(\Delta\text{FFM } (\text{kg}) \times 1,020 \text{ kcal}\right)\right] + 100$$

Using this method compensation of 0% indicates changes in body composition following the intervention matched expected changes based on exercise EE. A positive value indicates changes in body energy stores are less than expected, with a value of 100% indicating body composition remained the same. In contrast, a negative value indicates body energy stores are reduced beyond what would be expected based on exercise EE (46, 49).

Gastric Emptying Test Day Measurements

Gastric Emptying

Gastric emptying parameters were calculated using the ^{13}C-octanoic acid breath test (51), using an identical procedure to that described in detail previously (32). In brief, the egg yolk of a standardized pancake breakfast meal [400 kcal; 15 g (15%) PRO, 17 g (37%) Fat, 48 g (48%) CHO)] was labeled with 100 mg ^{13}C-octanoic acid (Cambridge Isotope Laboratories, Andover, USA). Participants consumed the meal together with 250 ml of water within 10 min. Breath samples were collected in 10 ml glass Exetainer tubes (Labco, Buckinghamshire, UK) prior to the breakfast, immediately after, and subsequently at 15 min intervals for 5 h after breakfast. Participants remained in sedentary activities throughout. No food or drinks were provided to participants during this time. ^{13}C enrichment of breath samples was measured by isotope ratio mass spectrometry (Hydra 20–20) and compared to a reference gas (5% CO_2, 75% N_2, 20% O_2 calibrated with a standard of ^{13}CO2). Data were analyzed according to Ghoos et al. (51). The conventional uncorrected time based parameters (t_{lag} and $t_{1/2}$) proposed by Ghoos et al. (51) and the parameters latency time (t_{lat}) and ascension time (t_{asc}) proposed by Schommartz et al. (52) were calculated.

Subjective Appetite Sensations

Subjective appetite sensations were measured throughout the test day using an electronic appetite rating system. Participants were asked to rate feelings of hunger, fullness and desire to eat on 100 mm visual analog scales, using an identical protocol to previous work (53). The satiety quotient (SQ) (54) was calculated for each sensation at breakfast and palatability ratings of both breakfast and lunch meals were assessed immediately post-meal using 100mm visual analog scales.

Ad Libitum Energy Intake

At the end of the gastric emptying test, participants were provided with an *ad libitum* pasta lunch meal identical to that described previously (53) (47% CHO, 35% FAT, and 18% PRO, and an energy content of 1.8 kcal/g) and water and instructed to consume as much as they wished until comfortably full. The amount (g) of food consumed was determined by weighing the meal before and after consumption and energy intake (kcal) calculated.

Statistical and Data Analysis

Data are presented as mean values and standard deviations (SD). Changes from pre- to post- exercise intervention were assessed using paired sample *t*-tests. Unless otherwise stated Pearson correlations were used to determine relationships between changes in key variables. Spearman correlations were used for non-parametric data. Area under the curve for appetite ratings was calculated using the trapezoidal rule. Following a similar approach to King et al. (55), in order to provide insight into individual variability in responses that may be attributed to the exercise intervention, normal day-to-day variability in the key outcome measures is considered by graphically presenting the findings in relation to our previous work examining the reproducibility of gastric emptying (32) and energy intake (53) in a similar population of men with overweight/obesity without intervention. Statistical analysis was carried out using PASW Statistics 18.0 (SPSS Inc., Chicago, IL) and statistical significance accepted at $p < 0.05$.

RESULTS

Eighteen men met the inclusion criteria and three withdrew during the intervention, resulting in fifteen males completing the study. Three participants did not complete the 4 week

exercise intervention–two due to time commitments and personal circumstances and one participant was excluded due to insufficient attendance at exercise sessions. Results are presented for 15 men (BMI: 29.7 ± 3.3, Age: 31.1 ± 8.4 yr) who completed all parts of the study.

Exercise Intervention Characteristics and Energy Compensation

Participants completed 96 (3.9)% of the prescribed number of exercise sessions, with all participants completing a minimum of 90% (18 of 20) of the exercise sessions. Mean RPE decreased by 1.7 units during continuous and 2.2 units during HII exercise respectively, when compared over the first 30 min from week 1 to week 4 ($p < 0.001$). Mean total time spent in prescribed exercise was 705 ± 43 min. Mean total energy expended in prescribed exercise calculated over the 4 week intervention was 7,803 ± 1,587 kcal. Mean energy compensation was −41 ± 136%, indicating that on average participant's energy stores were reduced to a greater extent than would have been expected based on exercise energy expenditure. However, individual values ranged from −315 to 214% indicating inter-individual differences in responses were highly variable. In total, five had positive values (range 5–214%) indicating energy compensation occurred and ten negative values (range −20 to −315%) indicating greater reductions in energy stores than expected.

Anthropometry, Body Composition, Blood Pressure, and Fitness

The small reductions in weight, BMI, body fat, and waist circumference at the end of the intervention were statistically significant (**Table 1**). Weight change ranged from −2.4 kg loss to + 0.8 kg gain, and as a percentage of initial body weight from −3.0% loss to 0.9% gain. Systolic and diastolic blood pressure were significantly reduced and there was a significant increase in VO_2max (mean 12.8% increase in ml.kg.min^{-1}; mean 11.6% increase in L.min^{-1}) (**Table 1**). Four participants did not meet the criteria for VO_2max at pre- and post- test, however the verification test indicated that they could not complete any additional stages. Mean RER (Pre: 1.15 ± 0.04, Post: 1.13 ± 0.06) and HR_{max} (**Table 1**) during the final 30 s of the last completed stage did not differ significantly between pre- and post-test.

Gastric Emptying and Blood Parameters

Gastric emptying, fasting ghrelin, glucose, insulin and HOMA-IR did not significantly differ between pre- and post-exercise intervention (**Table 2**).

Despite no mean changes, there was variability in changes in these outcome measures. Six individuals had a faster $t_{1/2}$ at post-test, ranging from 0.1 to 17.8% (0.2 to 32.0 min) faster, and nine individuals had a slower $t_{1/2}$ at post-test, ranging from 3.5 to 13.9% (5.5 to 25.0) min slower. However, most changes were within the intra-individual CV of 8% identified in our previous work (32) (**Figure 1**). Comparing the results to the natural variation previously documented, the changes in GE $t_{1/2}$ of 66% ($n = 10$) of participants fell within this normal range.

TABLE 1 | Participant anthropometric, body composition, cardiorespiratory fitness, and blood pressure characteristics pre- and post- 4-week exercise intervention ($n = 15$).

	Pre	Post	*P*-value
Weight (kg)	95.6 ± 13.0	94.7 ± 13.0	**<0.01**
BMI (kg.m^{-2})	29.7 ± 3.3	29.3 ± 3.2	**<0.001**
Body composition			
Body fat (%)	30.0 ± 6.8	29.0 ± 6.7	**0.01**
FFM (kg)	66.4 ± 7.1	66.7 ± 6.8	0.50
Waist (cm)	97.1 ± 9.6	94.9 ± 8.7	**0.03**
Hip (cm)	107.8 ± 7.3	107.2 ± 7.2	0.15
Fitness			
VO_2max (ml.kg.min^{-1})	34.3 ± 5.9	38.7 ± 5.9	**<0.001**
VO_2max (L.min^{-1})	3.25 ± 0.57	3.63 ± 0.52	**<0.001**
HR max (bpm)	183 ± 13	182 ± 8	0.51
Workload max (Watts)	270 ± 51	308 ± 48	**<0.001**
Ventilatory threshold (Watts)[a]	136 ± 35	187 ± 33	**<0.001**
Ventilatory threshold (%VO_2max)[a]	53 ± 11	61 ± 5	**0.001**
Blood pressure			
Systolic (mmHg)	122 ± 8	116 ± 9	**0.01**
Diastolic (mmHg)	79 ± 7	74 ± 9	**<0.01**

Data are means ± SD.
BMI, body mass index; FFM, fat free mass; VO_2max, maximum oxygen uptake; HR, heart rate.
[a]*Combined VT calculations are reported for n = 14 as the time of VT for one participant occured at <4 min, therefore the data was rejected as per (39). Bold highlights statistically significant values.*

TABLE 2 | Gastric emptying time based parameters, fasting ghrelin, glucose, insulin, and HOMA-IR Pre and Post 4 week exercise intervention ($n = 15$).

	Pre	Post	*P*-value
GE t_{lag} (min)	111 ± 17	110 ± 18	0.71
GE $t_{1/2}$ (min)	175 ± 22	179 ± 25	0.25
GE t_{lat} (min)	37 ± 9	35 ± 8	0.09
GE t_{asc} (min)	137 ± 17	144 ± 21	0.10
Fasting ghrelin (ng/L)	805.4 ± 337.6	760.8 ± 331.0	0.12
Fasting glucose (mmol/L)	5.49 ± 0.30	5.44 ± 0.22	0.39
Fasting insulin (mIU/L)	9.40 ± 4.66	8.70 ± 4.18	0.19
HOMA-IR	2.30 ± 1.16	2.11 ± 1.05	0.20

Data are means ± SD.
GE, gastric emptying; $t_{1/2}$, half time; t_{lag}, lag time; t_{asc}, ascension time; t_{lat}, latency time.

Appetite Ratings and *ad libitum* Test Meal EI

Subjective appetite ratings are shown in **Supplementary Figure 1** and did not differ significantly between pre- and post- exercise intervention for fasting, mean 5 h, 5 h AUC and breakfast satiety quotient ($p > 0.14$ for all). In addition, there were no significant differences between pre- and post-intervention for palatability ratings of the breakfast and lunch meals ($p > 0.09$ for all).

EI at the *ad libitum* lunch test meal was significantly higher following the exercise intervention (Pre: 712 ± 173 kcal, Post: 883 ± 159 kcal, $p < 0.001$), with a mean 27% increase from baseline. Variability in individual changes in energy intake are shown in

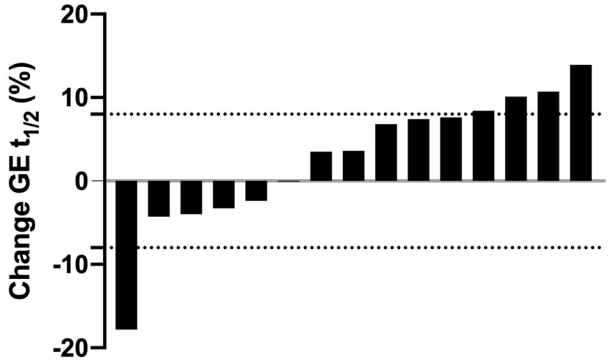

FIGURE 1 | Individual changes in gastric emptying half time (GE $t_{1/2}$) expressed as percentage change from baseline after the 4-week exercise intervention. Each bar represents an individual participant ($n = 15$). Values above zero indicate a longer (i.e., slower), values below zero indicate a shorter (i.e., faster) GE $t_{1/2}$ after the intervention. Dashed horizontal lines represent zones of natural variation in GE $t_{1/2}$ (± 8%) based on our previous work (32).

TABLE 3 | Mean physical activity characteristics at baseline and during week 4 (excluding prescribed exercise) of the 4 week exercise intervention ($n = 13$).

	Pre	Week 4	*P*-value
Physical activity			
Steps per day	6,714 ± 2,082	6,718 ± 2,399	0.99
AEE (kcal/day)	568 ± 196	579 ± 227	0.16
Time in activity			
Vigorous (min/day)	5 ± 4	5 ± 5	0.97
Moderate (min/day)	42 ± 18	42 ± 22	0.97

Data are means ± SD.
AEE, activity energy expenditure estimated from accelerometery.

Supplementary Figure 2, illustrating most changes were outside the intra-individual CV of 12% identified in our previous work (53). Comparing the results to the natural variation previously documented, the changes in energy intake of 73% ($n = 11$) of participants fell outside the normal range.

Non-exercise Activity

Due to two invalid accelerometery data sets, physical activity data is reported for $n = 13$. Wear duration was significantly less at post-intervention (pre: 893 ± 73 min and post 826 ± 72 min, $p = 0.03$), due to the time in prescribed exercise being excluded from calculations. Non-exercise activity did not significantly differ between pre-intervention and week 4 (**Table 3**). When controlling for the difference in wear time, there was a significant interaction effect for AEE ($p = 0.04$) and steps per day ($p = 0.01$) but not for time spent in moderate or vigorous activity. Including EE from the prescribed exercise sessions, there was a significant increase in average daily AEE of +303 ± 162 kcal over week 4 of the intervention compared to pre-intervention ($p = 0.007$).

Data was available for a complete 24 h period for $n = 11$ participants after both a HII and continuous exercise session in week 4. Non-exercise activity did not differ in the 24 h after a single continuous exercise session compared to after a HII session in week 4 (**Supplementary Table 1**; **Supplementary Figure 3**). Wear duration did not differ between conditions ($p = 0.14$).

Relationships Among Energy Compensation, Anthropometric, Body Composition, Gastric Emptying, Blood Markers, Physical Activity, Appetite, and *ad libitum* Energy Intake Changes

Energy compensation was inversely associated with change in AEE (including prescribed exercise) ($r = -0.61$, $p = 0.03$), indicating a greater increase in AEE in week 4 was associated with less energy compensation. Energy compensation was also associated with change in body fat [percent ($r = 0.84$, $p < 0.001$) and kg ($r = 0.97$, $p < 0.001$)], change in fasting insulin ($r = 0.56$, $p = 0.03$) and change in HOMA-IR ($r = 0.51$, $p = 0.05$), indicating lower energy compensation was associated with a greater reduction in body fat, insulin and HOMA-IR. However, energy compensation was not associated with changes in FFM (kg, $r = -0.43$, $p = 0.11$), gastric emptying or any other variables.

There was a trend toward a negative correlation between change in gastric emptying t_{asc} with average daily AEE (including AEE in the prescribed exercise sessions) ($r = -0.53$, $p = 0.06$). Change in t_{asc} was also negatively correlated with change in AEE outside of the prescribed exercise ($r = -0.67$, $p = 0.01$) and similar negative correlations were found between change in t_{asc} and changes in steps per day ($r = -0.65$, $p = 0.02$) and mean time in vigorous activity per day ($r = -0.64$, $p = 0.02$) outside of prescribed exercise. These findings indicate a greater increase in activity was associated with shorter (i.e., faster) gastric emptying time following the intervention. However, changes in gastric emptying were not correlated with changes in anthropometric, body composition, blood markers, VO_2max, appetite (5 h mean or AUC) or *ad libitum* test meal energy intake variables. Change in AEE (including prescribed exercise) was also inversely associated with change in body fat (kg) ($r = -0.58$, $p = 0.04$), indicating a greater increase in AEE in week 4 was associated with less energy compensation and a greater reduction in body fat at post-intervention. Change in AEE (excluding prescribed exercise) was not correlated with time spent in prescribed exercise (Spearman rho = 0.02, $p = 0.95$), indicating overall exercise participation was not associated with change in AEE outside of the intervention.

A decrease in fasting insulin and HOMA-IR from pre-to post-intervention was associated with a decrease in body fat (insulin: $r = 0.69$, $p = 0.004$; HOMA-IR: $r = 0.67$, $p = 0.006$). Fasting ghrelin was not associated with changes in other variables. Change in *ad libitum* test meal energy intake was associated with change in percentage body fat ($r = 0.52$, $p = 0.048$) but not with other variables, indicating an increase in energy intake was associated with a lesser reduction in body fat following the intervention.

DISCUSSION

The present findings demonstrate that in response to a 4-wk exercise intervention combining HII and continuous exercise

(1) gastric emptying, glucose, insulin, ghrelin, appetite ratings and non-exercise activity are unaltered despite an increase in *ad libitum* test meal energy intake; and (2) body composition, cardiorespiratory fitness and blood pressure are improved, in men with overweight and obesity. Compliance was high (≥90% completion of all sessions), the intensity and frequency of sessions were high, each session was supervised in the laboratory and the intervention resulted in a significant improvement in cardiorespiratory fitness. It can therefore be reasonably concluded from the present findings that in the short to medium term (4 weeks), in the absence of acute exercise effects gastric emptying is unaltered in response to exercise training in men with overweight and obesity.

In contrast, cross sectional studies have shown faster gastric emptying in active compared to inactive men (13). In a previous study demonstrating faster gastric emptying in marathon runners, the runners were training for a mean 4.9 years (13) and in our previous work, habitual exercisers were defined as individuals engaged in 4 or more exercise sessions per week for a minimum of 6 months (14). Therefore, gut adaptations (i.e., faster gastric emptying) in response to regular exercise may only occur after a much longer period of time than the 4-week intervention in the present study. Interestingly, the only significant correlates of changes in gastric emptying parameters over time were changes in activity assessed by accelerometer outside of the prescribed exercise sessions. A greater increase in activity between baseline and week 4 was associated with a faster gastric emptying time. In addition, there was a trend toward a greater increase in average daily AEE including time in prescribed exercise being associated with a shorter (i.e., faster) gastric emptying time. These findings are consistent with our previous cross-sectional evidence showing significant associations between greater activity, AEE and faster gastric emptying (14). In the present study, activity was restricted in the 48 h prior to gastric emptying testing, which could be one factor explaining why gastric emptying and appetite were unchanged overall. Further studies are warranted to investigate this and the temporal pattern of changes in gastric emptying and associations with appetite and daily EI with longer term interventions and more substantial weight loss.

The rationale for the 4 week duration of intervention was to investigate the effects of the exercise intervention on gastric emptying and compensatory responses before substantial changes in body composition were likely to occur and impact responses. Some evidence shows change in VO_2max is associated with energy compensation (47, 48). In contrast, the exercise intervention in the present study demonstrated on average a significant improvement in VO_2max without energy compensation or changes in gastric emptying. Week 4 exercise intervention responses may also be useful in determining longer term effects of exercise on weight loss and compensatory responses in individuals with overweight or obesity (56). However, a limitation of the current study is that the sample size limited further understanding of individual responses. Only 5 participants had positive energy compensation values indicating a degree of compensation for exercise energy expenditure. Further studies with a larger numbers of participants would assist to better understand whether alterations in gastric emptying may be a compensatory mechanism occurring in individuals identified as "compensators" compared to "non-compensators" with exercise intervention.

The 4-week exercise intervention also had no significant effect on fasting glucose, insulin, HOMA-IR or total ghrelin, and could provide further explanation behind why no changes in gastric emptying were observed. Excluding the effects of acute exercise may similarly be one explanation for the lack of changes in fasting insulin and HOMA-IR. Although, it has long been established that a single bout of exercise improves glucose metabolism acutely (57), the benefits of exercise appear to diminish within 48 to 72 h of the last exercise session (23, 58). The timing of the post-testing (≥ 48 h after last exercise) in the present study was selected to allow the effects of short-term exercise training in the absence of acute exercise effects to be established, and to allow comparison with previous studies examining the effects of exercise training on gut peptides (3, 20, 21, 59).

In addition, although changes in body composition were significant, the reductions in body fat were modest (mean ∼1% change) and it is likely that more substantial changes in body fat may be required to improve insulin sensitivity in response to chronic exercise. Indeed, we observed that changes in insulin and HOMA-IR were strongly associated with reductions in body fat. These findings support the contention that the chronic impact of exercise training on insulin may be mediated by reduced adiposity (57). The present findings are also consistent with evidence that fasting ghrelin levels appear to be unaffected by exercise training in the absence of significant concurrent weight loss (60).

Subjective appetite sensations were similarly unchanged after the 4-week intervention, consistent with some previous studies showing no change in appetite ratings following both 7 and 14 days of exercise in lean men and women (61, 62). However, in response to longer-term interventions, changes in subjective appetite ratings have been documented (5, 63) suggesting appetite ratings may only respond to longer duration interventions.

Despite no significant changes in appetite ratings, *ad libitum* energy intake at the lunch test meal increased (mean 171 kcal higher (27% increase), which equated to ∼32% of the average energy expended in a prescribed exercise session in week 4). Although AEE was not associated with lunch test meal energy intake in the present study, AEE has been previously identified as an independent predictor of mean daily energy intake and to have a small contribution to the drive to eat (64, 65). Others have shown partial compensation in energy intake of ∼30% for exercise induced EE following 14 days of high exercise levels in lean men (62). Although, in the latter study daily food intake was measured, the findings of a significant increase in energy intake at the *ad libitum* lunch in the present study could be indicative of a partial compensation in energy intake. Indeed, an increase in test meal energy intake was associated with a lesser reduction in body fat in response to the intervention. As gastric emptying was unchanged, other factors such as changes in leptin (66), cognitive factors such as attitudes and beliefs (e.g., exercise

makes you hungry), a desire for self-reward after exercise and misjudgements about the amount of energy expended relative to energy intake (67, 68) could have contributed to the change in *ad libitum* energy intake at the lunch test meal. It is therefore important to address a range of factors which may contribute to compensatory increases in energy intake and thus impede weight/fat loss when individuals commence an exercise program for weight management.

A second major aim of the present study was to examine the effects of the intervention on cardiorespiratory fitness and other adaptations to exercise. We observed a mean 13% increase in VO_2max [(+4.4 ml.kg.min^{-1}); 12% in L.min^{-1} (+0.38 L.min^{-1})]. Previous studies in men with overweight and obesity involving HII only interventions have reported a mean 8% (+0.25 L.min^{-1}) increase in VO_2peak following 2 weeks (23), 7% (+1.9 ml.kg.min^{-1}) increase following 4 weeks (34) and 2.8% (+0.84 ml.kg.min^{-1}) increase following 6 weeks (69) of HII training 3 days per week. Others (70) have reported a 13% (+0.4 L.min^{-1}) increase in VO_2max in overweight males following 12 weeks of HII training three times per week. Interestingly, the total prescribed exercise time in that study [720 min (70)] was similar to the present study (750 min). However, compared to the latter study we observed a similar improvement in VO_2max following just 4 weeks of training and with less total training sessions (20 vs. 36) and less HII sessions (10 vs. 36).

Limited research has examined the effects of combining HII and continuous exercise interventions in healthy individuals with overweight and obesity. However, this type of training has been shown to be well-tolerated in a small study of individuals with overweight and obesity (71). Moreover, in individuals with Type 2 diabetes, Mourier et al. (72) examined the effects of combined continuous (2 days per week) and HII exercise (once per week) for 8 weeks, and observed a substantial improvement in VO_2peak [41% (+9.4 ml.kg.min^{-1}) increase] and reductions in adiposity. The addition of continuous to HII exercise sessions, thus increasing the total exercise dose and energy expenditure, is one possible explanation for the improvements in VO_2max and body composition. Molecular mechanisms such as an increase in PGC1-α with combined interval and continuous exercise (73) could also potentially contribute to the significant changes in VO_2max observed. Taken together, the current findings demonstrate that a short-term intervention combining HII and continuous exercise has beneficial effects on cardiorespiratory fitness, body composition and blood pressure.

A final objective was to compare the effects of the intervention on non-exercise activity. We found no change in non-exercise activity between baseline and the final week of the exercise intervention. Although other studies using doubly labeled water to quantity total EE have shown reductions in non-exercise activity in women with overweight and obesity (24, 46), the present findings are consistent with a systematic analysis (74) and some longer term studies in adults with overweight and obesity (63, 75). The present data also suggest the prescription of both HII and continuous moderate intensity exercise are effective for increasing total daily activity levels during an exercise intervention in men with overweight and obesity.

We acknowledge that the sample size limits the ability to generalize the findings, although it is similar to other studies in this area (3, 23). The study was undertaken in males to minimize confounding effects of menstrual cycle on key outcomes. In some studies examining changes in non-exercise activity and energy intake to exercise training in females, compensation has been demonstrated (24, 46, 63), therefore results in females may differ and further studies in females are warranted. Exercise EE was not directly measured during all exercise sessions, as indirect calorimetry during each training session was not feasible. In addition a constant load exercise test to estimate EE was not undertaken. Test meal intake as assessed in the present study provides an objective measurement but does not necessarily reflect daily changes, which represents a limitation of the current study. For example, Myers et al. (63) demonstrated an increase in daily energy intake but not lunch or dinner test meal intake following exercise intervention in women with overweight and obesity. It should also be noted that there was no dietary intervention, similar to others assessing the impact of exercise without dietary intervention (23, 33–35) and there was similarly no control group (3, 23, 63). However, the study was well-powered to detect significant changes in the primary outcome measure based on our previous work examining the reproducibility of gastric emptying in this population without intervention (32), all exercise sessions were supervised in the laboratory, exercise compliance was high (96%) and the "booster" VO_2max test provided verification VO_2peak was indicative of a true maximal VO_2.

Adherence and perceived difficulty of exercise are important factors for sustaining long-term participation in physical activity. The exercise intervention used represented a considerable change in lifestyle for inactive individuals. Given that others have shown that health benefits can be achieved with less intense HII protocols (23), more research is needed to identify the optimal interval protocol for improving health outcomes. Although achievable, participants reported that the sessions, in particular the HII sessions, were more difficult at the beginning of the intervention. However, anecdotally some participants preferred HII and others continuous exercise. Others have shown untrained adults reported greater enjoyment after a single bout of HII as compared to continuous exercise (76). Moreover, HII exercise has been reported to be perceived as "more motivating" and continuous exercise "quite boring" (77). Combining both types of training may therefore further serve to provide variety.

In conclusion, firstly, 4 weeks of exercise training did not alter gastric emptying, glucose, insulin, ghrelin or subjective appetite ratings in the present study of inactive men with overweight and obesity. In the absence of acute exercise effects, these measures may only adapt to a greater volume of exercise or changes in other characteristics associated with regular exercise. Further longer term interventions are needed to characterize the temporal pattern of changes in gastric emptying with regular exercise and the underlying mechanisms. Secondly, the intervention

combining continuous and HII exercise had beneficial effects on cardiorespiratory fitness, body composition and blood pressure and appeared not to alter non-exercise activity. Randomized controlled trials of larger sample sizes directly examining the efficacy of a combination of continuous and HII exercise compared to continuous and HII exercise only interventions would be of interest for future investigations to determine whether a combination intervention is more effective than either intervention alone.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Queensland University of Technology Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KH, NB, and NK: conceptualisation, writing, review, and editing. KH: data collection, formal analysis, and writing—original draft. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by a Queensland University of Technology Postgraduate Research Award (QUTPRA).

ACKNOWLEDGMENTS

The authors are grateful to all of the participants in the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.654902/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Sports and Active Living

ORIGINAL RESEARCH
published: 15 June 2021
doi: 10.3389/fspor.2021.683327

High-Protein Energy-Restriction: Effects on Body Composition, Contractile Properties, Mood, and Sleep in Active Young College Students

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Background: It is often advised to ensure a high-protein intake during energy-restricted diets. However, it is unclear whether a high-protein intake is able to maintain muscle mass and contractility in the absence of resistance training.

Materials and Methods: After 1 week of body mass maintenance (45 kcal/kg), 28 male college students not performing resistance training were randomized to either the energy-restricted (ER, 30 kcal/kg, $n = 14$) or the eucaloric control group (CG, 45 kcal/kg, $n = 14$) for 6 weeks. Both groups had their protein intake matched at 2.8 g/kg fat-free-mass and continued their habitual training throughout the study. Body composition was assessed weekly using multifrequency bioelectrical impedance analysis. Contractile properties of the m. rectus femoris were examined with Tensiomyography and MyotonPRO at weeks 1, 3, and 5 along with sleep (PSQI) and mood (POMS).

Results: The ER group revealed greater reductions in body mass (Δ −3.22 kg vs. Δ 1.90 kg, $p < 0.001$, partial $\eta^2 = 0.360$), lean body mass (Δ −1.49 kg vs. Δ 0.68 kg, $p < 0.001$, partial $\eta^2 = 0.152$), body cell mass (Δ −0.85 kg vs. Δ 0.59 kg, $p < 0.001$, partial $\eta^2 = 0.181$), intracellular water (Δ −0.58 l vs. Δ 0.55 l, $p < 0.001$, partial $\eta^2 = 0.445$) and body fat percentage (Δ −1.74% vs. Δ 1.22%, $p < 0.001$, partial $\eta^2 = 433$) compared to the CG. Contractile properties, sleep onset, sleep duration as well as depression, fatigue and hostility did not change ($p > 0.05$). The PSQI score (Δ −1.43 vs. Δ −0.64, $p = 0.006$, partial $\eta^2 = 0.176$) and vigor (Δ −2.79 vs. Δ −4.71, $p = 0.040$, partial $\eta^2 = 0.116$) decreased significantly in the ER group and the CG, respectively.

Discussion: The present data show that a high-protein intake alone was not able to prevent lean mass loss associated with a 6-week moderate energy restriction in college students. Notably, it is unknown whether protein intake at 2.8 g/kg fat-free-mass prevented larger decreases in lean body mass. Muscle contractility was not negatively altered by this form of energy restriction. Sleep quality improved in both groups. Whether these advantages are due to the high-protein intake cannot be clarified and warrants further study. Although vigor was negatively affected in both groups, other mood parameters did not change.

Keywords: fat-free-mass, Tensiomyography, muscle quality, sports nutrition, proteolysis

OPEN ACCESS

Edited by:
Karsten Koehler, Technical University of Munich, Germany

Reviewed by:
Alexander Carswell, University of East Anglia, United Kingdom
Amy Knab, Queens University of Charlotte, United States

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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Sports and Active Living

Received: *20 March 2021*
Accepted: *18 May 2021*
Published: *15 June 2021*

Citation:
Roth C, Rettenmaier L and Behringer M (2021) High-Protein Energy-Restriction: Effects on Body Composition, Contractile Properties, Mood, and Sleep in Active Young College Students. Front. Sports Act. Living 3:683327. doi: 10.3389/fspor.2021.683327

INTRODUCTION

During voluntary weight loss, as much lean body mass as possible should be maintained (Artioli et al., 2010). This, referred to as high-quality weight loss (Churchward-Venne et al., 2013), leads to a better power-to-mass ratio (O'Connor et al., 2007; Turocy et al., 2011), improves efficiency of movement (Sundgot-Borgen and Garthe, 2011), and increases the likelihood of athletic success (Slater et al., 2005; Chappell et al., 2018). However, following low energy availability, muscle protein synthesis is reduced leading to a negative net protein balance, and thus, finally culminates in muscle mass loss (Carbone et al., 2013; Pasiakos et al., 2013). In this context, it has been suggested that higher protein intake (2.4 vs. 1.2 g/kg) might restore muscle protein synthesis (Longland et al., 2016; Macnaughton et al., 2016) due to amino acids being preferentially used for muscle protein synthesis instead of gluconeogenesis (Walberg et al., 1988; Wackerhage and Rennie, 2006), with a concomitant decrease in protein breakdown (Kim et al., 2016; Park et al., 2020). Greater amino acid availability results in a more pronounced positive protein balance (Pikosky et al., 2008; Gwin et al., 2020), leads to a muscle sparing effect and is, therefore, recommended as an efficient strategy to increase the likelihood of lean mass retention (Phillips, 2008, 2014; Manore, 2015; Murphy et al., 2015; Witard et al., 2019).

Various studies examining the impact of energy restriction in active individuals have been conducted (Karila et al., 2008; Pikosky et al., 2008; Morton et al., 2010; Wilson et al., 2012; Pasiakos et al., 2013; Rhyu and Cho, 2014; Huovinen et al., 2015). While most of the studies revealed that energy restriction was associated with a significant lean body mass loss (Karila et al., 2008; Pikosky et al., 2008; Morton et al., 2010; Pasiakos et al., 2013; Rhyu and Cho, 2014), ranging from 34% (∼-1200 kcal/day; Morton et al., 2010) to 84% (∼-2500 kcal/day; Karila et al., 2008) of the total mass lost per week, some studies reported no significant lean body mass change during energy restriction (Huovinen et al., 2015; Wilson et al., 2015). Since all of these studies differ in total energy deficit, protein intake, sleep duration, baseline body fat, and type of physical activity performed, which are all known to significantly influence lean body mass change (Heymsfield et al., 2011), the exact reasons for the inherent inter-study differences remain unclear. Although higher protein intake during energy deficit may lead to a more favorable lean body mass sparing when compared to lower intakes (Pikosky et al., 2008; Mettler et al., 2010; Wilson et al., 2015; Hudson et al., 2020), it is currently unclear whether a moderately energy-restricted high-protein diet alone is a sufficient stimulus to maintain lean body mass and muscle contractile properties in male college students in the absence of resistance training. Although rapid weight loss procedures have been shown to negatively affect neuromuscular performance (Zubac et al., 2020), a moderate energy restriction may elicit performance-enhancing effects (Pons et al., 2018).

Therefore, the primary aim of this study was to investigate whether a high-protein moderately energy-restricted diet can preserve lean body mass in college students in the absence of resistance training. Furthermore, we investigated if muscle contractility can be preserved during this type of energy restriction. Based on currently available evidence, we hypothesized that a) a high-protein moderately energy-restricted diet is able to preserve the lean body mass even in the absence of resistance training and b) contractile properties are not negatively altered throughout the study. In an attempt to clarify the observed inter-study differences, this study aimed to tightly assess moderator variables affecting lean body mass change (protein intake, sleep duration, body fat, physical activity). Since the majority of previously conducted studies only used pre-post measurements, no precise conclusion can be drawn regarding the time course of lean body mass change. Hence, this study used weekly body composition measurements which have been previously described solely for overweight and obese individuals (Heymsfield et al., 2011).

MATERIALS AND METHODS

Study Design

The two group, parallel research design was adapted from Mettler et al. (2010) and Philpott et al. (2019) and is illustrated in **Figure 1**. Once the participants were pair-matched using the variable muscle mass divided by body mass, they were randomly assigned (randomizer.org) to either the energy restriction group (ER, $n = 14$) or the eucaloric control group (CG, $n = 14$). The study protocol consisted of 1 week under eucaloric conditions (45 kcal/kg) for both groups followed by a 6-week intervention period in which the ER group only consumed 30 kcal/kg. The CG maintained their energy intake. Protein consumption was at 2.8 g/kg fat-free-mass (FFM) for both groups during the whole study period. While body composition was assessed weekly via multifrequency bioelectrical impedance analysis (MFBIA), contractile function (Tensiomyography and MyotonPRO), sleep status, and mood were measured in weeks 1, 3, and 5.

The study was approved by the local ethics committee (#2019-24, Goethe University Frankfurt, GER), was conducted in accordance with the ethical standards set by the declaration of Helsinki with its recent modification of Fortaleza (Brazil, October 2013), and met the ethical standards in sport and exercise science according to Harriss and Atkinson (2015). Moreover, the study was preregistered in the International Clinical Trials Registry Platform (WHO) with the registration number DRKS00017263.

Participants

An *a priori* power analysis was conducted using G*Power 3.1 (University Düsseldorf, Germany). The analysis determined that 28 participants were needed for a power of 0.80, with an effect size of $f = 0.22$ and an $\alpha = 0.05$. Given the fact that lean mass change differs between 0% (Huovinen et al., 2015), 30% (Morton et al., 2010), and up to 84% of the lost mass per week (Karila et al., 2008), no exact effect size calculation was possible. Therefore, we statistically calculated with 30% lean body mass loss and assumed a moderate effect. Accounting for MFBIA precision error and individual variability in lean body mass loss, we further lowered the effect size to detect possible lean mass alterations.

Thirty-five healthy males with no experience in resistance training, as assessed by a pre-study questionnaire, were recruited from local sports clubs and university courses (see **Figure 2**). One participant declined to participate and three participants were

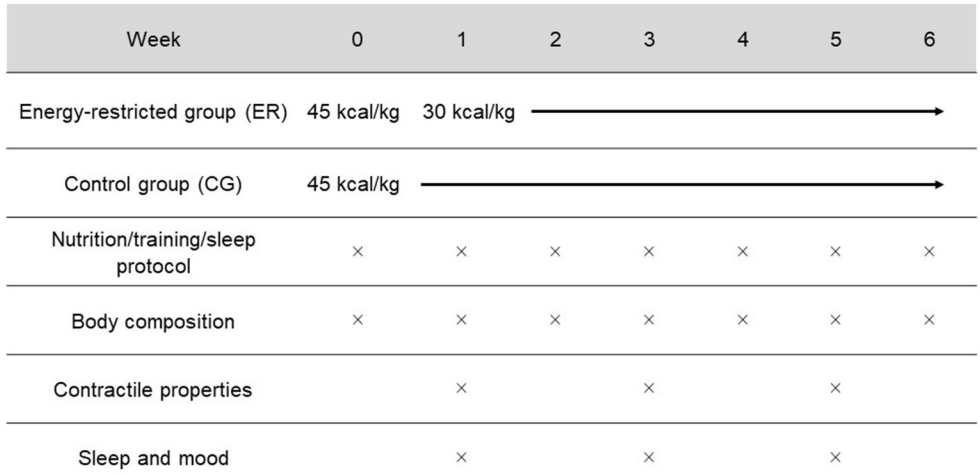

FIGURE 1 | Schematic overview of the study design. In week 0, all participants consumed 100% of their energy requirements (45 kcal/kg). For weeks 1–6, the ER group decreased their energy intake to 30 kcal/kg. Both groups consumed 2.8 g/kg FFM of protein and continued their habitual exercise during the study. As indicated by the × symbol, body composition was assessed weekly. Contractile properties, sleep, and mood were examined at weeks 1, 3, and 5.

excluded due to lacking protocol compliance (did not adhere to dietary intake). Finally, 28 healthy males (ER: age 26.57 ± 4.20 years; height 1.83 ± 0.05 m; body mass 82.26 ± 8.18 kg; CG: age 25.29 ± 2.97 years; height 1.81 ± 0.09 m; body mass 79.19 ± 6.43 kg) were used for data analysis. Due to hormonal fluctuations (Cumberledge et al., 2018), only male participants were included in order to increase reliability. The participants, who all reported that anabolic-androgenic drugs have never been consumed before, undertook at least two sport sessions per week. Since we only aimed for including lean participants, participants were excluded if their body fat was above 25%; this is the cut-off value for obesity, as suggested by Beals et al. (2019). During the study, the participants were asked to continue their habitual training. All participants were informed about the goal of the study as well as its conduction; in particular, interventional strains and requirements were highlighted. Every individual voluntarily agreed and gave written and informed consent to participate in the study.

Diet and Exercise

On each day during the study, all participants provided self-reported dietary intakes (energy, protein, carbohydrates and fats) using a smartphone app (MyFitnessPal®) as well as their daily body mass. For the latter, participants reported to the nearest 0.1 kg on their own digital scales wearing only underwear. The use of mobile apps for dietary self-reporting is considered to be reliable (Evenepoel et al., 2020). Every subject had either previously used this mobile app or was instructed and taught in a separate one-day workshop given by our lab, as suggested by Capling et al. (2017). In order to increase the compliance rate, the participants received cooking recipes and links to adequate webpages.

In the first week of the study (week 0), both groups had to match an energy intake of 45 kcal/kg. Since our participants reported to be highly active, we decided to stay slightly above the current recommendations of 45 kcal/kg FFM (Economos et al., 1993; Koehler et al., 2016). At the beginning of the intervention period, the ER group decreased their energy to 30 kcal/kg for 6 weeks to induce a moderate energy deficit (Chappell et al., 2018). For data analysis, energy availability was calculated as recommended (Heikura et al., 2018). Protein consumption was controlled during the maintenance and the intervention phase for both groups and was set at 2.8 g/kg FFM (Helms et al., 2014; Hector and Phillips, 2018; Witard et al., 2019). Due to (1) the anabolic effect of protein on muscle protein synthesis, as well as (2) a potential adaptation effect to higher protein intakes with a subsequent increased risk of protein catabolism (Millward, 2001), we aimed for the higher end of the current protein recommendations (Murphy et al., 2015; Bandegan et al., 2017). The remaining energy was individually distributed to carbohydrates and fats as preferred by the participants. Every type of consumed food and drinks (in g and ml respectively) had to be tracked in the nutritional diary. Supplements could be consumed *ad libitum*; however, the participants were asked to abstain from creatine. Compliance was checked weekly by screening all submitted protocols. If unclarities appeared (e.g., protein intake was too low), we kindly asked the participant to improve this issue during the following days. Participants were encouraged to honestly report any non-compliance.

All participants continued their habitual exercise throughout the study. Moreover, the participants provided a self-reported exercise diary on a daily basis as described (Lee et al., 2015). Since resistance training was prohibited during the study, all types of other sports were allowed. The participants were asked to provide sport-specific information for each training session including subjective intensity of the training as well as training duration. Baseline characteristics (week 0) are shown in **Table 1**.

Measurements

Body composition was assessed using MFBIA, 3-compartment model (Nutriguard-MS Vers. 2, Data-Input, Darmstadt, Germany). Examination was conducted as described in the

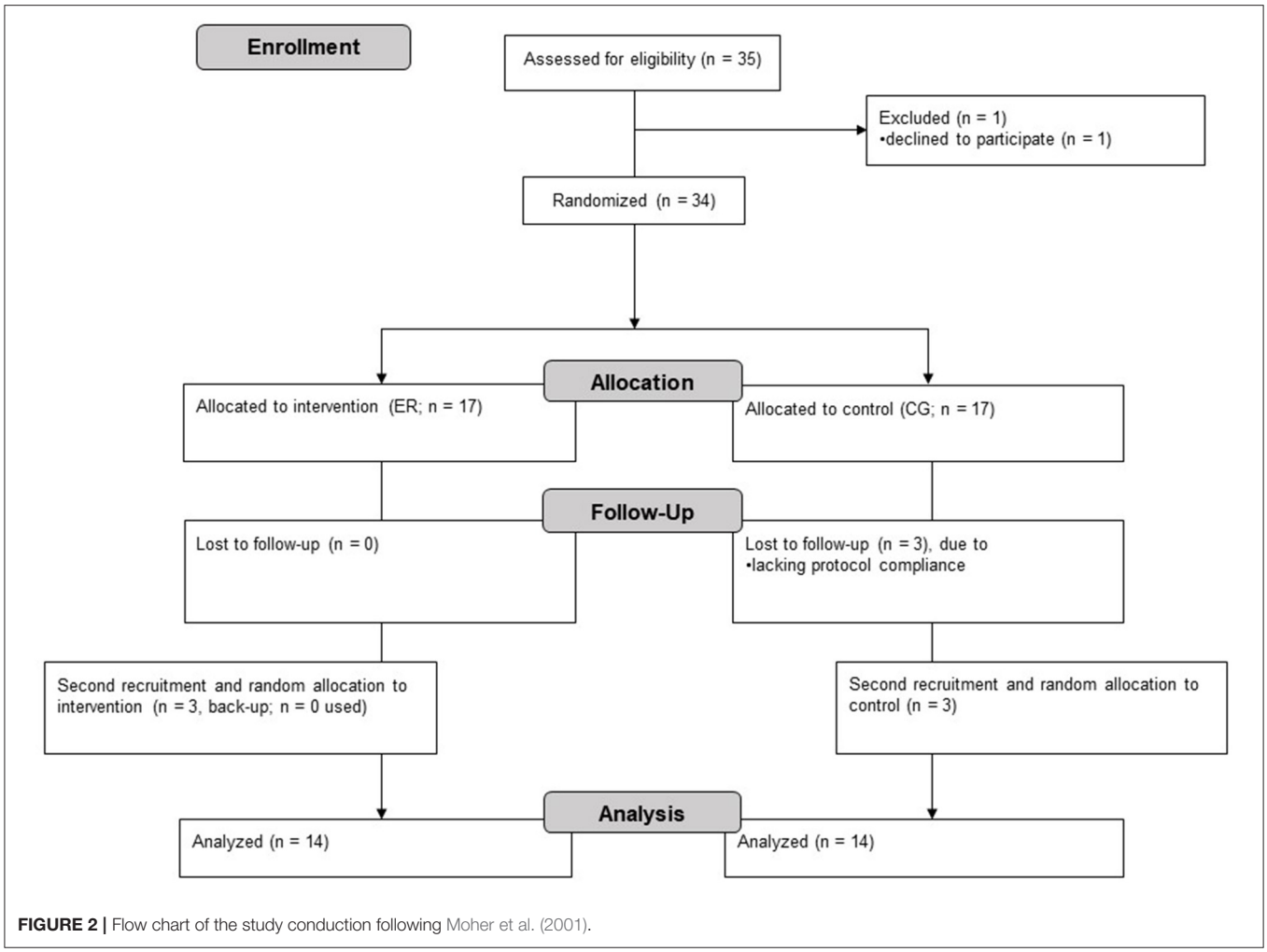

FIGURE 2 | Flow chart of the study conduction following Moher et al. (2001).

TABLE 1 | Baseline characteristics in the energy-restricted group (ER) and the control group (CG) during the maintenance week (week 0).

	ER	CG	*p*-value
Age (years)	26.57 ± 4.20	25.29 ± 2.97	0.358
Height (m)	1.83 ± 0.05	1.81 ± 0.09	0.836
Body mass (kg)	82.24 ± 8.18	79.19 ± 6.43	0.328
BMI (kg/m²)	24.68 ± 2.19	24.55 ± 2.54	0.890
Physical activity (minutes/week)	403.27 ± 292.30	389.00 ± 232.03	0.907
Lean mass (kg)	65.87 ± 6.19	64.04 ± 5.36	0.451
Fat mass (%)	20.12 ± 3.90	19.16 ± 3.48	0.534
Energy intake (kcal/day)	3355.88 ± 510.67	3355.61 ± 332.87	0.999
Protein intake (g/day)	182.20 ± 25.55	160.10 ± 22.36	0.036[#]

[#]*Indicates a significant baseline group difference ($p < 0.05$) during week 0 as assessed by independent t-test or Mann-Whitney U-test (data in means ± standard deviation).*

manufacturer's manual following the ESPEN guidelines (Kyle et al., 2004). Briefly, two adhesive electrodes (Bianostic AT, Data-Input, Darmstadt, Germany) were placed on the dominant side of the body: the dorsal surface of the hand and foot proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints. Another two electrodes were placed at the pisiform prominence of the wrist, with the proximal side covering half of the ulnar tubercle, as well as between the medial and lateral malleoli, with the proximal side covering half of the medial malleolus. The dominant side was determined by asking the participants for their dominant side and was maintained for every measurement. In this context, three frequencies (5, 50, and 100 kHz) were

used at a current of 800 μA. Uncertainties of resistance (R) and reactance (Xc) given by the manufacturer were depicted as ± 1 ohm and ± 1 ohm, respectively, whilst the precision of measurement was given as 0.5% and 2.0% differing from the value, respectively.

MFBIA (whole body) was tested weekly in a supine position. The same experienced examiner carried out the standardized measurements throughout the entire study period. Participants visited the lab after an overnight fast between 8 and 11 a.m. and emptied their bladder to control for hydration status between the different measurements (Turocy et al., 2011; Bosy-Westphal and Müller, 2014). This was verified by extracellular/intracellular water ratio which is described as a highly sensitive indicator of hydration status change (Wang et al., 2007; Inal et al., 2014; Brzozowska et al., 2019). For instance, deviating toward 1 would suggest a water shift to the extracellular space which is indicative of water loss. Furthermore, the participants were asked to abstain from physical activity the day before testing. Following every testing, a second measurement was conducted to ensure correct values. If the values deviated by more than 3 units digit, a third measurement was conducted and the mean values were calculated. In the context of tracking body composition changes, MFBIA is considered as a reliable tool (Moon, 2013; Bosquet et al., 2017) during hypercaloric (Schoenfeld et al., 2020b) and hypocaloric conditions (Antonio et al., 2019a) in an athletic population, producing similar values as Dual Energy X-ray Absorptiometry (DXA) in males (Golja et al., 2020). Moreover, MFBIA appears to be valid in detecting total body water changes (Utter et al., 2012).

Tensiomyography (TMG; TMG-BMC Ltd., Lublijana, Slovenia) was used to assess the contractile function of the m. rectus femoris (dominant side, supine position). TMG is a method to assess radial deformation of the muscle after a single electrical stimulus. Before the first measurement, the center of the anterior inferior iliac spine and the upper edge of the patella was defined, the thickest part of the muscle belly manually palpated and marked with a skin-friendly pen. Subsequently, a high-precision digital displacement sensor was applied perpendicularly to the muscle belly with a spring constant of 0.17 N mm^{-1} (Macgregor et al., 2018) and retracted into its housing by ∼2 cm. If necessary, the sensor position was slightly adjusted to locate an area with the greatest amount of muscle belly to sustain an optimal point (Šimunić, 2012). In order to ensure precise inter-day reliability, we strongly encouraged the participants to redraw the marked points following water or sweat-yielding events.

Muscle twitch was induced through a single 1-ms-wide electrical stimulus with the cathode placed distal and symmetrically to the anode (Zubac et al., 2017). The electrodes (self-adhesive; dura-stick plus, 50 × 50 mm), which had an inter-electrode distance of 5 cm as suggested by Piqueras-Sanchiz et al. (2020), were attached on shaved skin. A Blackrole® was deposited under the dominant leg to ensure a leg angle of 120° as suggested (Paula Simola et al., 2015; Sánchez-Sánchez et al., 2018). In order to identify peak muscle response, we progressively increased the intensity at a 10 mA interval every 30 s, beginning with 30 mA (Lohr et al., 2018; Wilson et al., 2018) up until there was no further increase in the amplitude or until maximal output was reached (110 mA) as recommended by Šimunić (2012). Only the curve with the highest maximum of radial displacement was included in the analysis (García-García et al., 2018). In addition to the five standard TMG parameters, which include the maximal radial muscle displacement (D_m), contraction time (T_c), delay time (T_d), sustain time (T_s), and half relaxation time (Tr), we calculated muscle contraction velocity (V_c) as D_m divided by the sum of T_d and T_c (Loturco et al., 2016) multiplied by 1000 (mm/s). Relative reliability (ICC) was excellent for D_m, T_c, V_c, and T_d during inter-day testing, with T_r being the least reliable parameter (Rodriguez Matoso et al., 2010; Šimunić, 2012; Ditroilo et al., 2013; Lohr et al., 2018, 2019).

MyotonPRO (MMG; Myoton Ltd., Tallinn, Estonia) was used to extend the muscle quality assessment. In general, MMG is utilized to evaluate viscoelastic characteristics of skeletal muscles and other soft tissues (Aird et al., 2012). MMG causes a light mechanical impulse (0.15 N for 15 ms) to the relaxed muscle and records the natural oscillation of myofascial tissue by a 3-axis digital acceleration sensor sampled at 3200 Hz (Gavronski et al., 2007; Viir et al., 2011). From this raw data, MMG calculates the parameters of stiffness (S, N/m), logarithmic decrement (D, without unit), frequency (F, Hz), relaxation time (R, ms), and creep (C, without unit).

MMG was placed perpendicularly on the same palpated point as described in the TMG section. Per measurement, we applied three measures in multiscan mode, producing five single measures with a 1 s interval. If two of the measures were equal, this value was taken; otherwise, a mean value was calculated. If the coefficient of variation was above 3%, this measure was repeated (Lohr et al., 2018). Most of the studies confirmed good to excellent inter-day reliability for S, D, and F when m. rectus femoris was examined (Bizzini and Mannion, 2003; Zinder and Padua, 2011; Aird et al., 2012; Mullix et al., 2012). Both MMG and TMG were assessed at weeks 1, 3, and 5.

The German version of the *Profile of Mood States* (POMS-G) was utilized to detect possible mood changes during the study period (McNair et al., 1981; Bullinger et al., 1990). A pathopsychological state might affect training performance and, hence, may have an effect on lean mass retention (Franchini et al., 2012; Sundgot-Borgen et al., 2013; Stults-Kolehmainen et al., 2014). Consequently, POMS-G was assessed at weeks 1, 3, and 5. The POMS-G is a frequently-used, reliable and valid questionnaire (Albani et al., 2005; Grulke et al., 2006). In contrast to the original version (McNair et al., 1992), POMS-G is a short form consisting of 35 items and 4 scales (depression-anxiety, fatigue, vigor, and hostility). Each item is assessed on a 7-point Likert scale and retrospectively examines mood state during the last 24 h. Due to its similarities to the English version, our findings can also relate to studies using the English version (Kellmann and Golenia, 2003).

Duration of *sleep* (sleep onset and hours of sleep per night, assessed with a sleep diary) and subjective sleep quality (PSQI-G) were assessed daily and at weeks 1, 3, and 5, respectively. While sleep has mediating effects on testosterone production and muscle protein synthesis (Leproult and van Cauter, 2011; Pejovic et al., 2013), we aimed to clarify the effect of a high-protein energy

restriction on sleep quality in healthy male college students. The PSQI is a reliable clinical sleep-behavior questionnaire which was also validated for the general population (Buysse et al., 1989). In contrast to the original version, the PSQI-G assesses the global sleep score in a 2 week interval (Riemann and Backhaus, 1996). The questionnaire contains 19 questions each using Likert scales from 0 to 3 and is categorized into seven sub-variables which are summed up to the PSQI-G score. Regarding cut-off values, scores >5 are associated with a poor sleep condition and ≤ 5 with a good sleep condition (Zhou et al., 2016). During the intervention, we used the standardized procedure as reported (Riemann and Backhaus, 1996).

Statistical Analysis

A general linear two-way mixed ANOVA with repeated measures [group (2) × time (3/6)] and pairwise comparisons (Bonferroni correction) was performed separately for each dependent variable (SPSS version 24.0, Chicago, IL, USA). When a significant group × time interaction was revealed or the Box's test exposed statistical significance, the simple main effects were examined separately using (a) repeated-measures ANOVA (time) and (b) univariate ANCOVA covarying for t_1 (group). Before tests were calculated, the research team did an (a) visual review of boxplots, (b) test of normal distribution with the Shapiro-Wilk's test, (c) Levene's test for homogeneity of variance, (d) Box's test of equality of covariance matrices, as well as (e) Mauchly's test of sphericity. Dependent *t*-tests were further carried out to evaluate changes between week 0 and week 1. All tests were based on a 5% level of significance. Data are presented as means ± standard deviation. When possible, effect sizes were reported.

RESULTS

Body Composition

A significant group × time interaction was found for body mass [$F_{(3.488,90.676)} = 14.604$, $p < 0.001$, partial $\eta^2 = 0.360$]. The simple main effect for time revealed a significant body mass loss in the ER group [$F_{(5,65)} = 12.745$, $p < 0.001$, partial $\eta^2 = 0.495$] between week 1, week 5, and week 6 and a body mass gain in the CG [$F_{(5,65)} = 6.033$, $p < 0.001$, partial $\eta^2 = 0.317$]. Additionally, significant between-group differences were exhibited beginning at week 2 [$F_{(1,25)} = 5.156$, $p = 0.032$, partial $\eta^2 = 0.171$]. Consequently, BMI changed significantly from week 1 to week 6 ($p < 0.001$; **Table 2**).

A significant group × time interaction was found for lean body mass [$F_{(5,130)} = 4.673$, $p < 0.001$, partial $\eta^2 = 0.152$; **Figure 3**]. While lean body mass significantly declined over time in the ER group [$F_{(5,65)} = 6.181$, $p < 0.001$, partial $\eta^2 = 0.332$], the CG increased lean body mass [$F_{(5,65)} = 4.369$, $p = 0.002$, partial $\eta^2 = 0.252$]. For the ER group, a significant difference was solely observed between week 3 and week 6 ($p = 0.002$). Contrarily, between-group differences revealed statistical significance at the beginning of week 3 [$F_{(1,25)} = 6.921$, $p < 0.05$, partial $\eta^2 = 0.217$]. The lean body mass change ranged from +1 kg to −5.2 kg in the ER group and, on average, accounted for 47% of the lost body mass. Hydration status as assessed by extracellular/intracellular water ratio was constant throughout the study in both groups ($p > 0.05$). Further MFBIA derived parameters are collated in **Supplementary Table 6**.

Similar to what has been reported for lean body mass, the body cell mass, representing the protein-rich and metabolically-active compartments of the body, showed a significant group × time interaction [$F_{(3.190,82.951)} = 5.740$, $p < 0.001$, partial $\eta^2 = 0.181$]. While the simple main effect for time also exhibited a significant decrease in the ER group [$F_{(5,65)} = 6.851$, $p = 0.003$, partial $\eta^2 = 0.345$] as well as a significant increase in the CG [$F_{(5,65)} = 4.078$, $p = 0.003$, partial $\eta^2 = 0.239$], between-group differences were found at the beginning of week 2 [$F_{(1,25)} = 4.871$, $p < 0.05$, partial $\eta^2 = 0.163$]. Pairwise comparisons over time located the meaningful differences in the ER group between week 3, week 5 and week 6 ($p < 0.05$). While we did not find a group × time interaction for extracellular mass ($p = 0.10$), the main effect for time revealed a change in both groups [$F_{(5,130)} = 2.592$, $p = 0.029$, partial $\eta^2 = 0.091$]. However, no significant between-group differences were observed for the extracellular mass ($p = 0.993$).

A significant group × time interaction was seen for total body water [$F_{(5,130)} = 4.681$, $p < 0.001$, partial $\eta^2 = 0.153$]. The simple main effect for time revealed a significant decline in total body water in the ER group [$F_{(5,65)} = 6.093$, $p < 0.001$, partial $\eta^2 = 0.319$] as well as a significant increase in the CG [$F_{(5,65)} = 4.259$, $p = 0.002$, partial $\eta^2 = 0.247$], with pairwise comparisons revealing statistical meaningful differences between week 3 and week 6 in the ER group ($p = 0.003$). Moreover, we identified a significant between-group difference for total body water change beginning with week 4 [$F_{(1,25)} = 4.676$, $p < 0.05$, partial $\eta^2 = 0.158$]. Total body water can be further divided into intracellular and extracellular water. Since both variables revealed a significant Box's test, only the simple main effects were interpreted. While the ER group showed a significant decrease of intracellular water over time [$F_{(5,65)} = 10.426$, $p < 0.001$, partial $\eta^2 = 0.445$], no change could be detected in the CG ($p = 0.335$). Pairwise comparisons showed significant differences in the ER group between week 1 and week 6, week 2 and week 6, week 3, week 5 and week 6 as well as week 4 and week 6 ($p < 0.05$). Furthermore, significant between-group differences were found at the beginning of week 5 [$F_{(1,25)} = 5.848$, $p = 0.023$, partial $\eta^2 = 0.190$]. Similar to the extracellular mass, extracellular water decreased only in the ER group [$F_{(5,65)} = 3.160$, $p = 0.013$, partial $\eta^2 = 0.196$], but did not in the CG ($p = 0.380$). Herein, no between-group differences were observed ($p > 0.05$).

The body fat percentage showed a significant group × time interaction [$F_{(5,130)} = 19.819$, $p < 0.001$, partial $\eta^2 = 0.433$]. The simple main effect for time exhibited a significant decrease in the ER group [$F_{(2.202,28.623)} = 14.632$, $p < 0.001$, partial $\eta^2 = 0.530$] as well as a significant increase in the CG [$F_{(2.080,27.036)} = 6.287$, $p = 0.005$, partial $\eta^2 = 0.326$]. We found a significant difference in the simple main effect for group beginning with week 2 [$F_{(1,25)} = 11.036$, $p < 0.05$, partial $\eta^2 = 0.306$].

Diet and Exercise

Food diary analysis showed that the participants in the ER group consumed less energy compared to the maintenance period ($p < 0.001$) and the CG ($p < 0.001$). Regarding energy and protein intake, compliance was >90% on average per group. In individual

TABLE 2 | Overview of body composition changes in the energy-restricted group (ER) and the control group (CG).

		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Δ
Body mass (kg)	ER	82.26 ± 8.18	80.58 ± 8.18§	80.14 ± 8.85$^{\#}$	79.93 ± 8.47$^{\#}$	79.13 ± 8.41$^{\#}$	77.92 ± 7.76$^{\#}$	77.36 ± 8.00$^{*x\#}$	−3.22
	CG	79.19 ± 6.43	77.49 ± 6.62	78.41 ± 6.34$^{\#}$	79.79 ± 6.19$^{\dagger\#}$	79.37 ± 6.81$^{\#}$	79.26 ± 6.58$^{\#}$	79.39 ± 6.09$^{x\#}$	1.90
BMI (kg/m²)	ER	24.68 ± 2.19	24.11 ± 2.41					23.13 ± 2.19$^{x\#}$	−0.98
	CG	24.55 ± 2.54	23.71 ± 2.46					24.29 ± 2.45$^{x\#}$	0.58
Lean body mass (kg)	ER	65.87 ± 6.19	64.81 ± 5.89§	64.82 ± 6.50	64.98 ± 6.18$^{\#}$	64.54 ± 5.85$^{\#}$	63.69 ± 5.78$^{\#}$	63.32 ± 5.84$^{*x\#}$	−1.49
	CG	64.04 ± 5.36	63.33 ± 5.33	63.93 ± 5.31	64.94 ± 5.12$^{\dagger\#}$	64.46 ± 5.63$^{\#}$	64.11 ± 4.96$^{\#}$	64.01 ± 5.14$^{\#}$	0.68
Body cell mass (kg)	ER	37.92 ± 3.69	37.69 ± 3.80	37.69 ± 4.03$^{\#}$	37.89 ± 3.76$^{\#}$	37.41 ± 3.71$^{\#}$	37.05 ± 3.72$^{\#}$	36.84 ± 3.82$^{*\#}$	−0.85
	CG	36.95 ± 3.44	36.66 ± 3.48	37.26 ± 3.71$^{\#}$	37.50 ± 3.44$^{\dagger\#}$	37.32 ± 3.74$^{\#}$	37.19 ± 3.49$^{\#}$	37.25 ± 3.74$^{\#}$	0.59
Body fat (%)	ER	20.12 ± 3.90	19.44 ± 4.50	18.91 ± 4.56$^{\#}$	18.58 ± 4.36$^{\dagger\#}$	18.24 ± 4.64$^{\#}$	17.85 ± 4.39$^{\#}$	17.70 ± 4.40$^{x\#}$	−1.74
	CG	19.16 ± 3.48	17.96 ± 3.90	18.33 ± 3.87$^{\#}$	18.72 ± 3.72$^{\dagger\#}$	18.74 ± 3.96$^{\#}$	19.92 ± 4.14$^{\#}$	19.18 ± 3.57$^{x\#}$	1.22
Intracellular water (l)	ER	28.32 ± 1.94	27.98 ± 1.90§	27.91 ± 2.03	28.09 ± 1.94	27.91 ± 1.86	27.65 ± 1.90$^{\#}$	27.40 ± 1.96^{*x}	−0.58
	CG	27.43 ± 2.22	27.25 ± 2.11	27.49 ± 2.20	27.79 ± 2.07	27.86 ± 1.76	27.82 ± 1.65$^{\#}$	27.80 ± 1.73	0.55
Extracellular water (l)	ER	19.92 ± 2.68	19.47 ± 2.44	19.49 ± 2.74	19.47 ± 2.60	19.35 ± 2.45	18.99 ± 2.36	18.96 ± 2.38	−0.51
	CG	19.42 ± 3.12	19.11 ± 2.28	19.32 ± 2.19	19.71 ± 2.50	19.32 ± 2.39	19.09 ± 2.04	19.07 ± 2.08	−0.04

*§Indicates a significant difference between week 0 and week 1 ($p < 0.05$); †indicates a significant difference between week 1 and week 3 ($p < 0.05$); *indicates a significant difference between week 3 and week 6 ($p < 0.05$). ^{x}indicates a significant difference between week 1 and week 6 ($p < 0.05$). $^{\#}$indicates a significant between-group difference as shown by the simple main effect for group ($p < 0.05$); Δ was calculated as week 6–1.*

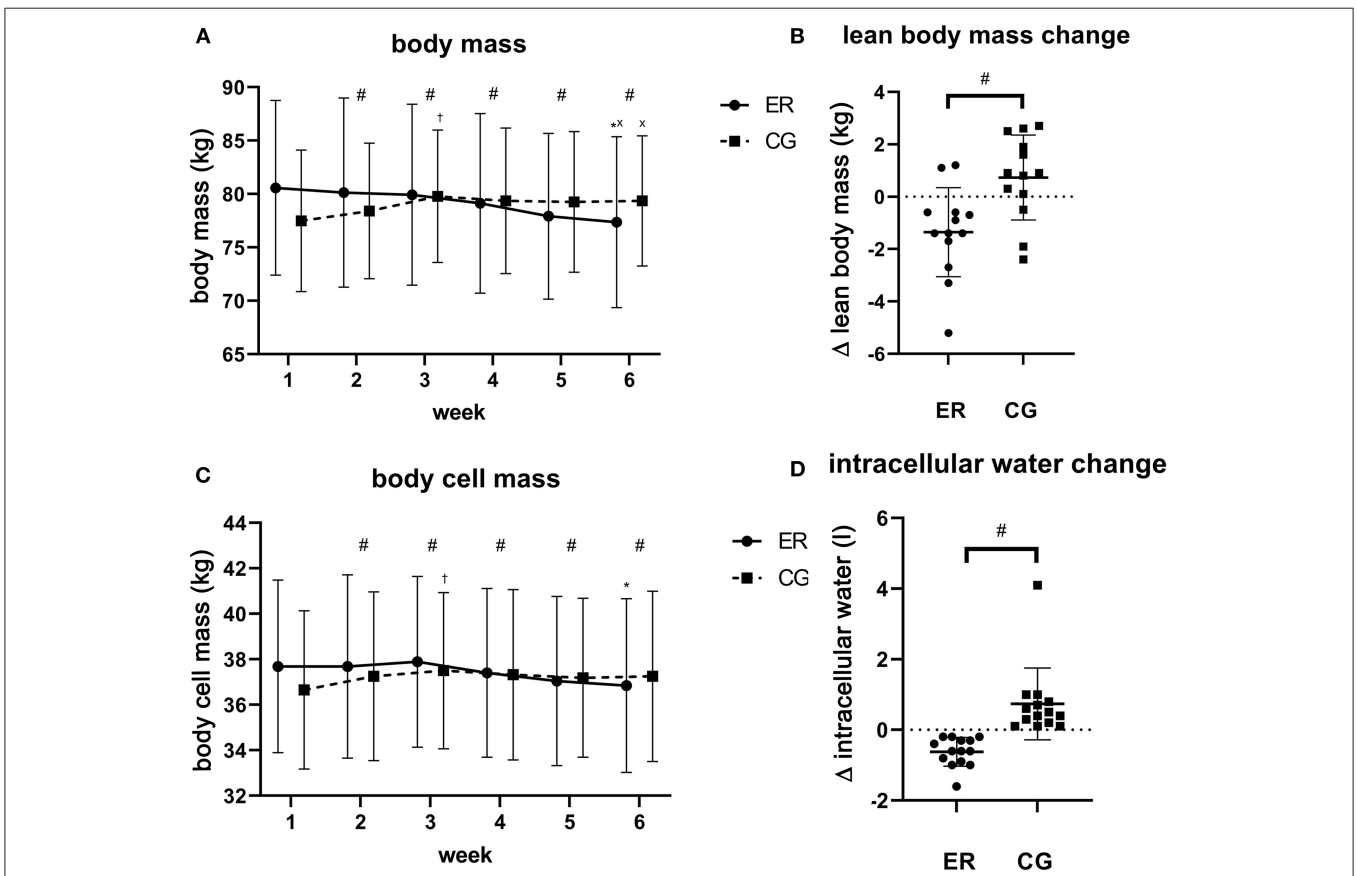

FIGURE 3 | Visual representation of measured parameters. The figure shows body mass change (kg) **(A)**, Δ lean body mass (week 6–1; kg) **(B)**, body cell mass change (kg) **(C)** as well as Δ intracellular water (week 6–1; l) **(D)**. Data is plotted as means ± standard deviation. $^{\#}$Illustrates a significant difference between groups ($p < 0.05$). †Indicates a significant difference between week 1 and week 3 ($p < 0.05$); *Indicates a significant difference between week 3 and week 6 ($p < 0.05$). ^{x}Indicates a significant difference between week 1 and week 6 ($p < 0.05$). ●, energy-restricted group (ER); ■, control group (CG).

numbers, the energy intake of the ER group equated to 29.65 ± 1.63 kcal/kg with an energy availability of 31.36 ± 3.13 kcal/kg FFM, respectively (**Table 3**). In contrast, energy intake of the CG equated to 42.64 ± 2.57 kcal/kg with an energy availability of 48.98 ± 3.36 kcal/kg FFM. Based on Hall's formula (Hall, 2008), the calculated energy deficit was ∼-535 kcal/day for the ER group and 316 kcal/day for the CG. Except for week 0, no significant differences were found for protein consumption ($p > 0.05$). Retrospectively, protein consumed was 2.77 ± 0.26 g/kg FFM for the ER group and 2.62 ± 0.33 g/kg FFM for the CG. While the ER group significantly reduced fat and carbohydrate intake between the maintenance and the intervention period ($p < 0.001$), significant between-group differences were spotted in the individual fat (ER: 0.95 ± 0.21 g/kg; CG: 1.45 ± 0.36 g/kg) and carbohydrate (ER: 2.89 ± 0.44 g/kg; CG: 4.79 ± 0.96 g/kg) intake throughout the study period ($p < 0.001$). During the study, the participants in both groups supplemented protein shakes, multivitamin supplements to avoid deficiencies, omega-3 and caffeine.

The participants continued their habitual training during the study. In summary, 14 different sports were practiced: gymnastics, bouldering, climbing, soccer, spikeball, bicycling, jogging, table tennis, swimming, volleyball, basketball, boxing, dancing, and paddleboarding. No significant differences in minutes of sport per week, as well as subjective intensity during training were found between the groups ($p > 0.05$). Training sessions per week varied for both groups between 1 and 6 sessions (ER: 4.46 ± 1.76; CG: 2.86 ± 1.29, $p = 0.012$).

Contractile Properties

For TMG, no significant differences were found for T_s (ER: Δ −4.82 ms; CG: Δ 16.63 ms), T_r (ER: Δ −16.65 ms; CG: Δ 16.90 ms) and T_d (ER: Δ 1.28 ms; CG: Δ 0.34 ms, all $p > 0.05$). Although group allocation had no effect on T_c (ER: Δ 3.04 ms; CG: Δ −0.47 ms), D_m (ER: Δ 0.91 mm; CG: Δ 0.66 mm), and V_c (ER: Δ 3.84 mm/s; CG: Δ 10.85 mm/s) change, there appears to be an increasing trend in the ER group ($p = 0.10$) as well as in the ER and the CG ($p = 0.066$) for T_c and D_m over time, respectively (**Figure 4**; **Supplementary Table 7**). Lastly, V_c significantly increased to week 3 but returned to baseline at week 5.

For MMG, no significant differences were found for stiffness (ER: Δ −4.42 N/m; CG: Δ −4.62 N/m), decrement (ER: Δ −0.04; CG: Δ −0.01), relaxation time (ER: Δ 0.37 ms; CG: Δ 0.08 ms) and creep (ER: Δ 0.02; CG: Δ 0.00, all $p > 0.05$). While frequency did not change over time (ER: Δ 0.00 Hz; CG: Δ 0.04 Hz, $p > 0.05$), the groups differed by trend ($p = 0.057$). An overview of the MMG values is found in **Table 4**.

Sleep and Mood Analysis

No significant differences were detected for sleep in hours per night and time to fall asleep ($p > 0.05$). While the PSQI-G score significantly decreased over time [$F_{(2,52)} = 5.568$, $p = 0.006$, partial $\eta^2 = 0.176$], no significant differences were found between the ER group (Δ −1.43) and the CG (Δ −0.64; $p = 0.247$).

Profile of mood states analysis did not reveal a significant difference for depression/anxiety (ER: Δ −2.36; CG: Δ 2.50), fatigue (ER: Δ −3.43; CG: Δ 1.22), and hostility (ER: Δ −3.64; CG: Δ 1.64; all $p > 0.05$). However, vigor decreased significantly over time [$F_{(2,52)} = 3.417$, $p = 0.040$, partial $\eta^2 = 0.116$] with no differences between the ER group (Δ −2.79) and the CG (Δ −4.71; $p = 0.583$; **Table 5**). In this context, sleeping hours per night correlated with vigor change ($r = 0.422$, $p = 0.025$).

DISCUSSION

Body Composition

In this study, we tested the effect of a high-protein moderate energy restriction on body composition change. Generally, maintaining muscle mass is an important health factor due to role of muscle as a primary site of postprandial glucose disposal, lipid oxidation and resting energy expenditure (Hector and Phillips, 2018). In the context of sports, temporary phases of energy restriction are used to reduce body mass while trying to maintain as much lean body mass as possible (Artioli et al., 2010). In particular, lean body mass retention is not only crucial for athletic performance (Wolfe, 2006), but also correlates with athletic success (Slater et al., 2005; Chappell et al., 2018).

In the ER group, lean body mass decreased significantly between week 3 and week 6 with an average total loss of −1.49 kg. According to Siedler et al. (2021), BIA day-to-day variance in lean body mass is as high as 0.9 kg. However, since the decrease in lean body mass is greater than what could be explained by BIA precision error, our data suggest real lean body mass loss in the ER group. With that said, we conclude that the investigated high-protein moderate energy restriction is likely not able to prevent lean mass loss in college students in the absence of resistance training. Consequently, our hypothesis is rejected. Notably, it is unknown whether protein intake at 2.8 g/kg FFM prevented larger decreases in lean body mass. Contrarily, lean body mass was not negatively altered in the CG. Since the CG increased body mass, this indicates a slight caloric surplus.

The energy-restriction-induced reduction of lean body mass is in accordance with the majority of studies (Karila et al., 2008; Pikosky et al., 2008; Morton et al., 2010; Wilson et al., 2012; Pasiakos et al., 2013; Rhyu and Cho, 2014), albeit conflicting results exist (Paoli et al., 2012; Huovinen et al., 2015; Wilson et al., 2015). Since caloric intake, total protein consumption, sex, and sleep duration were taken into account, the inter-study differences may, at least partly, be explained by the magnitude of mechanical tension the body is exposed (Callahan et al., 2021). In one of the studies reporting no significant lean mass change, Paoli et al. (2012) recruited elite artistic gymnasts using a keto-approach (−400 kcal/day, high-protein). With respect to their training regimen, an intense schedule of body weight exercises was carried out which might have led to a greater fiber recruitment of the loaded muscles. In turn, this could have acted as an anabolic stimulus and, in connection with the small energy restriction applied (Karila et al., 2008; Heymsfield et al., 2011), may have led to the retention of muscle mass. This seems to be in accordance with our study showing individual variation in lean mass change in the context of the different types of physical activity performed. Notably, given the fact that Paoli et al. (2012) studied elite athletes, we cannot rule out

TABLE 3 | Energy intake, dietary intake, and physical activity in the energy-restricted group (ER) and the control group (CG) during the study.

		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Energy (kcal/day)	ER	3355.88 ± 510.67	2396.82 ± 248.86$^{\S\#}$	2386.65 ± 279.88$^{\#}$	2362.62 ± 309.36$^{\#}$	2372.48 ± 290.45$^{\#}$	2351.33 ± 291.52$^{\#}$	2382.41 ± 249.81$^{\#}$
	CG	3355.61 ± 332.87	3356.78 ± 333.46$^{\#}$	3331.54 ± 346.68$^{\#}$	3340.70 ± 386.22$^{\#}$	3320.97 ± 328.05$^{\#}$	3336.28 ± 344.55$^{\#}$	3330.19 ± 515.20$^{\#}$
Protein (g/day)	ER	182.20 ± 25.55$^{\#}$	180.32 ± 24.26	178.59 ± 26.88	179.44 ± 22.16	179.92 ± 20.03	180.09 ± 22.47	190.35 ± 22.75
	CG	160.10 ± 22.36$^{\#}$	166.81 ± 26.15	166.78 ± 24.34	168.88 ± 28.89	171.16 ± 25.66	172.19 ± 26.62	160.47 ± 31.99
Fat (g/day)	ER	113.24 ± 25.50	79.22 ± 11.41$^{\S\#}$	75.86 ± 21.37$^{\#}$	76.13 ± 23.18$^{\#}$	78.30 ± 20.31$^{\#}$	71.26 ± 14.92$^{\#}$	71.64 ± 15.62$^{\#}$
	CG	109.12 ± 29.16	122.63 ± 29.29$^{\#}$	119.99 ± 30.15$^{\#}$	123.87 ± 38.59$^{\#}$	119.04 ± 32.21$^{\#}$	122.51 ± 35.93$^{\#}$	104.81 ± 34.63$^{\#}$
Carbohydrates (g/day)	ER	383.93 ± 92.21	233.77 ± 34.84$^{\S\#}$	239.46 ± 43.76$^{\#}$	230.80 ± 44.06$^{\#}$	231.31 ± 39.12$^{\#}$	236.45 ± 43.20$^{\#}$	226.39 ± 33.86$^{\#}$
	CG	388.95 ± 88.91	358.33 ± 93.79$^{\#}$	367.31 ± 75.96$^{\#}$	352.66 ± 84.81$^{\#}$	366.58 ± 71.76$^{\#}$	366.60 ± 91.18$^{\#}$	411.44 ± 77.61$^{\#}$
Physical activity (minutes/week)	ER	403.27 ± 292.30	343.08 ± 221.83§	294.17 ± 195.68	273.46 ± 175.55	255.83 ± 139.74	367.73 ± 342.91	257.62 ± 143.89
	CG	389.00 ± 232.03	221.43 ± 178.02§	268.08 ± 179.66	317.00 ± 195.83	235.83 ± 147.54	234.62 ± 172.22	231.07 ± 135.49

§*Significantly differed from week 0 ($p < 0.05$);* $^{\#}$*indicates a significant between-group difference ($p < 0.05$).*

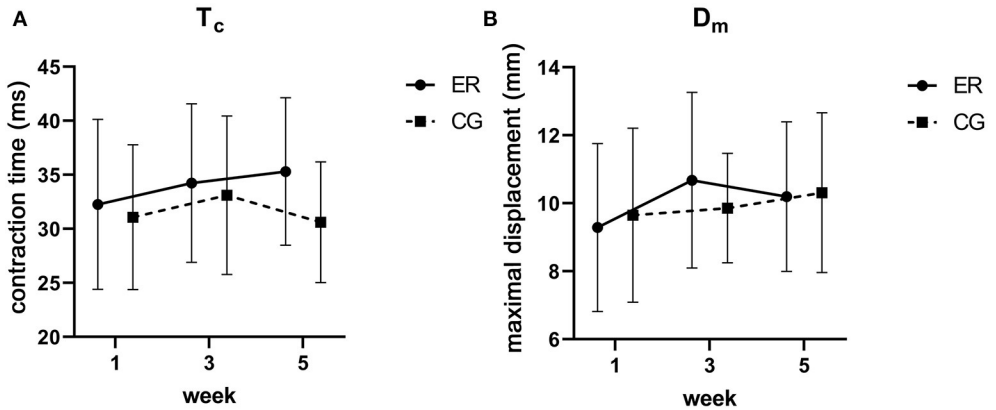

FIGURE 4 | Visual representation of contraction time [T_c in ms; **(A)**] and maximal displacement (D_m in mm) change **(B)**. Data is plotted as means ± standard deviation. • = energy-restricted group (ER), ■ = control group (CG).

TABLE 4 | Overview of the MyotonPRO analysis [energy-restricted group (ER), control group (CG)].

		Week 1	Week 3	Week 5
Stiffness (N/m)	ER	246.01 ± 25.20	243.30 ± 23.51	241.59 ± 26.60
	CG	258.70 ± 23.15	252.00 ± 27.69	254.08 ± 27.67
Decrement	ER	1.39 ± 0.19	1.41 ± 0.20	1.35 ± 0.22
	CG	1.42 ± 0.23	1.37 ± 0.23	1.41 ± 0.30
Frequency (Hz)	ER	13.98 ± 0.94	14.07 ± 0.88	13.98 ± 0.86
	CG	14.77 ± 1.11	14.59 ± 1.16	14.81 ± 1.18
Relaxation time (ms)	ER	21.93 ± 1.85	22.12 ± 1.81	22.30 ± 1.71
	CG	21.12 ± 1.59	21.20 ± 1.82	21.20 ± 1.80
Creep	ER	1.34 ± 0.09	1.35 ± 0.10	1.36 ± 0.10
	CG	1.30 ± 0.10	1.30 ± 0.10	1.30 ± 0.10

that strength and conditioning exercises were used additionally without being reported.

Since lean body mass in MFBIA depicts the fat-free compartments of the whole body with muscle mass only representing ∼50% (Serra-Prat et al., 2019), solely interpreting the lean body mass change may bias the results. Therefore, body cell mass, representing the protein-rich and metabolically-active compartments of the body (Kyle et al., 2004), i.e., the muscle and

TABLE 5 | Overview of the sleep and mood analysis [energy-restricted group (ER), control group (CG), PSQI-G (Pittsburgh sleep quality index–German)].

		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Sleep per night (hours)	ER	7.42 ± 0.87	7.70 ± 1.02[§]	7.71 ± 0.74	7.56 ± 0.63	7.63 ± 0.63	7.74 ± 0.80	7.67 ± 1.15
	CG	7.00 ± 0.96	7.25 ± 0.77	7.37 ± 0.65	7.45 ± 1.01	7.51 ± 0.94	7.38 ± 0.73	7.22 ± 1.30
Time to fall asleep (minutes)	ER	12.54 ± 7.19	13.58 ± 10.92	12.72 ± 9.77	14.46 ± 8.34	14.45 ± 14.89	18.01 ± 21.80	10.65 ± 6.74
	CG	18.89 ± 17.23	12.16 ± 8.36	12.18 ± 8.28	16.66 ± 19.89	11.40 ± 8.32	10.84 ± 6.40	7.05 ± 4.20
PSQI-G-score	ER		5.14 ± 1.75		3.93 ± 0.92		3.71 ± 1.27*	
	CG		5.07 ± 2.23		5.14 ± 1.51		4.43 ± 2.03*	
POMS-G								
Depression/anxiety	ER		24.86 ± 9.54		24.86 ± 9.45		22.50 ± 7.52	
	CG		23.86 ± 9.78		27.43 ± 12.43		26.36 ± 13.70	
Fatigue	ER		21.00 ± 6.19		21.21 ± 7.56		17.57 ± 8.67	
	CG		21.14 ± 6.70		20.14 ± 6.89		22.36 ± 8.02	
Vigor	ER		33.00 ± 6.26		31.50 ± 5.49		30.21 ± 8.05*	
	CG		32.21 ± 4.89		31.57 ± 7.94		27.50 ± 8.90*	
Hostility	ER		14.50 ± 6.98		13.93 ± 8.22		10.86 ± 5.48	
	CG		14.43 ± 6.05		16.79 ± 8.26		16.07 ± 9.47	

[§]*Indicates a significant difference between week 0 and week 1 ($p < 0.05$); *significantly differed to week 1 ($p < 0.05$).*

organ tissue, is probably the most sensitive marker for muscle loss in MFBIA. In accordance with what has been concluded for the lean body mass change, body cell mass linearly decreased over time in the ER group at the beginning of week 4. In this context, the herein depicted time course of muscle mass loss is in contrast to Heymsfield et al. (2011) who reported an almost linear muscle mass loss at the beginning of the hypocaloric phase in overweight individuals mainly based on the CALERIE study (Heilbronn et al., 2006; Redman et al., 2007; Rickman et al., 2011) and the study by Wood et al. (2007). Contrarily, Schoenfeld et al. (2020a) reported that lean mass loss predominantly occurred during the final weeks of the contest preparation. Since we cannot identify whether these differences might be attributed to the insensitivity of our MFBIA model, other moderator variables, or the potential protective properties of a high-protein dieting approach, this should be studied in future.

Muscle Contractile Properties

In this study, we tested the effect of a high-protein moderate energy restriction on muscle contractile properties which is, to our knowledge, the first study directly examining the impact of controlled dietary manipulations on TMG and MMG outcomes. In this context, we hypothesized that contractile properties are not negatively altered throughout the study.

Despite depicting high alterations in contractile properties (e.g., muscle force after electrical stimulation of the ulnar nerve) during severe caloric restriction (Lopes et al., 1982; Lennmarken et al., 1986), no group × time interaction was found for any tested variable. With that being said, we conclude that the high-protein moderately energy-restricted diet used in this study did not negatively alter muscle contractile properties. Consequently, our hypothesis is accepted. However, whether this advantage is due to the high-protein diet itself cannot be clarified with the present study and must be examined in future work. Notably, T_c, which is the contraction time in ms from 10 to 90% of D_m on the ascending curve (García-García et al., 2019), tended to increase over time and may reflect a muscle fiber type shift (Valencic and Knez, 1997; Dahmane et al., 2005; Šimunić et al., 2011; Zubac and Šimunić, 2017) in the context of region-specific muscle mass loss (Zubac et al., 2017; Paravlic et al., 2020). However, since different fiber type distributions highly influence the direction of the T_c shift (García-García et al., 2013), no exact conclusion can be drawn. Furthermore, a non-significant upwards trend of D_m in the ER group (9.8%) was spotted. In this context, D_m is seen as an indicator of muscle stiffness whereas a strong negative correlation between D_m and stiffness (Macgregor et al., 2018), as well as D_m and atrophy (Pišot et al., 2008, 2016) appears to exist. This was expanded by Šimunić et al. (2019) declaring D_m as a potential marker of early atrophy. However, since the same non-significant trend, i.e., stiffness loss, was also found in the CG (6.8%), no exact conclusion can be drawn.

Furthermore, the high-protein energy restriction did not show any significant effects on the MMG parameters. However, while the ER group remained at a constant frequency, we noted an upwards trend in the CG. This might reflect higher external loading (e.g., physical activity) since muscle tone amplitude decreases during bed rest (Pišot et al., 2008; Demangel et al., 2017; Schoenrock et al., 2018). Although we cannot rule out that the potential between-group difference is attributed to mechanical tension (Rusu et al., 2013; Schoenrock et al., 2018) or day-to-day variability, physical activity (minutes of sport per week) did not differ between groups. Therefore, we cautiously argue that the greater carbohydrate intake and hence, higher glycogen and intracellular water levels led to a comparably higher muscle tone. As already hypothesized by the following authors (Shiose et al., 2016; Cholewa et al., 2019), carbohydrate loading may increase subcutaneous tension and, thereby, stretches the skin over the evaluated muscle.

Sleep

Sleep is critical for recovery, performance and lean mass retention (Knufinke et al., 2018; Wang et al., 2018). In our intervention, sleeping hours per night and sleep onset did not change throughout the study as measured by sleep diary. Although diaries might be more accurate than questionnaires, they are prone to recall bias (Halson, 2019) and hence, must be cautiously interpreted. The PSQI-G score, indicative of subjective sleeping quality, decreased significantly in both groups; however, this trend was higher, though not significant, in the ER group compared to the CG. These findings are in contrast to data reported by Driver et al. (1999) who concluded that caloric restriction does not elicit a significant effect on sleep quality in healthy, non-obese men. However, the participants of Driver et al. (1999) only consumed 87 g of protein per day on average and hence, consumed more than 50% less protein compared to our study. The sleep-improving properties of higher protein consumption is described by other authors (Lindseth et al., 2013) and is probably explained by the improved tryptophan to large-chain neural amino acids (Trp-to-LCNAA) ratio. Mediated by a higher insulin secretion, tryptophan is transported across the blood chain barrier and hence, stimulates the synthesis and function of neurotransmitters (e.g., serotonin) as a dietary precursor (Wurtman et al., 2003). However, there seems to be a ceiling effect as seen in athletes who are used to a steady protein supplementation (Antonio et al., 2019b). With that being said, we conclude that a high-protein moderate energy restriction (ER group) may have beneficial effects on sleeping quality which might be greater by trend than a high-protein intake alone (CG). However, due to the lack of low-protein controls, this cannot be clarified and warrants further study.

Mood

Mood changes are constantly reported in athletic populations (Helms et al., 2019; Reardon et al., 2019). However, there appears to be a plethora of factors influencing mood changes ranging from predisposition, acute biological effects of semi-starvation, to stress due to body monitoring (Helms et al., 2019). Our data predominantly demonstrate no changes in the POMS-G scores. This indicates that neither the moderate energy restriction nor the constant diet, training and body mass tracking had a negative impact on the POMS-G-derived parameters of depression/anxiety, fatigue and hostility. In this context, mood stability might be attributed to the flexible and individual macronutrient profile in our study (Westenhoefer et al., 1999, 2013) and the short duration under energy restriction. Surprisingly, vigor decreased in both groups. In this context, both the ER group and the CG exhibited a significant drop by 10% and 9%, respectively. This is in accordance with most (Degoutte et al., 2006; Koral and Dosseville, 2009; Hulmi et al., 2016), but not all (Wilson et al., 2012) research. For example, in a study by Koral and Dosseville (2009) examining the contest preparation of judokas (−600 kcal/day), the authors reported decreased vigor for the energy restriction but not for the isocaloric controls. Although Koral and Dosseville (2009) attributed the decrease in vigor to body mass loss—supporting the drop revealed in the ER group—this explanation does not fit to the vigor drop shown in the CG in this study. A possible explanation for this might be the high dietary intake that our participants had to consume (45 kcal/kg) of which most of them were not accustomed (Burke et al., 2018).

Limitations

Nevertheless, our findings need cautious interpretation due to inherent limitations. Overall, the study relied on self-reported dietary intake. Although we controlled total protein intake, meal frequency (Iwao et al., 1996), protein dosage per meal (Loenneke et al., 2016), protein timing (Schoenfeld et al., 2013), and protein source (Gilbert et al., 2011) might also influence lean mass preservation during energy restriction.

In perspective of MFBIA, we found subsequent points worth mentioning. Firstly, hydration status was only assessed using extracellular/intracellular water ratio. Although examination was carried out after an overnight fast, studies intending to replicate our design may use exact measurements of hydration status (e.g., urine-specific gravity) and may also implement a refeeding period after the weight loss intervention to account for possible water fluctuations (Martin-Rincon et al., 2019), as well as their effect on the body cell mass calculation (Walter-Kroker et al., 2011). Secondly, adipose tissue consists of a large extracellular and a small fat-free cell mass per unit weight (Wang and Pierson, 1976; Abe et al., 2019) and, therefore, large amounts of adipose tissue loss may be automatically reported as lean tissue loss. Nevertheless, body cell mass quantification does not take adipocyte changes into account and is likely to be a better marker to decide whether real muscle loss has occurred. Since body cell mass is not only made up of skeletal muscle but also comprises organ tissue, this may also bias interpretation (Nose et al., 1983; Gallagher et al., 2017). Hence, future studies should use DXA or implement a combination of methods (DXA and MFBIA or sonography and BIA/DXA; Haun et al., 2018). Thirdly, regarding the BIA technique, 95% of the impedance is measured in the lower limbs. Thus, the depicted values are mainly derived as a snapshot of lower body changes (Ward, 2019); however, they do seem to be supported by the TMG data.

CONCLUSION

In conclusion, the present data show that a high-protein intake alone was not able to prevent lean mass loss associated with a 6-week moderate energy restriction in college students in the absence of resistance training. However, the data revealed that this form of energy restriction did not negatively affect muscle contractility. Sleep quality improved in both groups. This is probably explained by the improved tryptophan to Trp-to-LCNAA ratio; however, there seems to be a ceiling effect as seen in athletes who are used to a steady protein supplementation. Whether these advantages are due to the high-protein intake cannot be clarified due to the lack of low-protein controls and warrants further study. Although vigor was negatively affected in both groups, other mood parameters did not change. In summary, decreasing energy intake moderately while increasing protein consumption does not maintain lean body mass but does

maintain contractility in the absence of resistance training in male college students.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are publicly available. The data can be accessed at the International Clinical Trials Registry Platform (WHO) under the registration number DRKS00017263. Upon request, the data will be made available, without undue reservation.

ETHICS STATEMENT

The study involving human participants was reviewed and approved by the local ethics committee (#2019-24, Goethe University Frankfurt, GER) and was conducted in accordance with the ethical standards set by the declaration of Helsinki. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Data analysis was performed by CR and double-checked by LR. Data interpretation was performed by CR, LR, and MB. CR wrote the first draft of the manuscript. All authors contributed to the conception and design of the study, manuscript revision, and read and approved the final version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fspor.2021.683327/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Sports and Active Living

CORRECTION
published: 08 November 2021
doi: 10.3389/fspor.2021.762606

Corrigendum: High-Protein Energy-Restriction: Effects on Body Composition, Contractile Properties, Mood, and Sleep in Active Young College Students

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OPEN ACCESS

Edited by:
Vassilis Mougios, Aristotle University of Thessaloniki, Greece

Reviewed by:
Evelyn Parr, Australian Catholic University, Australia
Donny Michael Camera, Swinburne University of Technology, Australia

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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Sports and Active Living

Received: *22 August 2021*
Accepted: *29 September 2021*
Published: *08 November 2021*

Citation:
Roth C, Rettenmaier L and Behringer M (2021) Corrigendum: High-Protein Energy-Restriction: Effects on Body Composition, Contractile Properties, Mood, and Sleep in Active Young College Students. Front. Sports Act. Living 3:762606. doi: 10.3389/fspor.2021.762606

Keywords: fat-free-mass, Tensiomyography, muscle quality, sports nutrition, proteolysis

A Corrigendum on

High-Protein Energy-Restriction: Effects on Body Composition, Contractile Properties, Mood, and Sleep in Active Young College Students

by Roth, C., Rettenmaier, L., and Behringer, M. (2021). Front. Sports Act. Living 3:683327. doi: 10.3389/fspor.2021.683327

In the original article, there was a mistake in **Supplementary Table 7** as published. There was a computation error in V_c week 3, as well as a rounding error in T_r. The corrected table has been renamed "**Supplementary Table 2**" and appears below.

In the original article, there were rounding errors as well as wrongly used signs in **Table 2** as published. The corrected **Table 2** appears below.

In the original article, there was a mistake in the legend for **Supplementary Table 7** as published. Since the V_c values for week 3 were corrected due to a computation error, the sign for significance is no longer needed. The name of the table has been corrected to "**Supplementary Table 2**" and the corrected legend appears below.

Supplementary Table 2 Overview of the TMG analysis [energy-restricted (ER) and control group (CG)].

In the original article, the computation error in V_c as well as a rounding error in T_r have been corrected.

A correction has been made to **Results**, Contractile Properties, Paragraph 1:

For TMG, no significant differences were found for T_s (ER: Δ −4.82 ms; CG: Δ 16.63 ms), T_r (ER: Δ −16.63 ms; CG: Δ 16.90 ms) and T_d (ER: Δ 1.28 ms; CG: Δ 0.34 ms, all $p > 0.05$). Although group allocation had no effect on T_c (ER: Δ 3.04 ms; CG: Δ −0.47 ms), D_m (ER: Δ 0.91 mm; CG: Δ 0.66 mm), and V_c (ER: Δ 3.84 mm/s; CG: Δ 10.85 mm/s) change, there appears to be an increasing trend in the ER group ($p = 0.10$) as well as in the ER and the CG ($p = 0.066$) for T_c and D_m over time, respectively (Figure 4; **Supplementary Table 2**).

Supplementary Table 2 | Overview of the TMG analysis [energy-restricted (ER) and control group (CG)].

		Week 1	Week 3	Week 5
T_c (ms)	*ER*	32.27 ± 7.86	34.24 ± 7.33	35.31 ± 6.83
	CG	31.08 ± 6.70	33.12 ± 7.33	30.61 ± 5.82
T_s (ms)	*ER*	196.19 ± 64.26	178.37 ± 53.89	191.37 ± 51.65
	CG	152.18 ± 53.25	180.11 ± 50.65	168.81 ± 48.87
T_r (ms)	*ER*	135.85 ± 68.71	108.56 ± 49.62	119.22 ± 53.04
	CG	81.03 ± 49.34	105.10 ± 69.12	97.93 ± 66.99
D_m (mm)	*ER*	9.29 ± 2.47	10.68 ± 2.58	10.20 ± 2.20
	CG	9.65 ± 2.56	9.86 ± 1.61	10.31 ± 2.35
T_d (ms)	*ER*	27.82 ± 3.82	28.54 ± 2.46	29.10 ± 3.66
	CG	26.39 ± 2.29	27.19 ± 2.73	26.73 ± 2.36
V_c (mm/s)	*ER*	157.67 ± 45.23	173.09 ± 48.70	161.51 ± 43.15
	CG	171.67 ± 53.36	167.80 ± 41.74	182.52 ± 48.65

TABLE 2 | Overview of body composition changes in the energy-restricted group (ER) and the control group (CG).

		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Δ
Body mass (kg)	ER	82.24 ± 8.18	80.58 ± 8.18	80.14 ± 8.85$^{\#}$	79.93 ± 8.47$^{\#}$	79.13 ± 8.41$^{\#}$	77.92 ± 7.76$^{\#}$	77.36 ± 8.00$^{*x\#}$	−3.22
	CG	79.19 ± 6.43	77.49 ± 6.62	78.41 ± 6.34$^{\#}$	79.79 ± 6.19$^{\dagger\#}$	79.37 ± 6.81$^{\#}$	79.26 ± 6.58$^{\#}$	79.39 ± 6.09$^{x\#}$	1.90
BMI (kg/m²)	ER	24.68 ± 2.19	24.11 ± 2.41					23.13 ± 2.19$^{x\#}$	−0.98
	CG	24.55 ± 2.54	23.71 ± 2.46					24.29 ± 2.45$^{x\#}$	0.58
Lean body mass (kg)	ER	65.87 ± 6.19	64.81 ± 5.89§	64.82 ± 6.50	64.98 ± 6.18$^{\#}$	64.54 ± 5.85$^{\#}$	63.69 ± 5.78$^{\#}$	63.32 ± 5.84$^{*\#}$	−1.49
	CG	64.04 ± 5.36	63.33 ± 5.33	63.93 ± 5.31	64.94 ± 5.12$^{\dagger\#}$	64.46 ± 5.63$^{\#}$	64.11 ± 4.96$^{\#}$	64.01 ± 5.14$^{\#}$	0.68
Body cell mass (kg)	ER	37.92 ± 3.69	37.69 ± 3.80	37.69 ± 4.03$^{\#}$	37.89 ± 3.76$^{\#}$	37.41 ± 3.71$^{\#}$	37.05 ± 3.72$^{\#}$	36.84 ± 3.82$^{*\#}$	−0.85
	CG	36.95 ± 3.44	36.66 ± 3.48	37.26 ± 3.71$^{\#}$	37.50 ± 3.44$^{\dagger\#}$	37.32 ± 3.74$^{\#}$	37.19 ± 3.49$^{\#}$	37.25 ± 3.74$^{\#}$	0.59
Body fat (%)	ER	20.12 ± 3.90	19.44 ± 4.50	18.91 ± 4.56$^{\#}$	18.58 ± 4.36$^{\dagger\#}$	18.24 ± 4.64$^{\#}$	17.85 ± 4.39$^{\#}$	17.70 ± 4.40$^{x\#}$	−1.74
	CG	19.16 ± 3.48	17.96 ± 3.90	18.33 ± 3.87$^{\#}$	18.72 ± 3.72$^{\dagger\#}$	18.74 ± 3.96$^{\#}$	18.92 ± 4.14$^{\#}$	19.18 ± 3.57$^{x\#}$	1.22
Intracellular water (l)	ER	28.32 ± 1.94	27.98 ± 1.90§	27.91 ± 2.03	28.09 ± 1.94	27.91 ± 1.86	27.65 ± 1.90$^{\#}$	27.40 ± 1.96$^{*x\#}$	−0.58
	CG	27.43 ± 2.22	27.25 ± 2.11	27.49 ± 2.20	27.79 ± 2.07	27.86 ± 1.76	27.82 ± 1.65$^{\#}$	27.80 ± 1.73$^{\#}$	0.55
Extracellular water (l)	ER	19.92 ± 2.68	19.47 ± 2.44	19.49 ± 2.74	19.47 ± 2.60	19.35 ± 2.45	18.99 ± 2.36	18.96 ± 2.38	−0.51
	CG	19.42 ± 3.12	19.11 ± 2.28	19.32 ± 2.19	19.71 ± 2.50	19.32 ± 2.39	19.09 ± 2.04	19.07 ± 2.08	−0.04

In the original article, there was a rounding error in the body mass (ER group). A correction has been made to **Materials and Methods**, Participants, Paragraph Number 2:

Thirty-five healthy males with no experience in resistance training, as assessed by a pre-study questionnaire, were recruited from local sports clubs and University courses (see **Figure 2**). One participant declined to participate and three participants were excluded due to lacking protocol compliance (did not adhere to dietary intake). Finally, 28 healthy males (ER: age 26.57 ± 4.20 years; height 1.83 ± 0.05 m; body mass 82.24 ± 8.18 kg; CG: age 25.29 ± 2.97 years; height 1.81 ± 0.09 m; body mass 79.19 ± 6.43 kg) were used for data analysis. Due to hormonal fluctuations (Cumberledge et al., 2018), only male participants were included in order to increase reliability. The participants, who all reported that anabolic-androgenic drugs have never been consumed before, undertook at least two sport sessions per week. Since we only aimed for including lean participants, participants were excluded if their body fat was above 25%; this is the cut-off value for obesity, as suggested by Beals et al. (2019). During the study, the participants were asked to continue their habitual training. All participants were informed about the goal of the study as well as its conduction; in particular, interventional strains and requirements were highlighted. Every individual voluntarily agreed and gave written and informed consent to participate in the study.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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frontiers in Nutrition

ORIGINAL RESEARCH
published: 30 June 2021
doi: 10.3389/fnut.2021.683369

Physical Activity, Weight Loss, and Weight Maintenance in the DiOGenes Multicenter Trial

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In this secondary analysis of the DiOGenes study, we investigated whether physical activity (PA) contributes to diet-induced weight loss and helps to reduce subsequent regain. We also studied the associations of PA with changes in cardiometabolic variables. Adults with overweight were included and followed an 8-week low-calorie diet (LCD). When successful (>8% weight loss), participants were randomized to different *ad libitum* diet groups and were advised to maintain their weight loss over the 6-month intervention period. Body weight (BW), body composition, cardiometabolic variables and subjectively-assessed PA were measured at baseline, at the end of weight loss and at the end of the intervention. BW was reduced by the LCD (from 99.8 ± 16.7 to 88.4 ± 14.9 kg; $P < 0.001$). This reduction was maintained during the weight maintenance period (89.2 ± 16.0 kg). Total PA (sum score of the three subscales of the Baecke questionnaire) increased during the weight loss period (from 8.16 ± 0.83 to 8.39 ± 0.78; $P < 0.001$) and this increase was subsequently maintained (8.42 ± 0.90). We found no evidence that baseline PA predicted weight loss. However, a higher level of baseline PA predicted a larger weight-loss-induced improvement in total cholesterol, triglycerides, glucose and CRP, and in post-prandial insulin sensitivity (Matsuda index). Subsequent weight and fat mass maintenance were predicted by the post-weight loss level of PA and associated with changes in PA during the weight maintenance phase. In conclusion, despite the fact that higher baseline levels of PA did not predict more weight loss during the LCD, nor that an increase in PA during the LCD was associated with more weight loss, higher PA levels were associated with more improvements in several cardiometabolic variables. The positive effect of higher PA on weight loss maintenance seems in contrast to randomized controlled trials that have not been able to confirm a positive effect of exercise training programmes on weight loss maintenance. This analysis supports the notion that higher self-imposed levels of PA may improve the cardiometabolic risk profile during weight loss and help to maintain weight loss afterwards.

Keywords: obesity, exercise, weight regain, diet, metabolic health

OPEN ACCESS

Edited by:
Karsten Koehler, Technical University of Munich, Germany

Reviewed by:
Jennifer L. Scheid, Daemen College, United States
Barbara Strasser, Sigmund Freud Private University Vienna, Austria

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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *20 March 2021*
Accepted: *08 June 2021*
Published: *30 June 2021*

Citation:
van Baak MA, Hul G, Astrup A and Saris WH (2021) Physical Activity, Weight Loss, and Weight Maintenance in the DiOGenes Multicenter Trial. Front. Nutr. 8:683369. doi: 10.3389/fnut.2021.683369

INTRODUCTION

The beneficial effects of physical activity (PA) are sufficiently known and include improvements in physical fitness and well-being. In individuals with obesity PA levels are usually low, contributing to low levels of cardiorespiratory fitness and ill metabolic health. Many people with overweight or obesity try to lose weight by means of an energy-restricted diet. In well-structured weight-loss progammes many of them will be successful in losing a substantial amount of weight. However, in the long run maintaining this weight loss turns out to be very difficult (1–4). There is ample evidence from randomized clinical trials (RCTs) that adding an exercise program to a weight loss programme by means of energy restriction will result in extra weight loss [eg., (5–7)]. Combined diet and exercise interventions on the long-term have been found to result in more weight loss than diet alone (8, 9). In contrast, there is no evidence from RCTs that exercise training programmes help to prevent weight regain after weight loss (7, 10). On the other hand, a systematic review of weight control registries shows that an increased level of PA is the most consistent positive correlate of weight loss maintenance (11). Whether self-selected, habitual levels of PA contribute to more weight loss success and may help to prevent weight regain is less well studied.

We therefore performed a secondary analysis of the large multicenter European DiOGenes (Diet, Obesity and Genes) trial (12). Primary aim of this trial was to investigate the effect of different macronutrient compositions of an *ad libitum* diet on weight regain after successful weight loss. In this trial, adults with overweight or obesity were included.

The primary objective of this analysis was to investigate whether self-selected habitual levels of PA contribute to weight loss and weight loss maintenance success. Based on previous literature (5–7, 13), we hypothesized that higher levels of PA are associated with more weight loss during an energy-restricted diet and also with less subsequent weight regain. We therefore analyzed whether baseline PA predicts weight loss during an energy-restricted diet and whether the PA level after weight loss predicts weight maintenance. In addition we analyzed whether PA levels change over time in this weight loss/weight maintenance study, whether weight loss is associated with a change in PA during the weight loss phase, and whether weight maintenance is associated with a change in PA during the weight maintenance period. Where relevant these questions were also addressed for body composition, blood pressure and selected metabolic variables.

MATERIALS AND METHODS

Study Design

DiOGenes is a large multicenter European DiOGenes (Diet, Obesity and Genes) trial, which was conducted between 2006 and 2009 in eight academic centers across Europe (Denmark, Netherlands, Unites Kingdom, Germany, Czech Republic, Greece, Spain, Bulgaria). The trial was registered under ClinicalTrials.gov number NCT00390637. Overweight or obese adults and their children were recruited. After baseline measurements, the adults tried to lose weight by means of a low-calorie diet (∼800–1000 kcal/d) for 8 weeks. Participants having successfully lost at least 8% of their initial weight were randomized into one of five groups with different diet compositions: healthy diet, high protein/high glycemic index (GI), high protein/low GI, low protein/high GI, or low protein/low GI. Dietary intake during this period was *ad libitum*. Further details can be found in Larsen et al. (12). The primary aim of the study was to analyze the effect of diet composition on weight maintenance. These primary outcomes have been reported previously (14).

Subjects

In total 1,121 individuals [men(M) and women(F) with at least one child <18 years in their household] were included in the study. They were generally healthy. Details on in- and exclusion criteria can be found in (12). All participants gave informed consent prior to the study and the study was conducted according to the Declaration of Helsinki. 773 participants that completed the 8-week weight loss phase and had lost ≥8% of their initial body weight were randomized to the subsequent 6-month randomized *ad libitum* diet intervention, which was completed by 548 participants (14). However, not all data were available at all time points, especially the data on PA were missing in many participants. Therefore, the number of subjects will differ in the different analyses. Complete body weight and PA data were available for 193 participants (76M/117F).

Methods

Measurements where obtained at baseline, at the end of the 8-week weight loss period and at the end of the 6-month weight maintenance period. All measurements were performed in the morning after an overnight fast. Body weight was measured on calibrated digital scales (Seca 861, Hamburg, Germany) to the nearest 0.1 kg. Body composition was measured by dual-energy radiograph absorption (Lunar Radiation, Madison, WI) or bio-impedance (Quad-Scan 4000; Bodystat, Douglas, Isle of Man, United Kingdom) depending on study center. Blood pressure was measured in sitting position after 5 min of rest with a semi-automatic device (Omron). A fasting blood sample was drawn after a 10-min rest from the antecubital vein into vacutainers containing clot activator and gel for serum separation. After 10–30 min coagulation at room temperature, samples were centrifuged at 2500 x g for 15 min at room temperature. Within 30 min of centrifugation, serum was transferred to cryo vials and stored at −80°C until analysis. An oral glucose tolerance test was performed subsequently from which the Matsuda index for postprandial insulin sensitivity was calculated. All blood samples were analyzed in the same lab. Serum total cholesterol, HDL cholesterol, and TG concentrations were quantified by enzyme immunoassays (Ortho-Clinical Diagnostics, Johnson & Johnson) for the Vitros 5.1 FS analyzer. LDL and VLDL cholesterol concentrations were calculated using Friedewald's equation (15). Serum glucose was measured by a colorimetric assay (Ortho-Clinical Diagnostics) for the Vitros 950 analyzer

TABLE 1 | Anthropometrics and scores for physical activity categories and total physical activity at the three measurement time points ($N = 193$).

Variable	Baseline mean	Baseline SD	End of weight loss mean	End of weight loss SD	End of weight maintenance mean	End of weight maintenance SD	*P* value[a]
BW (kg)	99.8	16.7	88.4[b]	14.9	89.2[b]	16.0	0.000
BF% (%)	39.8	8.4	35.4[b]	8.9	34.7[b]	8.7	0.000
FM (kg)	39.8	11.6	31.4[b]	10.5	31.2[b]	10.5	0.000
FFM (kg)	60.0	12.8	57.0[b]	11.7	58.1[b,c]	12.1	0.000
Work score	2.74	0.34	2.76	0.34	2.76	0.37	0.355
Leisure time score	2.78	0.65	3.02[b]	0.63	3.04[b]	0.67	0.000
Sport score	2.64	0.37	2.60	0.40	2.61	0.37	0.306
Total score	8.16	0.83	8.39[b]	0.78	8.42[b]	0.90	0.000

[a]P value from repeated measurements ANOVA; [b]significantly different from baseline (post-hoc paired t-test with Bonferroni correction, P < 0.001); [c]significantly different from end of weight loss (post-hoc paired t-test with Bonferroni correction, P < 0.001). BW, body weight; BF%, percent body fat; FM, fat mass; FFM, fat free mass.

and serum insulin was measured by an immunoassay (Siemens Healthcare Diagnostics) for the ADVIA Centaur XP. Serum CRP was quantified by a high sensitivity immunoassay (hsCRP, Ortho-Clinical Diagnostics) for the Vitros 5.1 FS analyzer with a detection limit of 0.1 mg/L. CRP values >10 mg/L were taken as indications of acute inflammation and the CRP values were excluded from the dataset. Homeostasis model of assessment-insulin resistance (HOMA-IR) was calculated as [fasting glucose (mmol/L) x fasting insulin (mIU/L)]/22.5. The Baecke questionnaire on PA (16) was filled in online at the same time points. Participants were asked to report their PA over the last month. The Baecke questionnaire distinguishes between PA during work, leisure time and sports. For each activity category a mean score was calculated. By adding up the three scores total PA was calculated. The Baecke questionnaire has been validated against energy expenditure measured by objective methods, such as the doubly labeled water technique and tri-axal accelerometry (17, 18).

Data Analysis

Data are presented as mean ± SD. Changes in PA and anthropometric and metabolic variables over time were analyzed by means of repeated measurements ANOVA. Simple correlations were tested according to Pearson. Regression analysis was used to study predictors of weight and body composition changes with or without adjustment for potential confounders. To analyze whether baseline PA predicted the changes in anthropometric and metabolic variables over the weight loss period, the value of the outcome variable (anthropometric or metabolic variables) at the end of the weight loss period was the dependent variable. As independent variables the baseline values of the outcome variable and total physical activities score were included (model 1). In model 2 we adjusted model 1 for the weight change during the weight loss phase to see whether the association was independent of weight loss. In model 3 we additionally adjusted for sex. A similar approach was used to analyze whether PA at the end of the weight loss phase predicted the changes during the weight maintenance period. SPSS version 25 was used for the statistical analysis.

RESULTS

Changes in PA, Body Weight, Body Composition, Blood Pressure, and Metabolic Variables Over Time

As to be expected, ANOVA analyses showed that body weight (BW), body fat percentage (%BF) and fat mass (FM) were significantly reduced by the energy-restricted diet (BW from 99.8 ± 16.7 to 88.4 ± 14.9 kg; %BF from 39.8 ± 8.4 to 35.4 ± 14.9 %; FM from 39.8 ± 11.6 to 31.4 ± 10.5 kg; all $P < 0.001$) and the reductions were maintained during the weight maintenance period (**Table 1**). Fat free mass (FFM) was also reduced during the weight loss period (from 60.0 ± 12.8 to 57.0 ± 11.7 kg; $P < 0.001$), but it recovered partially during the weight maintenance phase (to 58.1 ± 12.1 kg; $P < 0.05$ vs baseline). Total PA, expressed as the sum score of the three sub scales of the Baecke questionnaire (work, leisure time, and sport) increased during the weight loss period (from 8.16 ± 0.83 to 8.39 ± 0.78; $P < 0.001$) and this increase was maintained during the weight maintenance period (**Table 1**). The increase in total activity was mainly due to an increase in leisure time activity. Work and sport activity did not change significantly (**Table 1**).

Blood pressure and metabolic variables were all significantly improved at the end of the weight loss phase (**Table 2**). Blood pressure, total, LDL-, and HDL-cholesterol had returned to baseline levels after the weight maintenance phase. Triglycerides, glucose, insulin, and HOMA-IR also increased during the weight maintenance phase, but remained lower than baseline. The Matsuda index and CRP levels maintained the weight-loss-induced levels (**Table 2**). Data on males and females separately can be found in the **Supplementary Material**.

Association Between Baseline PA and Changes During the Weight Loss Phase

The association of baseline PA with weight loss during the weight loss phase is shown in **Figure 1**. The association is positive ($r = 0.132$, $P = 0.000$), suggesting that those with higher baseline PA lost less weight. However, multiple regression analysis showed no statistically significant evidence for an association between baseline PA and weight at the end of the weight loss phase,

TABLE 2 | Blood pressure and metabolic parameters at the three measurement time points ($N = 107$).

Variable	Baseline mean	Baseline SD	End of weight loss mean	End of weight loss SD	End of weight maintenance mean	End of weight maintenance SD	*P* value[a]
SBP (mm Hg)	125	14	116[b]	14	123[c]	14	0.000
DBP (mm Hg)	77	11	72[b]	10	76[c]	10	0.000
total cholesterol (mmol/L)	4.7	1.0	4.0[b]	0.9	4.8[c]	0.9	0.000
LDL-cholesterol (mmol/L)	2.9	0.9	2.5[b]	0.7	3.0[c]	0.9	0.000
HDL-cholesterol (mmol/L)	1.2	0.3	1.1[b]	0.2	1.3[c]	0.3	0.000
Triglycerides (mmol/L)	1.3	0.6	1.0[b]	0.4	1.2[b,c]	0.5	0.000
Glucose (mmol/L)	5.1	0.5	4.8[b]	0.4	4.9[b,c]	0.4	0.000
Insulin (μIU/L)	11.7	7.1	6.7[b]	6.1	7.8[b,c]	6.5	0.000
HOMA_IR	3.1	1.9	1.7[b]	1.7	2.0[b,c]	1.5	0.000
Matsuda index	4.9	2.5	8.5[b]	4.0	8.2[b]	4.0	0.000
CRP (mg/L)	3.3	2.5	2.3[b]	2.4	1.9[b]	2.4	0.000

[a] P value from repeated measurements ANOVA; [b] significantly different from baseline (post-hoc paired t-test with Bonferroni correction, $P < 0.01$); [c] significantly different from end of weight loss (post-hoc paired t-test with Bonferroni correction, $P < 0.05$). SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, HOMA index for insulin resistance; CRP, C-reactive protein.

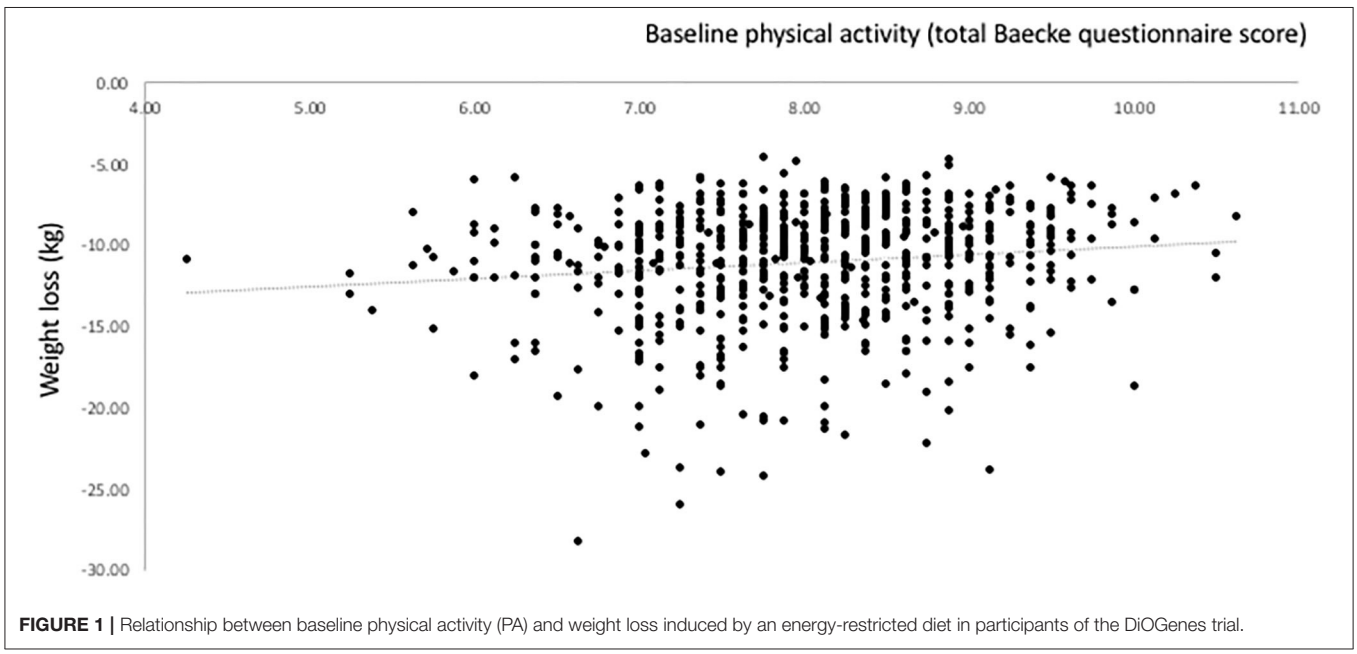

FIGURE 1 | Relationship between baseline physical activity (PA) and weight loss induced by an energy-restricted diet in participants of the DiOGenes trial.

when baseline weight was included as a covariate (**Table 3**). Furthermore, regression analyses for the outcome variables FM, SBP, DBP, insulin, HOMA-IR and HDL cholesterol showed no significant influence of baseline PA. However, baseline PA significantly predicted the change in CRP, total cholesterol, triglycerides, glucose, and the Matsuda index. The change in FFM and LDL-cholesterol also tended to be predicted by baseline PA ($P < 0.10$). A higher PA at baseline was associated with larger improvements in these variables, also when adjusted for weight loss and sex (**Table 3**).

We also analyzed whether a change in PA during the weight loss phase of the study was associated with weight loss. No correlation was found for body weight ($r = -0.052$, $P = 0.306$, $N = 395$), nor for the change in FM or the change in FFM.

The Level of PA After Weight Loss and Weight Maintenance Success

The relationship between the level of PA at the end of the weight loss phase and the subsequent weight changes are shown in **Figure 2**. The association is negative, indicating that the higher the PA level at the end of weight loss, the smaller the weight regain or the larger the further weight loss. In a multiple linear regression analysis, PA at the end of the weight loss phase significantly predicted body weight at the end of the 6-month intervention period adjusted for body weight at the end of weight loss (B −0.675, SE 0.277, $P = 0.015$, $N = 421$) (**Table 4**). There was no interaction with the diet group to which the participant was randomized. Negative associations were present for FM and FFM as well, although not statistically significant (both $P > 0.05$). No significant associations with CRP, glucose,

TABLE 3 | Results of the regression analyses with total physical activity at baseline as the independent variable and different variables at the end of the weight loss phase as the dependent variable.

		Model 1*			Model 2**			Model 3***		
Dependent Variable	r^2	**B**	**SE**	***P***	**B**	**SE**	***P***	**B**	**SE**	***P***
BW (kg)	0.969	0.008	0.116	0.946				0.021	0.114	0.856
BF%	0.767	0.015	0.190	0.939	−0.097	0.188	0.608	−0.220	0.186	0.238
FM (kg)	0.883	0.099	0.204	0.628	−0.013	0.188	0.944	−0.048	0.188	0.799
FFM (kg)	0.887	−0.270	0.189	0.155	−0.318	0.189	0.093	−0.332	0.186	0.075
SBP (mm Hg)	0.435	0.052	0.430	0.903	−0.087	0.431	0.839	−0.087	0.431	0.840
DBP (mm Hg)	0.490	0.392	0.299	0.190	0.291	0.300	0.332	0.292	0.298	0.329
total cholesterol (mmol/L)	0.495	−0.050	0.027	0.070	−0.065	0.027	0.018	−0.065	0.027	0.017
LDL-cholesterol (mmol/L)	0.535	−0.031	0.023	0.174	−0.042	0.023	0.066	−0.043	0.023	0.065
HDL-cholesterol (mmol/L)	0.553	0.006	0.008	0.405	0.005	0.008	0.493	0.006	0.008	0.422
Triglycerides (mmol/L)	0.289	−0.053	0.016	0.001	−0.058	0.016	0.000	−0.059	0.016	0.000
Glucose (mmol/L)	0.310	−0.078	0.018	0.000	−0.082	0.018	0.000	−0.081	0.018	0.000
Insulin (μIU/L)	0.666	−0.075	0.216	0.731	−0.178	0.217	0.411	−0.182	0.216	0.401
HOMA-IR	0.585	−0.050	0.068	0.464	−0.076	0.069	0.268	−0.077	0.069	0.261
Matsuda index	0.365	0.449	0.134	0.001	0.510	0.134	0.000	0.513	0.133	0.000
CRP (mg/L)	0.509	−0.200	0.091	0.028	−0.198	0.092	0.031	−0.195	0.092	0.034

**model 1 adjusted for the baseline value of the variable; **model 2 additional adjustment for the weight loss; ***model 3 additional adjustment for sex. r^2, coefficient of determination of model 1; B, regression coefficient; SE, standard error; P, P value. BW, body weight; BF%, percent body fat; FM, fat mass; FFM, fat free mass; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, HOMA index for insulin resistance; CRP, C-reactive protein.*

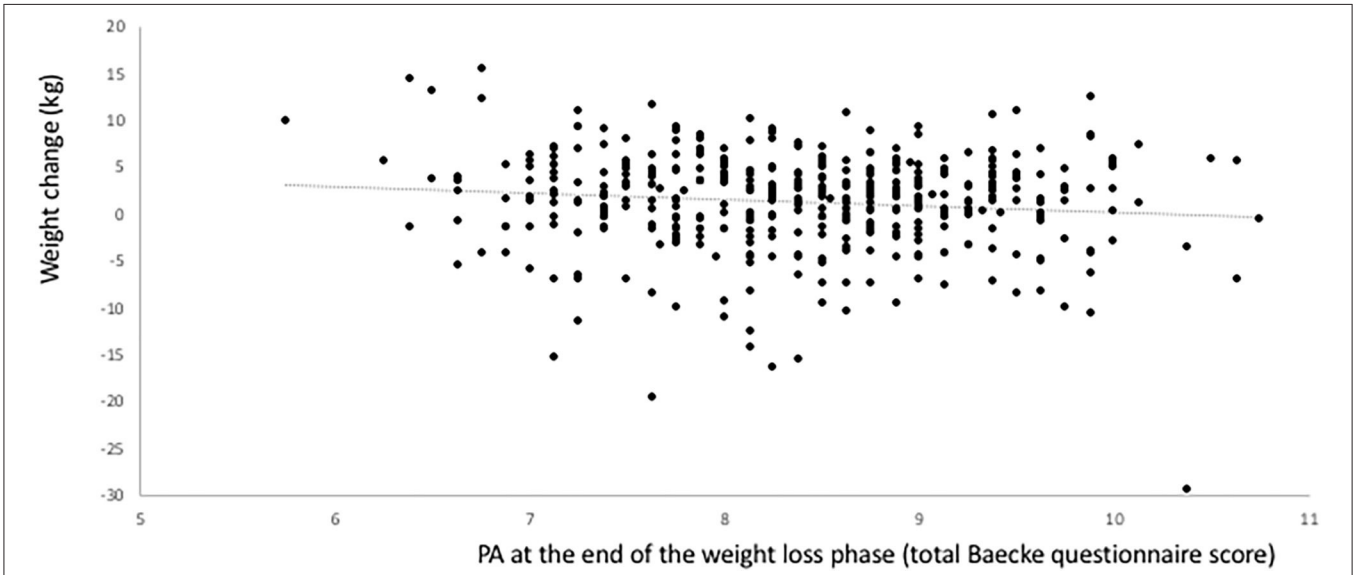

FIGURE 2 | Relationship between the level of physical activity at the end of the weight loss phase (start of the weight maintenance period) and subsequent weight changes during the weight maintenance phase in the DiOGenes trial.

HOMA-IR, insulin, LDL, Matsuda, HDL, TG, SBP, and DBP were detected and there was no interaction with diet in any of the associations.

We also analyzed whether the change in PA over the weight maintenance period was associated with changes in weight or body composition. A significant negative association was found for body weight and FM, but not for FFM.

DISCUSSION

In this secondary analysis of the DiOGenes trial, we found that the level of PA at baseline did not predict weight loss induced by an energy-restricted diet. However, weight-loss induced improvements in CRP, total cholesterol, triglycerides, glucose and the Matsuda index were more pronounced in participants with

TABLE 4 | Results of the regression analyses with total physical activity at the end of the weight loss phase as the independent variable and the variable at the end of the weight maintenance phase as the dependent variable.

	Model 1*				Model 2**			Model 3***		
Dependent variable	r^2	**B**	**SE**	***P***	**B**	**SE**	***P***	**B**	**SE**	***P***
BW (kg)	0.890	−0.677	0.284	0.017				−0.613	0.276	0.027
BF%	0.810	−0.187	0.236	0.429	0.100	0.193	0.605	−0.104	0.190	0.582
FM (kg)	0.801	−0.384	0.297	0.196	0.129	0.182	0.477	0.045	0.182	0.806
FFM (kg)	0.929	−0.337	0.198	0.090	−0.192	0.182	0.291	−0.066	0.174	0.705
SBP (mm Hg)	0.381	−0.378	0.595	0.526	−0.049	0.581	0.932	0.342	0.571	0.550
DBP (mm Hg)	0.484	−0.079	0.427	0.854	0.129	0.419	0.759	0.284	0.415	0.494
total cholesterol (mmol/L)	0.352	−0.026	0.042	0.537	0.004	0.042	0.918	0.015	0.042	0.717
LDL- cholesterol (mmol/L)	0.422	0.000	0.037	0.998	0.027	0.037	0.469	0.038	0.037	0.304
HDL-cholesterol (mmol/L)	0.493	0.004	0.013	0.738	0.001	0.013	0.945	−0.007	0.013	0.600
Triglycerides (mmol/L)	0.298	−0.050	0.025	0.043	−0.031	0.024	0.199	−0.016	0.024	0.511
Glucose (mmol/L)	0.298	0.004	0.023	0.858	0.015	0.023	0.513	0.019	0.023	0.401
Insulin (μIU/L)	0.688	−0.501	0.331	0.131	−0.401	0.332	0.228	−0.330	0.332	0.321
HOMA-IR	0.661	−0.127	0.088	0.151	−0.100	0.089	0.262	−0.078	0.088	0.375
Matsuda index	0.345	0.218	0.186	0.242	0.116	0.179	0.515	0.070	0.179	0.695
CRP (mg/L)	0.252	0.045	0.111	0.686	0.094	0.110	0.394	0.030	0.109	0.787

**model 1 adjusted for the baseline value of the variable; **model 2 additional adjustment for the weight regain; ***model 3 additional adjustment for diet group and sex. r^2, coefficient of determination of model 1; B, regression coefficient; SE, standard error; P, P value. BW, body weight; BF%, percent body fat; FM, fat mass; FFM, fat free mass; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, HOMA index for insulin resistance; CRP, C-reactive protein.*

higher baseline PA, independent of the change in body weight. On the other hand, the PA level reported at the end of the weight loss phase significantly predicted weight and FM maintenance, but not FFM maintenance or the change in any of the other measured variables. No interaction with diet composition during the weight maintenance phase was found.

Despite the fact that the DiOGenes trial did not include an intervention on PA levels of the participants, the results show an increase in self-reported total PA during the weight-loss phase, which was maintained during the weight maintenance phase. The increase was mainly due to an increase in leisure time PA as measured by the Baecke questionnaire, whereas the sport index did not change. The leisure-time PA index of the Baecke questionnaire is based on the frequency of television viewing, walking, and cycling and on the time spent walking or cycling for transportation. The sports index is derived from the two most frequently played sports with an estimation of their intensity and the number of hours per week and of months per year these two sports are played by the subject, an estimation of the level of PA compared with that of the subject's age peers, the frequency of sweating during leisure time, and the frequency of playing sports. The scores on the leisure time index and sport index were not correlated at baseline ($r = -0.005$, $P = 0.883$, $N = 917$), suggesting that they clearly measure different aspects of PA.

Although, in contrast to the full DiOGenes population ($n = 548$) (14), there was no statistically significant average weight regain in participants with complete data for PA, weight and body composition presented in **Table 1**, whereas the interindividual variation was large as shown in **Figure 2**.

Two systematic reviews, based on RCTs, addressed the question whether adding an exercise intervention to an energy-restricted diet intervention would improve total weight loss individuals with obesity (19, 20). In the meta-analysis by Cheng et al. which focused on postmenopausal women with obesity, the diet plus exercise intervention groups reduced their body weight by −1.2 kg more than the diet-only groups. Weight loss was on average approximately 6.5 kg in the diet-only group, with study durations varying between 12 weeks and 1 year (19). In the meta-analysis of Sardeli et al., which included elderly individuals with obesity, resistance training did not result in extra weight loss, but prevented the FFM loss induced by the energy-restricted diet. Average weight loss was around 4 kg with study durations varying between 12 and 24 weeks (20). Apart from the specific populations that were addressed in these reviews, it is obvious that both total weight loss and rate of weight loss were considerably higher in the DiOGenes study than in the studies included in these two systematic reviews. It is possible that the larger energy restriction and larger weight loss in the DiOGenes study explains why there was no association between the PA level at baseline or the spontaneous increase in PA during the weight loss intervention and body weight loss: the effect of the energy restriction may have been pre-dominant and the role of PA too subtle to induce measurable effects. It is therefore intriguing that we did find that participants who were more active at baseline did show more improvements in CRP, total cholesterol, triglycerides, glucose and the Matsuda index. This may suggest that a more active lifestyle makes the body more sensitive to weight-loss induced changes in these parameters.

In contrast to the lack of effect of baseline PA on weight loss, we found a negative association between the level of PA at the end of the 8-week weight loss phase and the weight and FM regain over the subsequent 6 months. None of the other variables was

associated with the level of PA at this time point. During the 6-month weight maintenance period participants were asked to try to at least maintain their body weight loss and not regain weight. In addition, the effect of different diet compositions, which varied in macronutrient composition, was tested. The association between PA and weight regain was independent of diet composition. A somewhat similar analysis was performed previously in the STORM trial (13). This trial investigated whether continued treatment with sibutramine had an effect on weight regain (18-months follow-up) after a 6-month period of weight loss induced by a combination of an energy-restricted diet and sibutramine treatment. PA was also measured by the Baecke questionnaire. A secondary analysis showed that a higher average PA level over the follow-up period was associated with better weight maintenance, expressed as weight regain/weight loss (13). This was the same in the DiOGenes trial ($r = 0.124$, $P = 0.041$). On the other hand, a systematic review and meta-analysis of randomized clinical trials published in 2014 that investigated the effect of exercise interventions on weight loss maintenance concluded that there was no effect of different forms of exercise training on weight regain after weight loss based on 4 RCT's (10). Two of the RCT's applied aerobic training and the other two resistance training. A more recent systematic review (7) was able to include one additional RCT applying a 1-year resistance training in post-menopausal women after weight loss (21), which also showed no effect on weight, FM, and FFM regain. Nevertheless, it cannot be excluded that there is a difference between self-selected levels of PA and those driven by an exercise intervention where intrinsic motivation and adherence may be a problem. This was also hinted at by Foright et al. (22).

As suggested in Foright et al. (22), the beneficial effect of higher levels of PA on weight maintenance may be related to a reduction of the weight-loss-induced gap between energy intake and energy expenditure that promotes weight regain. The mechanisms underlying this effect are not fully clear, but may include effects of exercise on GI signals, increased capacity for *de novo* lipogenesis in the liver and a reduction in adipose tissue, increased insulin response to a glucose load, increased dietary fat oxidation in the muscle and preservation of muscle mass and increases in leptin and insulin sensitivity in the brain (22). A recent systematic review and meta-analysis suggests that exercise interventions do not lead to measurable increases in daily energy intake in people with overweight or obesity (23), which would support some of the suggested mechanisms. Another proposed mechanism is a higher FFM associated with a higher level of PA, which leads to a higher level of resting energy expenditure. Indeed, the baseline level of PA was positively correlated with FFM, after adjustment for baseline weight and sex (B 0.505, SE 0.218, $P = 0.021$).

The volume of exercise or PA needed for prevention of weight regain after weight loss is not clear. Current recommendations vary between 200 and 450 min/week (15, 24, 25), but these are mainly based on cross-sectional, non-randomized and retrospective studies. A recently published RCT (26) compared the effects of three different partially supervised exercise programs (150, 220, or 300 min/week) in combination with weekly behavioral counseling on weight regain over 12 months after a weight loss program where participants had lost $\geq$5% of their initial weight. No difference in weight regain was found among the groups. The lack of a control group does not allow conclusions about whether or how much weight regain was prevented. The authors concluded from these results that it is likely that less exercise is needed than currently recommended for prevention of weight regain (26). From the current study no conclusions can be drawn about the effective volume of self-selected PA, because the questionnaire used to assess PA does not allow quantification of the volume of PA. This is clearly an area that needs further study.

Although this analysis has the advantage that it is based on a considerable number of participants, a major limitation is that PA was derived from a questionnaire. Although the Baecke questionnaire has been validated against total energy expenditure as measured by the doubly labeled water technique and other objective techniques on a group level (17, 18), this does not necessarily mean that the reported values are reliable on an individual level. Moreover, it was not validated specifically in a population with overweight and obesity. Although subgroups of the DiOGenes population participated in measurements of total daily energy expenditure by means of doubly labeled water or the IDEEA device [Intelligent Device for Energy Expenditure and PA (IDEEA)], these measurements turned out to be too incomplete to validate the questionnaire data. Thus, our results should only be regarded as a starting point for further research with more objective measurement of PA. Furthermore, the DiOGenes trial was designed as a randomized clinical trial. However, this secondary analysis is observational and cause-effect conclusions cannot be drawn from the associations reported. Nevertheless, the results give rise to some interesting hypotheses that could be tested in new studies. The first suggestion is to test whether a higher level of baseline PA makes the body more sensitive to weight-loss-associated changes in several metabolic variables, such as plasma glucose, total cholesterol, triglycerides and CRP concentrations and postprandial insulin sensitivity (Matsuda index). The second is to test whether a higher level of self-selected PA after weight loss is associated with better weight loss maintenance than an imposed exercise regimen.

In conclusion, this secondary observational analysis of the DiOGenes RCT found no evidence that baseline PA predicted weight loss induced by a low-calorie diet. However, a higher level of baseline PA predicted a larger weight-loss-induced improvement in plasma levels of total cholesterol, triglycerides, glucose and CRP, and in post-prandial insulin sensitivity (Matsuda index). Subsequent weight and FM maintenance were predicted by the post-weight loss level of PA and associated with changes in PA during the weight maintenance phase. Despite the fact that RCT's so far have not been able to confirm a positive effect of exercise training programmes on weight loss maintenance, this analysis supports the notion that self-imposed levels of PA may help to maintain weight loss.

DATA AVAILABILITY STATEMENT

Access to the data presented in this article can be requested from: m.vanbaak@maastrichtuniversity.nl.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by local ethics committees in 8 European countries. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MB conceived the idea for the analysis. MB and GH performed the analysis and wrote the manuscript. AA and WS read and commented on the manuscript and conceived the DiOGenes project. WS was the coordinator of the full DiOGenes project and AA the coordinator of research line 1 of the DiOGenes project in which the research on which this manuscript is based was carried out. MB was the principal investigator of the Maastricht center and GH was one of the investigators. All authors contributed to the article and approved the submitted version.

FUNDING

The DiOGenes project was supported by a grant from the European Commission (FP6-2005-513946).

ACKNOWLEDGMENTS

The authors wish to thank all DiOGenes RTD1 investigators for their work and contributions to the project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.683369/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 14 September 2021
doi: 10.3389/fnut.2021.688295

Free-Living Energy Balance Behaviors Are Associated With Greater Weight Loss During a Weight Loss Program

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Edited by:
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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *30 March 2021*
Accepted: *28 July 2021*
Published: *14 September 2021*

Citation:
Myers A, Camidge D, Croden F, Gibbons C, Stubbs RJ, Blundell J, Finlayson G and Buckland N (2021) Free-Living Energy Balance Behaviors Are Associated With Greater Weight Loss During a Weight Loss Program. Front. Nutr. 8:688295. doi: 10.3389/fnut.2021.688295

Introduction: Free-living movement (physical activity [PA] and sedentary behavior [SB]) and eating behaviors (energy intake [EI] and food choice) affect energy balance and therefore have the potential to influence weight loss (WL). This study explored whether free-living movement and/or eating behaviors measured early (week 3) in a 14-week WL programme or their change during the intervention are associated with WL in women.

Methods: In the study, 80 women ($M \pm SD$ age: 42.0 ± 12.4 years) with overweight or obesity [body mass index (BMI): 34.08 ± 3.62 kg/m^2] completed a 14 week WL program focused primarily on diet (commercial or self-led). Body mass (BM) was measured at baseline, and again during week 2 and 14 along with body composition. Free-living movement (SenseWear Armband) and eating behavior (weighed food diaries) were measured for 1 week during week 3 and 12. Hierarchical multiple regression analyses examined whether early and early-late change in free-living movement and eating behavior were associated with WL. The differences in behavior between clinically significant weight losers (CWL; ≥5% WL) and non-clinically significant weight losers (NWL; ≤3% WL) were compared.

Results: The energy density of food consumed [$\beta = 0.45$, $p < 0.001$] and vigorous PA [$\beta = -0.30$, $p < 0.001$] early in the intervention (regression model 1) and early-late change in light PA [$\beta = -0.81$ $p < 0.001$], moderate PA [$\beta = -1.17$ $p < 0.001$], vigorous PA [$\beta = -0.49$, $p < 0.001$], total energy expenditure (EE) [$\beta = 1.84$, $p < 0.001$], and energy density of food consumed [$\beta = 0.27$, $p = 0.01$] (regression model 2) significantly predicted percentage change in BM. Early in the intervention, CWL consumed less energy dense foods than NWL [$p = 0.03$]. CWL showed a small but significant increase in vigorous PA, whereas NWL showed a slight decrease in PA [$p = 0.04$].

Conclusion: Both early and early-late change in free-living movement and eating behaviors during a 14 week WL program are predictors of WL. These findings

demonstrate that specific behaviors that contribute to greater EE (e.g., vigorous PA) and lower EI (e.g., less energy-dense foods) are related to greater WL outcomes. Interventions targeting these behaviors can be expected to increase the effectiveness of WL programs.

Keywords: weight loss, energy balance, appetite, energy intake, free-living physical activity

INTRODUCTION

Obesity is a global public health concern in both developed and developing countries affecting over 1.9 billion adults worldwide (1). In England, the rate of obesity has almost doubled in the past 20 years with 63% of adults being classified as overweight or obese in 2018 (2). Overweight and obesity increase the risk of developing life-limiting conditions, such as cancer, cardiovascular disease, and type II diabetes, and can significantly reduce quality of life (3, 4). Weight management strategies are required to combat the obesity epidemic (5); however, for many, weight loss (WL) is difficult and weight regain following successful WL is common (6). Less than 20% of individuals with overweight or obesity were able to maintain a body mass (BM) reduction of 10% after 1 year (7). As such, there is a need to understand energy balance behaviors that influence successful WL.

Globally, 42% of adults report engaging in weight management attempts, with higher prevalence in Europe/Central Asia (61.3%) and in individuals with overweight/obesity and in women (8). The most commonly reported WL strategies were dieting and exercise (8, 9). Despite widespread efforts to lose weight, trends in overweight and obesity levels continue to rise. A large proportion of individuals with overweight and obesity find it difficult to achieve WL through lifestyle interventions (e.g., diet and/or exercise) alone (10). In a systematic review and meta-analysis of commercial WL diets, McEvedy et al. (11) found that 57% of individuals who commenced WL programs lost <5% of their initial BM when intention-to-treat data were analyzed. Even among those who completed the WL programs, 37% lost <5% of their initial BM. Achieving ≥5% WL is considered to be clinically significant because this amount of WL for individuals with a body mass index (BMI) of >25 kg/m^2 is associated with numerous health benefits, such as reduced blood pressure, cholesterol, and blood glucose which, in turn, reduces the risk of long-term conditions such as cardiovascular disease (12).

Studies have shown that individuals respond differently to WL interventions. The WL response can vary considerably between individuals to the same diet (13) or exercise (14, 15) intervention. Exploring the factors that are associated with clinically (≥5%) and non-clinically significant amounts of WL can help identify predictors of WL success and lead to the development of more effective WL strategies. Previous research has identified several psychological, physiological, and behavioral characteristics associated with WL success (16). These include baseline BM, early WL (17, 18), intervention adherence (19, 20), eating behavior traits, such as Three-Factor Eating Questionnaire Hunger, Disinhibition, and Restraint (21), appetite sensations (22), appetite-regulating hormones (23), fat consumption (24), exercise self-efficacy (25), resting energy expenditure (26), and physical activity (PA) (27).

Less research has explored whether energy balance behaviors, both at baseline and change from baseline, are predictive of WL success (28). Two behavioral components are integral to the energy balance equation: movement behaviors (PA and sedentary behavior [SB]) and eating behaviors (energy intake [EI] and food choice which is reflected in the macronutrient composition of the diet). These behaviors affect energy balance and therefore have the potential to influence WL, yet their association with the degree of WL success has received little attention. The key issue is that objective and accurate measures of energy balance behaviors, particularly in WL situations, are relatively hard to achieve. The identification of pre-existing energy balance behaviors and changes in those behaviors during the intervention that is predictive of WL success could inform personalized WL strategies for those in need of additional support.

The purpose of this study was to assess (i) the relationship among free-living movement behaviors, eating behaviors, and change in BM, (ii) whether free-living movement and eating behaviors existing at the beginning of the intervention, and their change during the intervention, significantly predict WL, and (iii) whether there were significant differences in free-living movement and eating behaviors between those who achieved clinically significant WL (≥5%) and those who did not (≤3%).

METHODS

Participants

The study was conducted as a secondary analysis of data collected from a trial that is published in more detail elsewhere (29) (ClinicalTrials.gov #NCT02012426). The secondary analysis study protocol was pre-registered on Open Science Framework (https://osf.io/fptwn). Based on the recommendations for the minimum sample size for detecting relationships between variables (30) and previous research (31), it was estimated that 61–84 participants would be sufficient to assess the overall relationships among free-living movement behaviors, eating behaviors, and change in BM (estimated $r = 0.30$–0.35, 0.8 power, and 0.05 alpha). Women with overweight or obesity were recruited by advertisement from the University of Leeds. The inclusion criteria were the following: provided written informed consent, healthy women, aged 18–65 years, BMI between 28 and 45 kg/m^2, reporting an interest in weight loss and not actively participating in a commercial WL program, not increased PA levels in the past 4 weeks, able to eat most everyday foods, fruits, and vegetables. The exclusion criteria were the following: significant health problems, taking any medication or supplements known to affect appetite or weight,

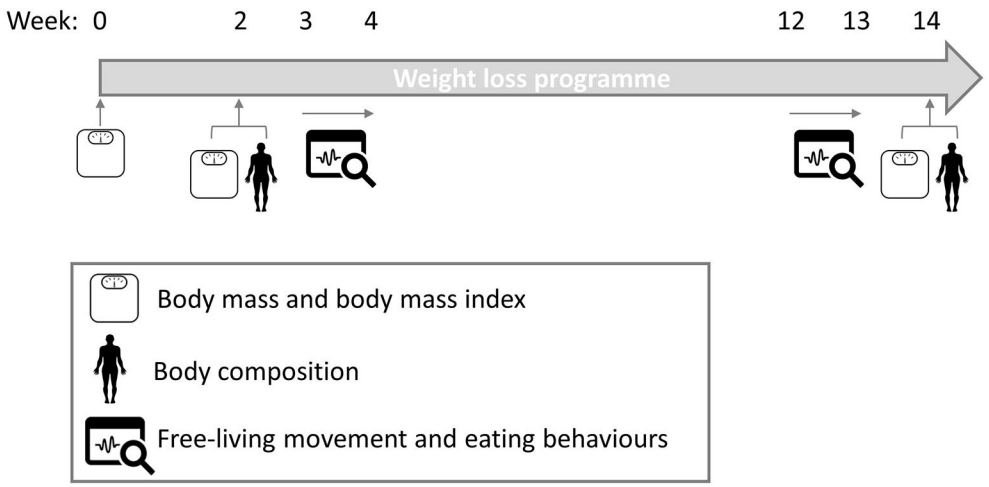

FIGURE 1 | Schematic diagram of study design.

pregnant, planning to become pregnant or breastfeeding, history of anaphylaxis to food, known food allergies or food intolerance, smokers and those who have recently ceased smoking (within the last 3 months), participants receiving systemic or local treatment likely to interfere with evaluation of the study parameters, those who have taken part in a commercial WL program in the last 2 months, individuals who work in appetite or feeding-related areas, unable to consume foods used in the study, individuals who have had bariatric surgery, history of an eating disorder, presence of untreated hypothyroidism, and insufficient English language skills to complete the study questionnaires.

Design

The study was a non-randomized, parallel-group design to assess whether behavioral characteristics (early and early-late change) were associated with ≥5% (clinically significant WL) or ≤3% (not clinically significant WL) WL following a 14 week WL program focused primarily on diet. The trial started with a 2 week run-in period followed by 12 weeks of trial monitoring. The purpose of the run-in period was to ensure the uptake and commitment of participants toward the programs. **Figure 1** provides a schematic overview of the study design. Self-reported compliance to the WL program was assessed each week by responding to "How well have you managed to stick with the weight control programme?" on a 100 mm visual analog scale (VAS) anchored at each end with "Not at all well" and "Very well". During weeks 2 and 14, participants visited the laboratory in the Human Appetite Research Unit at the University of Leeds for measurement days. For all laboratory visits, participants were instructed to fast from 10:00 pm the previous night and to abstain from strenuous exercise and alcohol consumption for at least 24 h before. Compliance was checked on arrival via self-report. The participants received payment of £250 on the completion of the study to reimburse them for their time and expenses. The study procedures and all study materials were reviewed and approved by the School of Psychology Research Ethics Committee at the University of Leeds (14-0090). The study was conducted between September 2014 and December 2015.

Weight Management Program

The participants either followed a commercial program [Slimming World (32)] or a self-led program [NHS Choices (33)] for the 14 week intervention period. The commercial program encouraged *ad libitum* intake of low energy-dense foods as part of a balanced diet, with weekly weigh-ins, group support, WL goals, and access to online support. The self-led program group accessed free online resources and a self-led diet program that recommends reducing calorie intake by 600 kcal/day. Both the programs encouraged individuals to self-monitor and increase engagement in PA by gradually increasing moderate-intensity aerobic exercise and resistance exercise to meet the Chief Medical Officer PA guidelines of 150 min/week of moderate-intensity PA. The effects of the different programs on BM have been reported previously (29).

For this secondary analysis, there were no specific research questions pertaining to the type of program used to induce WL. Rather, the study sought to assess behavioral characteristics associated with the degree of WL success. As such, data were analyzed with both WL program groups combined (commercial and self-led), and program type was controlled for in all analyses.

Body Mass, Body Mass Index, and Body Composition

Baseline (week 0): BM was measured on the first day of the weight management program with the use of electronic scales (commercial program group: recorded as part of their first weigh-in at a support group meeting; self-led group: recorded by a researcher at the research unit). Weight was measured with shoes and heavy clothing removed and height was measured using a stadiometer (Seca Ltd., Birmingham, UK) without shoes.

Week 2 and 14: BM and body composition (fat mass [FM] and fat-free mass [FFM]) were measured using the BOD POD (Body Composition Tracking System, Life Measurement, Inc., Concord, CA, USA), which uses air displacement plethysmography (34). The participants wore tight clothing and a swim cap to allow for an accurate measure of body volume.

Free-Living Eating Behavior

Participants completed a 7 day weighed food diary during weeks 3 and 12 (35). Electronic scales and training were provided to ensure detailed descriptions (e.g., brands) and consumed weights of foods and beverages were reported. Total EI, macronutrient composition, and energy density were calculated from the food diary. Energy density was calculated from the contribution of all food and milk (excluded all other drinks) (total EI divided by total weight intake) based on criteria previously used (36). Data were analyzed using a computerized food composition database called WISP 4.0 (Tinuviel Software 2013).

Free-Living Movement Behavior

Free-living PA and SB were measured during weeks 3 and 12 using the SenseWear Armband mini (SWA; BodyMedia, Inc., Pittsburgh, PA, USA), as has previously been described (37). The participants were instructed to wear the SWA on the posterior surface of their upper non-dominant arm for a minimum of 22 h/day for $\geq$6 days (except for the time spent showering, bathing, or swimming). For the SWA data to be valid $\geq$22 h of data per day had to be recorded for at least 5 days (midnight to midnight) including at least 1 weekend day. SB was classified as $\leq$1.5 metabolic equivalents (METs), light PA 1.6–2.9 METs, moderate PA 3–5.9 METs, and vigorous PA $\geq$6 METs (38).

Classification of Clinically Significant and Non-Clinically Significant Weight Losers

To identify differences that could account for individual variability in weight loss, the participants were grouped based on their BM change between baseline (week 0) and week 14. The participants were classified as clinically significant weight losers (CWL; $n = 41$) if they lost $\geq$5% and non-clinically significant weight losers (NWL; $n = 33$) if they lost $\leq$3% of their initial BM (18). Those who lost 3.1–4.9% of their initial BM ($n = 6$) were excluded from the analyses leaving two groups that exhibited a different weight loss response.

Change in Body Mass and Free-Living Movement and Eating Behaviors

Week two BM and BMI and week three free-living movement and eating behaviors will be referred to as "early" and week 14 BM and BMI and week 12 free-living movement and eating behaviors will be referred to as "late". "Early-late change" in free-living movement and eating behaviors refers to the difference between week 3 and week 12 measures.

Statistical Analysis

Data are reported as $M \pm SD$ (95% *CI*: lower, upper) throughout unless otherwise stated. All the variables were checked for outliers and normality was assessed using Shapiro-Wilk's test. The missing data were imputed using the last observation carried forward (LOCF) method (39). The analyses were conducted on the participants who completed the trial (completer analyses) and on an intention-to-treat basis (LOCF).

In this study, analysis of covariance (ANCOVA) controlling for the program type were performed to determine whether there was a significant change in baseline, early, and late measures of BM or BMI; or in early and late total daily free-living PA, SB, energy intake, macronutrient intake, or energy density. To explore the relationship among free-living movement behaviors, eating behaviors, and change in BM, partial correlation analysis was conducted controlling for program type. The assumptions of multiple regression were checked before conducting hierarchical multiple regression analysis, controlling for program type, to assess whether WL was significantly predicted by early (week three) and early-late change (difference between week three and week 12) in free-living PA, SB, energy intake, macronutrient intake, or energy density. A linear regression analysis was conducted to explore whether self-reported program compliance over the 14 weeks intervention significantly predicted weight change. The difference in baseline sample characteristics between the CWL ($\geq$5% reduction in BM) and NWL ($\leq$3% reduction in BM) were assessed using univariate ANCOVA controlling for program type. To assess group differences in changes in BM or BMI, 3 (week: zero, two, and 14) x 2 (groups: CWL and NWL) mixed ANCOVAs controlling for program type were performed. Univariate ANCOVAs were then conducted to explore the significant interaction effects. To compare changes in free-living PA, SB, energy intake and macronutrient intake data between groups, 2 (week: two and 14) x 2 (group: CWL and NWL) mixed ANCOVAs were performed controlling for program type. The effect of the covariate was only reported where significant. Greenhouse-Geisser probability levels were used to adjust for sphericity, only if appropriate. All main effects and interaction effects were examined with Bonferroni *post-hoc* tests. Partial eta squared (η^2) is reported for effective sizes and interpreted as follows: small, 0.01; medium, 0.06; large, 0.14 (40).

Statistical analysis was performed using IBM SPSS for Windows (Chicago, IL, USA, Version 24) and significance was set at $p < 0.05$ except for tests with multiple comparisons, in which case, a more conservative p-value was used to account for multiple comparisons [0.05 divided by the number of comparisons (three comparisons = $p < 0.017$)]. The analysis plan was registered prior to conducting the data analysis using the secondary data preregistration template on the Open Science Framework (OSF; https://osf.io/fptwn).

RESULTS

Study Population and Attrition Rates

In total, 613 individuals (291 commercial program) responded to the various recruitment methods. Of those, 517 were excluded for

TABLE 1 | Association between early (week three) and early-late change (week three to week 12) movement and eating behaviors and change in body mass (BM) between baseline and week 14.

	Total EE (kcal/d)	Light PA (min/d)	Moderate PA (min/d)	Vigorous PA (min/d)	SB (min/d)	Total EI (kcal/d)	Carbohydrate (%)	Fat (%)	Protein (%)	Energy density (kcal/g)
	Early (week 3)									
ΔBM (%)	0.06	−0.19	−0.10	**−0.32****	0.10	**0.28****	**−0.25***	0.20	−0.08	**0.37*****
	Early-late change (Δ)									
ΔBM (%)[1]	0.12	−0.11	−0.18	**−0.38****	**0.27***	−0.05	0.04	0.15	−0.08	0.08
ΔBM (%)[2]	0.13	−0.08	−0.19	**−0.37****	**0.25***	−0.04	0.01	0.10	−0.12	0.80

Data are partial correlations controlling for program type.

*Asterisks indicate that the differences are significant (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$).*

BM (%) is a percentage change from baseline to week 14.

[1]*Completer sample.* [2]*LOCF sample.*

Early (week 3) movement behavior correlations n = 77; early (week 3) eating behavior correlations n = 80; early-late change in movement behavior correlations for completer sample n = 65; early-late change in eating behavior correlations n = 77. Bold values indicate the correlation is significant.

various reasons and 96 (49 commercial programs) were recruited to the study. A further 16 either withdrew from the study or were excluded resulting in a final sample of 80 (37 commercial programs). Of the 80 participants who completed the study, 41 lost ≥5% of their initial BM. The details of the recruitment process and reasons for exclusion and attrition from the study have been reported previously (29). In this primary article, an additional two participants were excluded ($n = 78$) as there were delays with some elements of data collection, but those data are not being reported in this article, so the participants were retained. The participants were aged 42.0 ± 12.4 years with a BMI of 34.08 ± 3.62 kg/m^2. Average self-reported compliance (How well you have managed to stick with the weight control program?) across the 14-week program for those who completed the study was 48.0 ± 20.9 mm. During the 3rd week, the average SWA wear time was 1,412.1 ± 21.3 min/day (98.1%) and during the 12th week, the average SWA wear time was 1,416.5 ± 12.4 min/day (98.4%). The data from the SWA were missing for 15 participants because they either did not want to wear the SWA ($n = 1$), they did not comply with the wear procedure ($n = 11$), or the data file was lost/corrupted ($n = 3$). In addition, food diary data were missing for three participants because they did not complete the food diary.

Pooled Data

Changes in Body Mass, Body Mass Index, and Body Composition

When whole sample data were analyzed, there was a significant reduction in BM at each time point [$\eta p^2 = 0.284$; $p < 0.001$]: baseline [91.46 ± 12.61 kg (88.65, 94.27 kg)], week two [89.11 ± 12.61 kg (86.31, 91.91 kg)], and week 14 [87.05 ± 13.01 kg (84.16, 89.95kg)], *post-hoc* results between baseline and week 2, baseline and week 14, and week 2 and week 14 were all $p < 0.001$. Therefore, there was a significant reduction in BMI at each time point [$\eta p^2 = 0.274$; $p < 0.001$]: baseline [34.08 ± 3.64 kg/m^2 (33.27, 34.89 kg/m^2)], week two [33.20 ± 3.60 kg/m^2 (32.40, 34.00 kg/m^2)] and week 14 [32.44 ± 3.88 kg/m^2 (31.57, 33.30 kg/m^2)], *post-hoc results* between baseline and week two, baseline and week 14, and week two and week 14 were all $p < 0.001$. There was a significant interaction between week and program type for BM [$p = 0.001$] and BMI [$p = 0.001$].

There was a significant reduction in FM [$\eta p^2 = 0.22$; $p < 0.001$] from week two [41.46 ± 9.97 kg (39.03, 43.90 kg)] to week 14 [39.55 ± 10.30 kg (37.04, 42.07 kg)] and a significant interaction between week and program type for FM [$p = 0.009$]. There was no significant change in FFM [$\eta p^2 = 0.01$; $p = 0.48$] from week two [48.01 ± 5.97 kg (46.56, 49.47 kg)] to week 14 [48.03 ± 5.99 kg (46.57, 49.49 kg)].

Changes in Free-Living Movement and Eating Behaviors

There was a significant increase in percentage fat intake [$\eta p^2 = 0.05$; $p = 0.05$] from week three [32.76 ± 4.47% (31.75, 33.77%)] to week 12 [34.19 ± 5.77% (32.88, 35.50%)]. There were no other significant changes in any of the other free-living movement or eating behaviors [largest $\eta p^2 = 0.04$; smallest $p = 0.10$]. There was a significant interaction between week and program type for light PA [$p = 0.03$]. LOCF analyses did not differ (data not shown).

Behavioral Predictors of Body Mass Change

Partial correlations showed that higher vigorous PA [$p = 0.01$], higher percentage CHO intake [$p = 0.03$], lower total EI [$p = 0.01$], and lower energy-dense [$p < 0.001$] foods consumed early in the intervention (week three) were associated with a greater reduction in BM. An increase in vigorous PA [$p < 0.001$] and a decrease in SB [$p = 0.03$] from early (week three) to late (week 12) in the intervention were also associated with greater WL. All other movements and eating behavior variables were not significantly associated with BM changes (see **Table 1**).

Hierarchical linear regression analyses were conducted to evaluate the prediction of percentage BM change from movement and eating behaviors. Program type was controlled for and entered as a covariate in the first step of each regression model (forced entry). The movement and eating behavior variables [total energy expenditure (EE), light, moderate, and vigorous PA, SB, total EI, macronutrient composition, and energy density] were entered in step two using the stepwise method. Two separate hierarchical

TABLE 2 | Hierarchical linear regression analyses predicting change in percentage BM between baseline and week 14 from week three movements and eating behaviors and from early-late change in movement and eating behaviors.

Model	Variables	B (95% CI)	SE B	β	p	F	R^2	ΔR^2
Predictor variables: Early (week 3) movement and eating behaviors								
1	–	–	–	–	–	9.95	0.29	0.09
	Constant	−11.62 (−16.07, −7.17)	2.23	–	<0.001	–	–	–
	Program type	−0.43 (−2.44, 1.59)	1.01	−0.05	= 0.67	–	–	–
	Energy density (kcal/g)	5.69 (2.79, 8.58)	1.45	0.45	<0.001	–	–	–
	Vigorous PA (min/d)	−0.38 (−0.63,−0.13)	0.12	−0.30	= 0.003	–	–	–
Predictor variable: Early-late change (Δ) in movement and eating behaviors								
2	–	–	–	–	–	25.03	0.73	0.03
	Constant	−3.13 (−4.15,−2.11)	0.51	–	<0.001	–	–	–
	Program type	−0.85 (−2.17, 0.48)	0.66	−0.09	= 0.21	–	–	–
	Δ Vigorous PA (min/d)	−0.47 (−0.62,−0.32)	0.07	−0.49	<0.001	–	–	–
	Δ Total EE (kcal/d)	0.03 (0.03, 0.04)	0.01	1.84	<0.001	–	–	–
	Moderate PA (min/d)	−0.13 (−0.17,−0.10)	0.02	−1.17	<0.001	–	–	–
	Δ Light PA (min/d)	−0.05 (−0.07,−0.04)	0.01	−0.81	<0.001	–	–	–
	Energy density (kcal/g)	2.83 (0.59, 5.08)	1.12	0.18	= 0.01	–	–	–

Unstandardized beta (B), SE for the unstandardized beta (SE B), standardized beta (β), N = 63.
Model two was conducted on the completer sample.

multiple regressions were conducted to determine the unique contributions of early (model one) and early-late change (model two) in movement and eating behaviors to percentage BM change.

Model one revealed that the energy density of foods consumed and vigorous PA early in the intervention (week three) significantly predicted 29.0% of the variance in percentage BM change (as shown in **Table 2**). The results from model two demonstrated that early-late change in light PA, moderate PA, vigorous PA, total EE, and energy density of foods consumed significantly predicted 73% of the variance in percentage BM change. These hierarchical linear regression analyses demonstrate that higher week three vigorous PA and an increase in light, moderate, and vigorous PA were associated with greater WL. Conversely, higher week three energy density and an increase in total EE and energy density were associated with less WL. The LOCF analyses results did not differ (as shown in **Supplementary Table 1**).

Individual Variability in Body Mass Change

Analysis of Clinically Significant Weight Losers and Non-Clinically Significant Weight Losers Sample Characteristics

There was considerable individual variability in BM change among the participants ranging from −18.02 to +3.20 kg (−17.68 to +3.50%) with six (8.1%) participants gaining weight. Self-reported program compliance over the 14-week intervention significantly predicted weight change [$F(1, 64) = 33.19$, $p < 0.001$, $R^2 = 0.34$]. CWL reported significantly greater compliance with the program compared with NWL [CWL: 58.6 ± 17.4 mm (52.9, 64.3 mm); NWL: 35.3 ± 17.3 mm (28.7, 42.0 mm), $t(64) = 5.38$, $p < 0.001$].

Between Group Comparison of Changes in Body Mass

There were no differences between CWL and NWL early in the intervention for BM ($\eta p^2 = 0.005$; $p = 0.55$), BMI ($\eta p^2 = 0.027$; $p = 0.16$), FM ($\eta p^2 = 0.019$; $p = 0.29$), or FFM ($\eta p^2 = 0.001$; $p = 0.83$). There was a main effect of week for BM ($\eta p^2 = 0.269$; $p < 0.001$) and *post-hoc* tests showed that BM differed significantly between all three time points [*post-hoc* results all $p < 0.001$] (as shown in **Table 3**). There was also a week x group interaction on BM [$\eta p^2 = 0.526$; $p < 0.001$] that revealed CWL lost significantly more weight between all three time-points compared with NWL: baseline and week two [CWL: −3.09 ± 1.10 kg (−3.44, −2.75 kg); NWL: −1.52 ± 1.10 kg (−1.90, −1.13 kg), $\eta p^2 = 0.338$; $p < 0.001$]; baseline and week 14 [CWL: −7.21 ± 2.54 kg (−8.00, −6.42 kg); NWL: −1.12 ± 2.55 kg (−2.01, −0.23 kg), $\eta p^2 = 0.588$; $p < 0.001$]; and weeks two and 14 [CWL: −4.12 ± 2.39 kg (−4.86, −3.37 kg); NWL: 0.40 ± 2.40 kg (−0.44, −1.23 kg), $\eta p^2 = 0.469$; $p < 0.001$]. There was a significant interaction between week and program type for BM [$p = 0.02$]. Refer to Section 2 of the **Supplementary Materials** for between group comparison of changes in BMI and body composition.

Between Group Comparison of Change in Free-Living Movement and Eating Behaviors

Early in the intervention (week three), the energy density of the foods consumed by CWL was significantly lower than the energy density of the foods consumed by NWL [$\eta p^2 = 0.071$; $p = 0.03$] (as shown in **Table 4**). However, there were no statistically significant differences between groups in early (week three) measures of total EE, light PA, moderate PA, vigorous PA, SB, total EI, percentage carbohydrate intake, percentage fat intake, and percentage protein intake [largest $\eta p^2 = 0.071$; smallest $p = 0.08$].

TABLE 3 | Change in body mass (BM) and body mass index (BMI) between baseline, week 2 and week 14.

	Group	*n*	Baseline	Week 2 (early)	Week 14 (late)	Change (Δ)
BM (kg)	CWL	41	91.06 ± 13.15 (86.97, 95.15)	87.97 ± 13.03 (83.91, 92.02) [a]	83.05 ± 12.99 (79.80, 87.90) [b]	−7.21 ± 2.54 (−8.00,−6.42)***
	NWL	33	92.94 ± 13.19 (88.36, 97.52)	91.43 ± 13.07 (86.89, 95.97) [a]	91.82 ± 13.05 (87.29, 96.35) [b]	−1.12 ± 2.55 (−2.01,−0.23)
BMI (kg/m^2)	CWL	41	33.68 ± 3.75 (32.51, 34.86)	32.52 ± 3.66 (31.38, 33.66) [c]	31.00 ± 3.69 (29.85, 32.15) [d]	−2.68 ± 0.97 (−2.98, −2.38)***
	NWL	33	34.95 ± 3.77 (33.64, 36.35)	34.37 ± 3.67 (33.10, 35.65) [c]	34.52 ± 3.71 (33.23, 35.80) [d]	−0.43 ± 0.98 (−0.77, −0.09)

Data are adjusted M ± SD (95% CI).
Data are estimated marginal means adjusted for program type.
The change represents the difference between baseline and week 14.
*Asterisks indicate the differences are significant (*** $p < 0.001$) and when necessary superscript letters are used to indicate differences between the groups, i.e., the same letter is used for any pair when there is a significant difference observed (if bold $p < 0.01$, otherwise $p < 0.05$).*

TABLE 4 | Change in energy expenditure (EE), free-living physical activity [from light to vigorous physical activity (PA)], sedentary behavior (SB), energy intake, and macronutrient composition between week 3 and week 12.

	Group	*n*	Week 3 (early)	Week 12 (late)	Early-late change (Δ)
Total EE (kcal/d)	CWL	37	2606.36 ± 356.07 (2489.19, 2723.54)	2552.10 ± 370.84 (2430.06, 2674.14)	−54.27 ± 251.18 (−136.92, 28.39)
	NWL	24	2530.44 ± 357.23 (2384.47, 2676.41)	2487.28 ± 372.06 (2335.26, 2639.31)	−42.16 ± 252.00 (−146.13, 59.81)
Light PA (min/d)	CWL	37	201.64 ± 73.88 (177.33, 225.95)	191.29 ± 73.88 (165.79, 216.80)	−10.35 ± 66.17 (−32.13, 11.43)
	NWL	24	178.96 ± 74.13 (148.67, 209.25)	154.05 ± 77.75 (122.28, 185.81)	−24.91 ± 66.40 (−52.04, 2.22)
Moderate PA (min/d)	CWL	37	75.71 ± 48.11 (59.88, 91.55)	89.18 ± 55.79 (70.82, 107.54)	13.46 ± 39.97 (0.31, 26.62)
	NWL	24	67.31 ± 48.27 (47.59, 87.03)	65.40 ± 55.97 (42.53, 88.27)	−1.91 ± 40.11 (−18.29, 14.48)
Vigorous PA (min/d)†	CWL	37	2.22 ± 3.87 (0.95, 3.94)	4.23 ± 5.07 (2.56, 5.90)	2.01 ± 4.64 (0.49, 3.54)*
	NWL	24	1.39 ± 3.88 (-0.19, 2.98)	0.80 ± 5.09 (-1.28, 2.88)	−0.60 ± 4.65 (−2.50, 1.31)
SB (min/d)	CWL	37	717.77 ± 99.48 (685.03, 750.51)	706.99 ± 109.61 (670.93, 743.06)	−10.78 ± 100.30 (−43.78, 22.23)
	NWL	24	746.45 ± 99.81 (705.67, 787.23)	768.45 ± 109.97 (723.52, 813.38)	22.00 ± 100.63 (−19.12, 63.12)
Total EI (kcal/d)	CWL	40	1538.27 ± 448.30 (1396.83, 1679.72)	1536.40 ± 435.97 (1398.85, 1673.95)	−1.87 ± 395.53 (−126.63, 122.90)
	NWL	31	1702.77 ± 449.69 (1111.40, 1863.94)	1595.28 ± 437.33 (437.33, 1752.02)	−107.49 ± 396.72 (−249.67, 34.21)
Carbohydrate intake (%)	CWL	40	46.04 ± 6.30 (44.06, 48.03)	44.71 ± 8.17 (42.13, 47.28)	−1.34 ± 6.48 (−3.38, 0.71)
	NWL	31	43.37 ± 6.32 (41.10, 45.63)	41.91 ± 8.20 (38.98, 44.85)	−1.45 ± 6.50 (−3.78, 0.88)
Carbohydrate intake (kcal/d)	CWL	40	708.22 ± 96.91 (677.73, 738.80)	686.92 ± 125.52 (647.29, 726.41)	−21.30 ± 175.08 (−79.88, 107.44)
	NWL	31	738.49 ± 107.62 (699.84, 776.97)	668.58 ± 130.81 (621.84, 715.48)	−69.91 ±175.70 (−110.15, 15.82)
Fat intake (%)	CWL	40	32.01 ± 4.56 (30.58, 33.45)	32.84 ± 5.88 (30.99, 34.70)	0.83 ± 5.70 (−0.97, 2.63)
	NWL	31	33.44 ± 4.57 (31.80, 35.08)	35.58 ± 5.90 (33.47, 37.69)	2.14 ± 5.72 (0.09, 4.19)
Fat intake (kcal/d)	CWL	40	492.40 ± 70.14 (470.38, 514.53)	504.55 ± 90.34 (476.13, 533.13)	12.15 ± 182.72 (−41.81, 73.52)
	NWL	31	569.41 ± 77.82 (541.48, 597.33)	567.60 ± 94.12 (533.94, 601.26)	−1.81 ± 183.35 (−80.65, 50.81)
Protein intake (%)	CWL	40	19.06 ± 3.00 (18.11, 20.01)	18.95 ± 3.61 (17.81, 20.09)	−0.12 ± 4.11 (−1.41, 1.18)
	NWL	31	19.64 ± 3.02 (18.56, 20.72)	19.99 ± 3.62 (18.70, 21.30)	0.36 ± 4.13 (−1.12, 1.84)
Protein intake (kcal/d)	CWL	40	293.19 ± 46.15 (278.57, 307.79)	291.15 ± 55.46 (273.63, 308.66)	−2.04 ± 71.16 (−27.12, 17.78)
	NWL	31	334.42 ± 51.42 (316.03, 352.81)	318.90 ± 57.75 (298.32, 339.79)	−15.52 ± 71.40 (−45.90, 5.30)
Energy density (kcal/g)†	CWL	40	1.24 ± 0.27 (1.15, 1.32) [a]	1.36 ± 0.30 (1.26, 1.45)	0.12 ± 0.31 (0.02, 0.22)
	NWL	31	1.39 ± 0.31 (1.29, 1.48) [a]	1.47 ± 0.30 (1.36, 1.58)	0.08 ± 0.31 (−0.03, 0.19)

Data are adjusted M ± SD (95% CI).
Data from the SenseWear Armband were missing for 15 participants because they either did not want to wear the SWA, they did not comply with the wear procedure or the data file was lost/corrupted. Food diary data were missing for three participants because they did not complete the food diary.
Asterisks indicate early-late change is significant ($p < 0.05$).*
†*indicates the main effect of group is significant; and when necessary superscript letters are used to indicate differences between the groups, i.e., the same letter is used for any pair when there is a significant difference observed (if bold $p < 0.01$, otherwise $p < 0.05$).*

For early-late change in the movement and eating behaviors, CWL showed a small but significant increase in vigorous PA, whereas NWL showed a slight decrease [significant week x group interaction, $\eta p^2 = 0.072$; $p = 0.04$, as shown in **Table 4**]. The main effect of group for vigorous PA was significant; CWL [3.23 ± 3.87 min/day (1.95, 4.50 min/day)] performed more vigorous PA on average than NWL [1.10 ± 3.88 min/day (−0.49, 2.68 min/day), $\eta p^2 = 0.070$; $p = 0.04$]. On average, the energy density of foods consumed was lower in CWL [1.30 ± 0.24 kcal/g (1.22, 1.37 kcal/g)] compared with NWL [1.43 ± 0.24 kcal/g (1.34, 1.52 kcal/g), $\eta p^2 = 0.070$; $p = 0.03$]. There were no other main effects of week or group and no other week x group interactions for movement behaviors or eating behaviors.

The LOCF sample analyses (as shown in **Supplementary Table 2**) were much the same with the addition of a significant main effect of group for percentage fat intake; CWL [32.34 ± 4.49% (30.95, 33.74%)] consumed less fat on average than NWL [34.71 ± 4.51% (33.14, 36.27%), $\eta p^2 = 0.064$; $p = 0.03$].

DISCUSSION

The results of this study demonstrate that early and early-late change in both free-living movement and eating behaviors are associated with weight loss following a weight loss program focused primarily on diet. When whole sample data were analyzed, higher vigorous PA, higher percentage CHO intake, lower total EI, and lower energy density foods consumed early in the intervention (week three) were associated with a greater reduction in BM. In addition, an increase in vigorous PA and a decrease in SB from early (week three) to late (week 12) in the intervention were also associated with greater WL. Free-living movement and eating behaviors were also predictive of BM change. Consuming lower energy-dense foods and engaging in greater vigorous PA early in the intervention significantly predicted greater weight loss. As did early-late increases in light PA, moderate PA, vigorous PA, decreases in total EE and energy density of foods consumed. When participants were categorized based on their WL response, those who experienced the most successful weight loss (CWL) consumed lower energy-dense foods early in the intervention and on average, they showed a significant early-late increase in vigorous PA and performed more vigorous PA on average. Collectively, these findings demonstrate that specific behaviors that contribute to greater EE [e.g., vigorous PA (31)] and lower EI [e.g., less energy dense foods (29)] were related to better WL outcomes.

The current findings showing that the amount of PA is related to WL align with previous research which found that higher PA prior to engaging in an aerobic exercise intervention (41) and greater PA levels during a WL program were associated with greater WL (42, 43). Research examining whether energy balance behaviors (i.e., movement and eating behaviors), early in the intervention, and the change in those behaviors during an intervention, are predictive of WL success following a WL program primarily focused on diet is lacking. The present study confirms the findings from Vaanholt et al. (44) in women with overweight or obesity; free-living PA (particularly vigorous PA) early in the intervention and early-late change significantly predicted weight loss. Interestingly, early-late change in behavior predicted more of the variability is BM change (73%) than the model using early intervention data (29%). This suggests that change in behavior during a WL program is a more important determinant of weight loss success. To optimize WL, strategies to monitor energy balance behaviors during a weight loss intervention could identify individuals who may benefit from additional support. Light PA and moderate PA early in the intervention were not significant predictors of WL, but an early-late change in those behaviors was predictive of WL. Encouraging participants to replace SB with light PA and moderate PA early in the intervention could be one potential strategy to promote WL (45). Particularly, since a decrease in SB during the intervention was associated with greater WL. Interestingly, an early-late increase in total EE, which is heavily influenced by RMR which, in turn, is dependent on BM, was predictive of poorer WL outcomes in the current study. This finding appears counter-intuitive at first, but a probable explanation is that the total EE algorithm within the SWA software was influenced by the individuals who gained weight. BM is part of the SWA algorithm for estimating EE and an increase in BM (with an associated increase in resting metabolic rate) would result in an increase in total EE with no change in PA. Another possible explanation is that increased EE was driving an increase in EI, as proposed previously (46, 47), resulting in poorer WL.

Individuals who experienced the most WL (CWL) had significant differences in PA behavior profiles compared with those who experienced less WL (NWL). The CWL group significantly increased their vigorous PA whereas the NWL group showed a slight reduction. Furthermore, those who lost more weight performed more vigorous PA on average. Previous research has highlighted the role of vigorous PA in weight management (48). In this study, the increase in vigorous PA in the CWL group was small (∼2 min/day) and would have minimal impact on energy expenditure. However, the increase in vigorous PA could be large enough to positively impact other health outcomes. A recent review concluded low-volume high-intensity interval training protocols, with a similar amount of vigorous PA to the increase observed in the CWL group, has no effect on body fat or BM, a tendency to improve FFM (although not statistically significant) and favorable effects on various health outcomes, such as cardiorespiratory fitness (49). The observed increase in vigorous PA may reflect concerted efforts to increase purposeful structured exercise rather than incidental PA and potentially resulted in greater compliance with the WL diet as has been previously reported (50). Indeed, the CWL group self-reported significantly higher compliance with the program.

The consumption of lower energy-dense foods and lower total EI early in the intervention were associated with greater WL. Furthermore, consumption of lower energy-dense foods early in the intervention and an early-late decrease in energy-dense foods was predictive of successful WL. These findings are in line with previous research concluding that the consumption of a diet lower in energy-dense foods may be an effective strategy for managing body weight (51). Those who achieved clinically significant WL also exhibited different eating behavior to those who did not. Early in the intervention and on average, the CWL group consumed lower energy-dense foods compared with the NWL group. This supports previous findings demonstrating consumption of a low energy-dense diet leads to weight loss through improved appetite control and reduced EI (29). There was also a trend toward the main effect of week and group for percentage fat intake such that percentage fat intake was higher in the NWL group and there was an early-late increase in the percentage fat intake (but not absolute fat intake). The increase in fat intake during the intervention could indicate a decrease in adherence to the WL diet (52), a weakening

in restraint/increase in disinhibition (21) or a compensatory response to prevent further WL (53). The types of fat participants consumed were not measured, therefore it is not possible to comment on the quality of fats consumed by participants in this study (54).

This study supports previous research reporting that early WL is an important marker of program success and long-term WL outcomes (18). In the current study, those who lost ≥5% of their initial BM (CWL) showed a statistically significant reduction in BM at each time point (including baseline to week two), whereas those who lost ≤3% of their initial BM (NWL) did not. This provides further support for the use of early non-response to WL programs as a marker for identifying individuals who could benefit from additional support. Unick et al. (18) recommend the use of adaptive or stepped care interventions to provide an individualized program for those in need of additional help. Additional research is needed to explore the optimal time point to intervene, the threshold for identifying those in need of additional support, and the type of intervention that is most effective for boosting WL in early non-responders. In the present study, strategies early in the intervention to improve compliance, promote PA (particularly vigorous PA), and reduce the consumption of energy dense foods may have promoted greater WL in those with poorer WL outcomes.

Subtracting the EI data from the estimates of EE suggests that the CWL were in an energy deficit of ∼1068–1016 kcal/day, while the NWL were in an energy deficit of ∼827–892 kcal/day. Assuming EI and EE remained the same, that would give an energy deficit of ∼100,000 kcal for the CWL and ∼85,000 kcal for the NWL over the study. Assuming 1 kg of BM (70:30 fat/lean tissue) is equivalent to 7,000 kcal (55), based on the observed BM changes, the predicted energy deficit would be 50,470 kcal and 7,840 kcal for the CWL and NWL, respectively. These calculations highlight the well-documented issue of underreporting inherent with self-reported dietary intake, particularly in people with obesity (56, 57). Interestingly, those who lost less weight underreported their EI to a greater extent (∼780 kcal/day) than those who lost more weight (∼55 kcal/day). It is acknowledged that the calculations are not accurate (58); however, the 7,000 kcal rule provides an indication of the energy deficit required to produce the observed weight losses vs. the energy deficit calculated from the EI and EE data. These considerations highlight the potential problems with self-report dietary variables as the potential predictors of weight outcomes. If it is assumed that the energy deficits estimated from the PA assessments in combinations with body weight are likely to be more accurate than those using self-report dietary intakes, this would mean that EI was underreported by approximately 30% in the NWL group and by about 2–3% in the CWL group. This has implications for the combined predictor and the group comparisons presented above. Interestingly, it also implies that those who are more successful at WL are able to more accurately assess their true EI over time. These considerations are part of an on-going analysis of dietary misreporting as a predictor of successful WL.

There are several limitations inherent in this study that should be acknowledged. First, due to the restrictions around participant recruitment, PA and EI were not fully captured at baseline and some adaptations may already have occurred in the first 2 weeks of the intervention. Changes identified between early and late measurement periods may have reflected a regression back to baseline, for example, the increase in percentage fat intake, limiting the interpretability of these findings. However, early and early-late changes in PA and EI were still predictive of WL success. Second, while the data suggests adherence to the WL program may play a role in WL outcomes, there are limitations with this measure. There is no accepted or feasible method of measuring adherence to WL programs (18). In this study, adherence was self-reported and may have been confounded by participants knowing whether they lost weight each week. PA promotion was a component of the WL programs and accelerometer-based measures of free-living PA were positively correlated with adherence (data not presented) supporting the validity of the adherence measure. Third, WL was induced using two different WL programs (commercial and self-led), but because there were no specific research questions pertaining to program type, WL data were analyzed with both WL program groups combined and program type was controlled for in all the analyses. However, the two WL programs were inherently different. It is therefore difficult to disentangle whether reduced energy density was a predictor of WL success, given the group that was placed on the low energy dense diet lost the most weight (29), or whether it was due to the face-to-face support that group received, or a combination of both. Fourth, although it is an accepted and widely used energy density calculation, the Wrieden method did not account for the calories consumed in drinks (other than milk). Fifth, the stage of the menstrual cycle was not controlled for and therefore a confounding effect on energy intake cannot be ruled out (59). Finally, to overcome the attrition in this study, which is common in WL interventions (60), missing data was imputed using the last observation carried forwards method. This method has previously been implemented in WL trials (61) and the limitations of this approach have previously been acknowledged (62). A strength of the current study is the measurement of PA (for ∼23 h/day) and EI under free-living conditions over a complete week during the early and late stages of the intervention with strong and sensitive measurement methods.

Conclusion

Early and early-late change in free-living movement and eating behaviors during a 14-week WL program are predictors of WL. These findings demonstrate that specific behaviors that contribute to greater EE (e.g., vigorous PA) and lower EI (e.g., less energy-dense foods) are related to more successful WL outcomes. Interventions targeting these behaviors may increase the effectiveness of WL programs. Additional research is needed to explore the threshold for identifying those in need of additional support, the optimal time point to intervene, and

the type of intervention that is most effective for boosting WL in those in need of additional support.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The current datasets are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to Anna Myers, a.myers@shu.ac.uk.

ETHICS STATEMENT

This study involved human participants and was reviewed and approved by The School of Psychology Research Ethics Committee at the University of Leeds (14-0090). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AM, NB, CG, JB, and GF designed the research. NB, DC, and FC conducted the trial. AM processed the physical activity data, performed statistical analyses, and wrote the manuscript. All authors read and approved the final manuscript.

FUNDING

The trial was funded by Slimming World UK. Slimming World UK supported recruitment. The funder had no role in the analysis or writing of this article.

ACKNOWLEDGMENTS

This trial was registered on ClinicalTrials.gov #NCT02012426. This article is dedicated in memory of our co-author, colleague, and friend, Diana Camidge. Diana passed away shortly after the completion of this study, after fighting a very rare neurodegenerative disease (Gerstmann-Sträussler-Scheinker syndrome). Treatments are under development and a foundation in her name is raising funds to help fight the illness, www.dianacamidgefoundation.org. We thank the women who participated in this trial. We are also grateful to Dr. Stephen Whybrow (University of Aberdeen) for analyzing the food diaries.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.688295/full#supplementary-material

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frontiers in Nutrition

SYSTEMATIC REVIEW
published: 18 November 2021
doi: 10.3389/fnut.2021.734267

The Effects of Exercise on Appetite in Older Adults: A Systematic Review and Meta-Analysis

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Background: The effect of physical activity and exercise on hunger and satiety has been well-studied in younger adults, but the influence of aging is less understood. While some evidence suggests that acute bouts of exercise induce a compensatory eating drive, long-term activity may improve satiety sensitivity. The objective of this study was to investigate the effects of exercise on appetite in older adults.

Methods: We systematically reviewed available literature investigating the effect of exercise on appetite in older adults adults (CRD42020208953). PubMed, PsycINFO, Academic Search Complete, the Sports Medicine & Education Index, and Web of Science, were searched for peer-reviewed articles published in English with no date restriction. Included studies implemented a primary exercise or physical activity intervention with a control group, on a generally healthy population ≥60 years of age. Selected studies included at least one appetite outcome. Risk of bias was assessed using the 11-point Physiotherapy Evidence Database (PEDro) tool. Standardized mean difference summary statistics (Hedge's g effect sizes) and 95% confidence intervals were reported.

Results: We identified 15 reports (13 studies) which met all inclusion criteria (5 resistance training, 3 aerobic, 6 mixed modalities). Studies included 443 participants (Age = 68.9 ± 5.2, 82.3% female) and had generally "good" bias scores (PEDro = 6.4 ± 0.88). Random effects meta-analyses revealed that the exercising group showed statistically significant reductions in glucose [SMD = −0.34 (95% CI: −0.67, −0.02), $p < 0.05$, PEDro =6.4 ± 0.45] and leptin [SMD = −0.92 (95% CI: −1.28, −0.57), $p < 0.00001$, PEDro = 6.2 ± 0.75].

Discussion: This systematic review revealed that exercise and physical activity may modulate resting hunger and satiety in older adults. Decreases in fasting leptin and glucose hormones suggest that exercise promotes satiety sensitivity in adults aged 60+. This review highlights that engaging in exercise and activity programs may provide a meaningful avenue for improving chronic and functional disease burden in later life by promoting appetite control and balanced energy intake. Recommendations for future research include investigations of appetite in response to varied exercise modalities within more diverse and representative samples of older adults.

Keywords: exercise, appetite, aging, leptin, satiety

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Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *30 June 2021*
Accepted: *19 October 2021*
Published: *18 November 2021*

Citation:
Hubner S, Boron JB and Koehler K (2021) The Effects of Exercise on Appetite in Older Adults: A Systematic Review and Meta-Analysis. Front. Nutr. 8:734267. doi: 10.3389/fnut.2021.734267

INTRODUCTION

The number of older adults worldwide is projected to more than double by 2050, with those aged 65 and older reaching 1.5 billion within the next three decades (1). This demographic shift is happening concurrently with changes in work and lifestyle, specifically a global trend toward sedentary behavior (2). Overall, this inactivity negatively affects health, directly relating to a higher prevalence of chronic cardiovascular and metabolic disease (2). As such, physical activity and exercise are promoted to ameliorate physical, psychosocial, emotional, and cognitive wellness (3, 4). Worldwide physical activity guidelines recommend that older adults engage in moderate to vigorous-intensity aerobic activity a minimum of three times weekly, irrelevant of chronic disease status; guidelines also suggest integrating strengthening activities and multicomponent balance and fall prevention exercises and limiting sedentary time (4). Still, despite these recommendations, up to 70% of adults in developed countries report insufficient physical activity (2, 5). Low levels of physical activity and exercise ultimately increase disease risk, morbidity, and all-cause mortality (2).

Comorbidity is often compounded by overweight, obesity, and frailty, which are complex issues in older adult populations given age-related changes in body composition, adiposity, and appetite (2, 6–8). Exacerbated by low activity, older adults may experience unique weight-related challenges due to physiologically-driven, natural redistribution and increases in subcutaneous fat (6, 7). Increased adiposity can be concomitant with sarcopenia, the loss of muscle mass and strength associated with aging (6). Consequentially, efforts to *lower* body mass can affect poor nutrition and simultaneous fat and muscle loss, diminishing the prospective benefit of weight reduction (6, 7, 9). Further, age-related declines in metabolic and sensory systems, particularly those related to olfaction and gustation, can cause an "anorexia of aging" characterized by reduced eating drive and energy intake (10–12). Efforts to *maintain* body mass can also be attenuated by both undernutrition and malnutrition, precipitating sarcopenia and frailty (8, 12). Ultimately, biological changes complicate weight control in older adults, and management of energy intake remains key to mitigating morbidity and mortality (6, 7, 9, 12).

Across populations, physical activity and exercise are fundamentally associated with health outcomes and behaviors, extending to the management of body composition and energy intake (13). In young adults, it has been documented that physical activity and exercise may induce a compensatory response in energy intake, which can impact the long-term success of fitness regimens (13–16). Evidence from this population has demonstrated that, while acute exercise causes a brief, transient decrease in appetite, it likewise stimulates the release of orexigenic hormones and reduces anorexigenic processes, ultimately upregulating hunger and eating drive (13–16). Notwithstanding these acute hormonal changes and the associated physiological response, some research has failed to demonstrate the expected increase in post-exercise energy intake (13, 17). However, the effects of exercise are dependent on many individual factors, therefore this absence may at least partially stem from the mix of populations and methods included across research (18, 19). This may also be a consequence of relying on self-report methods, primarily diet logs, for assessment (17, 20, 21).

Considering chronic exercise and physical activity, evidence from long-term studies in young adults indicates that improvements in body composition and function result in enhanced satiety sensitivity, thus promoting metabolic balance and relative appetite control (18, 19, 22, 23). Further, sustained increases in activity have been evidenced to alter fasting levels of appetite-regulating hormones and metabolites (e.g., leptin, glucose, insulin, ghrelin, adiponectin) and heighten post-prandial satiety (13, 24). This suggests that fidelity to regular activity programs improves appetite control, helping to regulate accompanying increases in hunger drive (13). Prolonged exercise has also been linked to increased energy and protein intake, as well as improved muscle maintenance in aging populations, both of which can help mitigate adiposity, sarcopenia, and downstream disease risk (25–28). However, like acute exercise, there are bodies of opposing literature suggesting no effect of long-term physical activity on appetite, or that these relationships are inconsistent (13, 21, 29–32). Similarly, this is likely attributable, in part, to population and methodological variations between studies.

Overall, the relationships between exercise, physical activity, and appetite are poorly researched in older adults, with a marked paucity of aging-specific data (13, 17, 30). Previous research (3, 9, 13, 17, 21, 22, 27–36) on the effects of exercise and physical activity on appetite in aging populations is inconsistent and appears to be unclear. The purpose of this systematic review and meta-analysis was to assess the existing literature on exercise and physical activity interventions in older adult populations, with the aim of better understanding how they may impact hunger and satiety in aging groups. The authors hypothesized that activity and exercise-induced consequences of hunger, satiety, and compensation differentially affect aging adults. Additionally, despite the potential for negative outcomes, exercise and physical activity might lead to net improvements in appetite-regulating pathways and resultant health. The goal of this research was to elucidate known effects and identify areas for future investigation in aging populations.

METHOD

A systematic literature review and meta-analysis were performed following the guidelines set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [PRISMA, **Supplementary Tables 1**, **2** (37)]. Scope, methods, and aims for the study were decided upon a priori by the study team and are presented in a systematic review protocol (Prospero, 2020 CRD42020208953, *Blinded for Review*).

Literature Search Strategy

Electronic database searches were conducted in PubMed, APA PsycINFO, Academic Search Complete, the Sports Medicine & Education Index, and Web of Science. The search of Web of

Science included the Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index, and Emerging Sources Citation Index. Database searches were conducted in July 2020 and June 2021. Search results included all available reports from inception of the respective database to 5 June 2021.

The search strategy combined the following relevant terms: (1) *Title*: older*, elder*, senior*, or geriat*, AND (2) *Title*: exercise[Mesh]/exercis*, exert*, energ*, fitness*, activit*, isometric, anaerobic, aerobic, or isotonic, AND (3) *Title, Abstract, or Topic*: leptin, ghrelin, peptide tyrosine tyrosine, peptide yy, pyy, appetite, hunger, adiponectin, satiet*, GLP-1, GLP 1, glucagon like peptide 1, glucagon-like peptide 1, CCK, cholecystokinin, NPY, neuropeptide Y, AgRP, agouti-related peptides, orexin, pro-opiomelanocortin, pro opiomelanocortin, CART, cocaine-and-amphetamine regulated transcript, cocaine and amphetamine regulated transcript, CRH, corticotropin-releasing hormone, corticotropin releasing hormone, diet*, eating, ate, consum*, or food*.

Search terms were also used to exclude studies with titular indications of younger participants or non-human subjects. Specific relevant terms included: (1) *Title*: adolesce*, young adult*, youth, child*, or infant*, OR (2) *Title*: mice, mouse, pig, or rat. Database limiters/filters were used to exclude non-English publications and gray literature where appropriate.

The title/abstract screen and the full-text screen were performed by two reviewers (SH and MG). Disagreement and ambiguities in eligibility criteria were resolved by a third reviewer (JB) and consensus among authors. Results from the database search were managed in EndNote X9 (38) and Zotero 5.0 (39) citation software. Deviating from the pre-specified protocol, hand searching was performed, consisting of assessment of (1) included articles' reference sections and (2) relevant reviews and meta-analyses, identified via the database search. Hand searching was completed to ensure review of potentially missing reports, including any additional manuscripts associated with the included articles. This was deemed necessary given the small number of reports, in addition to the identification of several pairs of articles based upon the same studies, discussed later in further detail.

Study Selection Criteria

The specific research question for this systematic review and meta-analysis was "does exercise or physical activity influence hunger, appetite, or satiety outcomes in healthy adults aged 60 and older?" Studies on aging populations which implemented exercise or physical activity interventions and included hunger and satiety outcomes were included in this study. Considering the participants, interventions, comparators, and outcomes (PICOS) systematic review and meta-analysis design: (1) Participants were healthy adults 60 years and older. Studies which included participants with obesity and/or sarcopenia were included, and additional sub-analyses were proposed for these reports. (2) All studies included physical activity and/or exercise interventions. Interventions were classified as aerobic, resistance, or mixed modality. (3) At minimum, studies included an exercise-only group/condition and a control group/condition. To aid group comparison, controls were either a unique control group or the intervention group at rest, normal activity, or low activity (e.g., walking). (4) Included reports presented outcomes related to hunger-regulating hormones (e.g., ghrelin, leptin), compensatory eating (e.g., energy intake), and/or satiety (e.g., appetite). Although not explicitly indicated in the original PROSPERO registration, insulin/glucose was added as an outcome due to its frequency of reporting. Insulin and glucose are important signals within the orexigenic cascade, so inclusion is warranted (13, 24). In order to retain the pre-specified search strategy, additional database searching for these outcomes was not conducted; however, if studies identified by the original search included these measures, they were considered for inclusion. Studies were restricted to articles published in peer-reviewed journals in English. Given the limited subject data, most study designs were eligible for consideration with minor exceptions, detailed subsequently.

Studies were excluded if non-human subjects or populations younger than 60 years were included. Additionally, reports with unclear outcomes or those that were qualitative, gray literature (e.g., book chapters, dissertations, etc.), case studies, or unpublished/incomplete manuscripts were ineligible. Reports that included persons with appetite or activity-modulating health/physical conditions (e.g., diabetes, eating disorders, joint replacements) were excluded. Similarly, studies with interventions which included modifying lifestyle programs, supplements, or other similar components were excluded in absence of representative exercise-only and control-only groups. Interventions with maintenance/management diets based on participants' normal energy intake were eligible for inclusion as they were not considered to be 'modifying' programs.

Although previous systematic reviews have been conducted examining concepts similar to this question, the scope and goals of these reviews differed significantly and, by result, their search criteria varied considerably compared to the present study (13, 35, 40). Further, this review specifically aims to provide a summary of available research on the relationships between exercise, physical activity, and appetite within a reasonably healthy older adult population.

Data Extraction

Data was extracted by a single reviewer (SH). For studies that met all criteria, the following data were extracted: (1) sample (number of participants, mean age, proportion female); (2) intervention [exercise(s) and control(s)]; (3) study timing (length of study, number/frequency measures); and (4) hunger and satiety measures (pre- and post-test means, changes from baseline (change score), standard deviations, and correlation coefficients, when reported). In studies where more than one exercise intervention group was reported, summary statistics were combined and evaluated as a single group in alignment with Cochrane recommendations, where N is the number of participants, M represents mean, and SD is standard deviation (41):

$$N_{Total} = N_1 + N_2$$

$$M_{Total} = \frac{N_1 M_1 + N_2 M_2}{N_1 + N_2}$$

$$SD_{Total} = \sqrt{\frac{(N_1 - 1)\, SD_1^2 + (N_2 - 1)\, SD_2^2 + \frac{N_1 N_2}{N_1 + N_2}\left(M_1^2 + M_2^2 - 2M_1 M_2\right)}{N_1 + N_1 - 1}}$$

In reports where pre- and post-test results were reported, the following equations (41) were used to calculate, *M* and *SD* of the change score, where *r* represents the correlation coefficient between baseline and endpoint values:

$$M_{Changes} = M_{Endpoint} - M_{Baseline}$$

$$SD_{Change} = \sqrt{(SD_{Baseline})^2 + (SD_{Endpoint})^2 - (2 * r * SD_{Baseline} * SD_{Endpoint})}$$

A convenience estimate of $r = 0.5$ was used in this study due to insufficiently reported data (14, 41). For studies presenting additional incomplete outcome data, procedures for estimation of missing data were performed (42), where necessary.

Risk of Bias

Two reviewers (SH, MG) independently assessed each article for risk of bias without blinding to authorship or journal. Disagreements in scores across reviewers were resolved by consensus and inclusion of a third reviewer as necessary (JBB). The Physiotherapy Evidence Database (PEDro) risk of bias tool was utilized for study assessment (pedro.org.au).

Data Analysis

Data management and summary statistics were conducted and produced in IBM SPSS Statistics 27.0 (43). Meta-analyses and associated data visualizations were conducted and produced in Review Manager (RevMan) (44). The statistical R package "metaphor" via MAVIS: Meta-analysis via Shiny was used to review meta-analyses, assess publication bias, and produce funnel plots, as appropriate (45). Mean effect summary statistics were represented by standardized mean differences (SMDs) and 95% confidence intervals (CIs). Treatment effects were measured as the net change in outcome values at baseline and endpoint. Individual and pooled Hedge's g effect sizes (SMDs) for small sample bias with random effects models were calculated. Random effects models were favored due to variance in the studies. SMD was used to judge the magnitude of the effect. Studies were meta-analyzed if there were three or more reports with valid data. The I^2 test for heterogeneity was used with standard cutoffs of <25% indicating low heterogeneity, 25–50% representing moderate heterogeneity, and >50% indicating high heterogeneity. Sensitivity analyses were used in all meta-analytic calculations to assess robustness. Sensitivity analyses were conducted by removing one study at a time to assess for the effects of sample size, individual effect sizes, and study quality (risk of bias), where appropriate. Where possible, sub-analyses were used to examine studies which only included either healthy-weight or overweight/obese participants. For significant pooled effects, the funnel plot, rank correlation test for funnel plot asymmetry, and Rosenthal's fail-safe N were assessed to test for publication bias (42). All alpha levels were set to $p < 0.05$ a priori.

RESULTS

Study Identification

The literature search strategy accumulated 4,449 total titles. After duplicate deletion, 2,456 unique articles were identified for preliminary screening. Title and abstract evaluation identified 79 potential manuscripts for full text screening. After screening full articles, 19 reports met all inclusion criteria (46–64). One additional article was identified via hand searching (65). Corresponding authors of studies which included apparent duplicate or missing data (precluding full interpretation of the results), were contacted for clarification. No corresponding authors responded to these inquiries. Reports with duplicate data from included studies were retained, however, five studies which could not be interpreted without further detail were eliminated (59, 60, 62–64). Ultimately, 13 unique studies were represented by 15 reports and were included in analyses (46–59, 61). Four reports with duplicate data were consolidated and analyzed as two individual studies. Further, (1) Butterworth et al. and Nieman et al. (46, 55), and (2) Markofski et al. and Timmerman et al. (54, 61) were jointly analyzed. Timmerman et al. was included in this review as a relevant study duplicate to Markofski et al., although there were no measurable appetite outcomes (61). The results of the literature search and study selection are reported as a PRISMA flow diagram in **Figure 1** (37).

Characteristics of Included Studies

The characteristics of each of the included reports are summarized in **Supplementary Table 3**. All identified studies were randomized controlled trials (RCT), apart from one quasi-randomized control trial (54, 61) and one counterbalanced cross-over study (51). A single study implemented an acute exercise bout (51); the remaining 12 interventions ranged in duration from 8 to 44 weeks (46–50, 52–58, 61, 65). Studies utilized a variety of exercise or physical activity interventions with varied frequency and intensity; for the purpose of this review, they were summarized as resistance training [$N = 5$ (47, 49, 51, 58, 65)], aerobic exercise [$N = 3$ (46, 55–57)], or mixed modality [$N = 5$ (48, 50, 52–54, 61)]. In three studies, participants were specifically instructed to maintain their normal diets (47, 48, 65). Six studies excluded obese participants (46–48, 53, 55, 56, 58).

Across the 13 studies, 443 total participants were included in final analyses. Of these, 245 subjects were part of an exercise group and 178 were in a control group. The remaining 20 participants were part of crossover study where they served as the intervention and control; these 20 participants' data were not meta-analyzed to avoid double counting (51). Included subjects had a mean age of 68.9 years (SD = 5.2) and were predominantly female (82.3%). The 13 distinct studies were conducted in 6 countries: 6 in the United States (46, 48, 53–55, 58, 61, 65); 2 in the United Kingdom (50, 51); 2 in Brazil (47, 57); and 1 each in Greece (49), South Korea (52), and Japan (56).

More than one exercise intervention group was described in four reports (49, 50, 57, 58). As previously illustrated in the method, the intervention groups in each of these articles were merged into four respective combined groups; this resulted in a single exercising intervention group and a single control

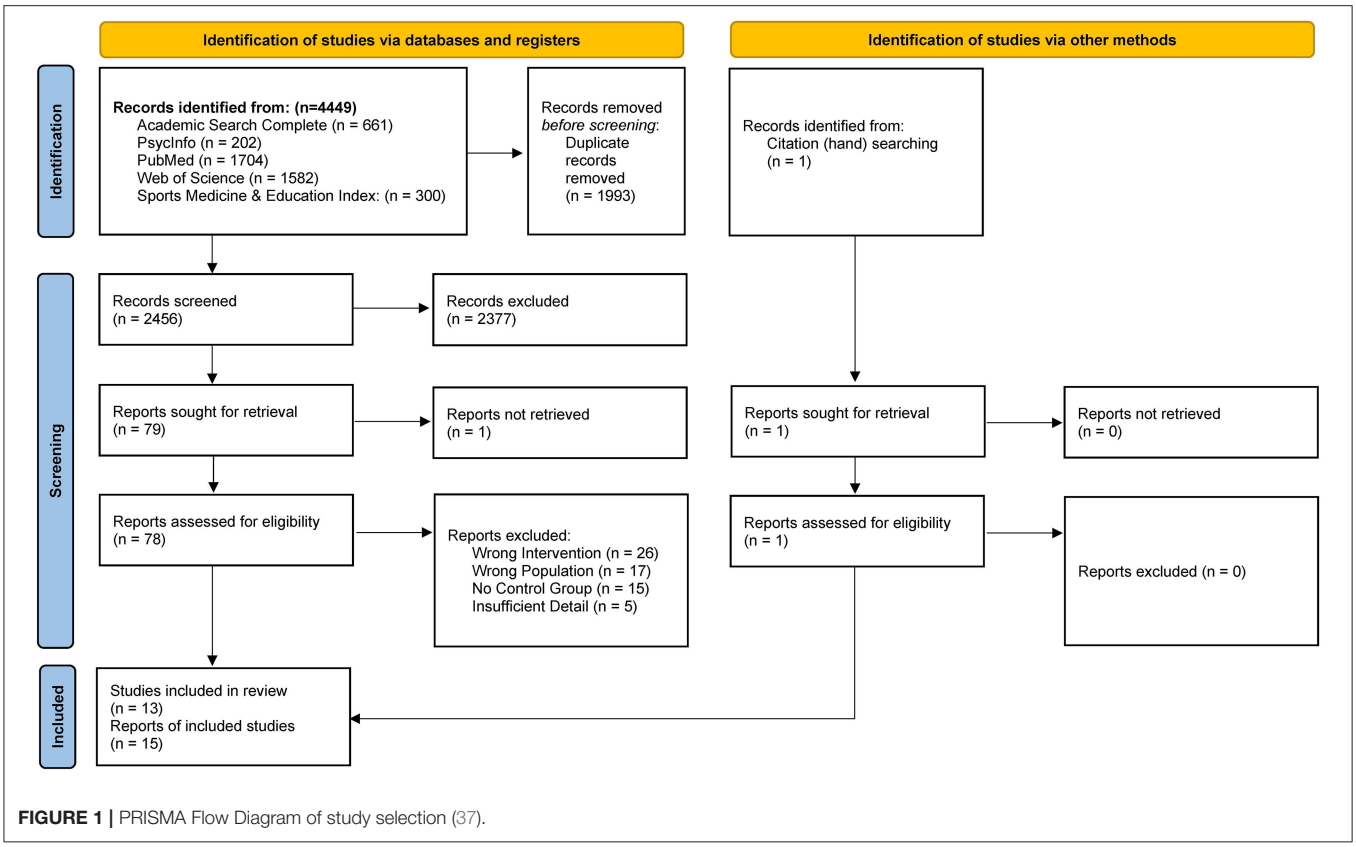

FIGURE 1 | PRISMA Flow Diagram of study selection (37).

group for each report. Development of the consolidated groups required combination of: (1) low, moderate, and high intensity exercise (49), (2) sedentary behavior fragmentation and light intensity activity (50), (3) dancing and walking interventions (57), and (4) high intensity and low intensity exercise (58).

Only 3 manuscripts provided change scores (48, 51, 53), while no studies provided a correlation coefficient (r value) in the absence of a change score. As such, an estimated r value of 0.5 was used to calculate change scores for 11 studies (46, 47, 49, 50, 52, 54–58, 65). Because of the small number of available change scores, no sensitivity analyses were performed to assess the effects of either the estimated data or imputed r value.

Risk of Bias of Included Studies

The risk of bias scores for included studies are reported in **Supplementary Table 4**. The risk of bias was generally "good" for the 15 included reports, with the average score equaling 6.4 (SD = 0.88) out of 11. Risk scores ranged from the upper limit of "fair" equaling 5 at the lowest (48, 54, 61), to the upper limit of "good" equaling 8 at the highest (50). In general, on items related to subject description, outcome reporting, and randomization, all included articles performed adequately. Specifically, for items related to subject eligibility and outcome reporting (PEDro #4, 10-11), all reports scored positively; between 11 and 14 articles earned points for randomization, baseline reporting, and subject measurements (PEDro #2, 4, 8-9). Conversely, reports failed to score on items related to (1) allocation concealment, (2) blinding of subjects, (3) blinding of therapists/administrators, and (4) blinding of assessors (PEDro #3, 5-7).

Meta-Analysis

Quantitative values for energy intake ($N = 6$), ratings of appetite ($N = 1$), glucose ($N = 5$), leptin ($N = 5$), and adiponectin ($N = 3$) were identified within the studies. Meta analyses with effect size calculations were possible for energy intake (46–48, 50, 55, 58), adiponectin (49, 54, 56, 61), glucose (49, 52, 53, 56, 57), and leptin (49, 52–54, 61, 65). In applicable studies, energy intake was reported in kcal/day (48, 50, 55, 58) and kcal/day/kg (47) of body mass. Glucose was reported as fasting glucose in mg/dl (52, 56, 57) and mmol/L (49, 53). Serum leptin and high molecular weight adiponectin were reported across studies as values of ng/ml and μg/ml, respectively. Because some outcomes were measured in different units across trials, the SMD was calculated for all measures. Ultimately, 18 individual effect sizes were calculated, in addition to the 4 random pooled effects (SMDs) for each outcome of interest. Sub-analyses produced an additional 4 SMDs.

The meta-analysis for energy intake included data from 5 studies and 177 subjects (46–48, 50, 58). Average PEDro score for included studies was "good", equaling 6.6 (SD = 1.02). Despite homogeneity amongst the studies ($I^2 = 0\%$), no significant intervention effect was identified. Sub-analyses on studies which only included non-obese participants were similarly non-significant for any effect of the intervention on energy intake (46–48, 55, 58). Similarly, meta-analysis of

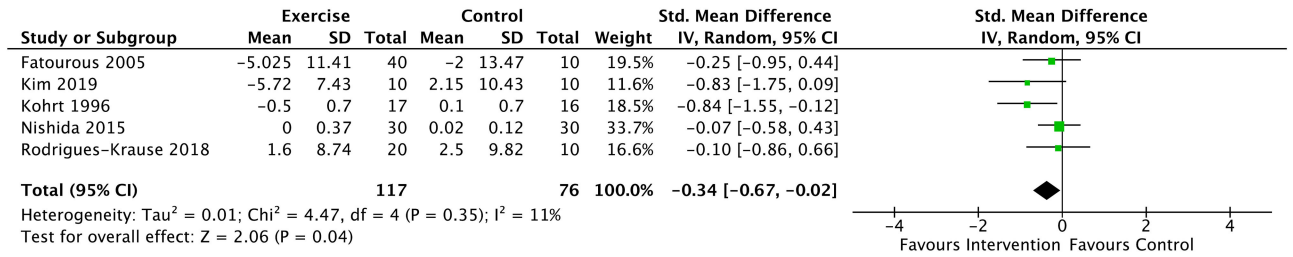

FIGURE 2 | Forest plot for the effect of exercise on glucose. Results favor lower scores in experimental group. Model is statistically significant with low heterogeneity (I^2= 11%), suggesting reliability of the small effect size (−0.34).

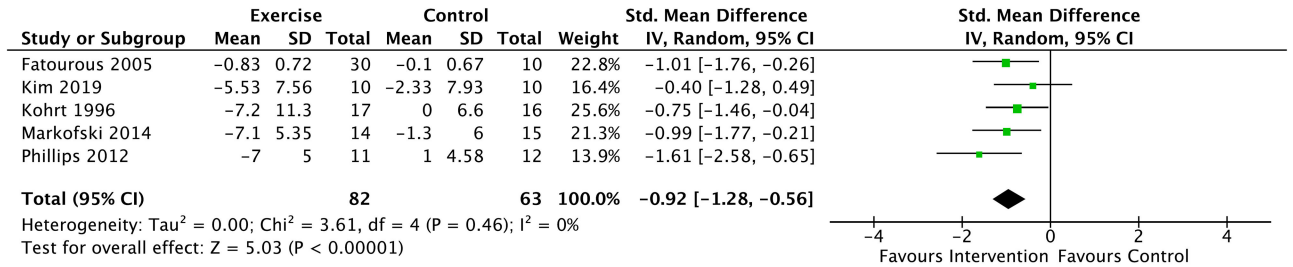

FIGURE 3 | Forest plot for the effect of exercise on leptin. Results favor lower scores in experimental group. Model is statistically significant and homogeneous, suggesting reliability of the large effect size (−0.92).

adiponectin data revealed no significant intervention effect. Analyses of adiponectin included results from 3 studies with a total of 140 participants and a "good" overall PEDro score (M = 6, SD = 0.82); contrary to energy intake, there was very high heterogeneity between these studies (I^2= 92%) (49, 54, 56). Forest plots for energy intake and adiponectin are available in **Supplementary Figures 1**, **2**.

Significant pooled effects were noted for both glucose and leptin in favor of the intervention (exercise/physical activity) group. Data from 5 studies and 193 participants were included for glucose estimates (49, 52, 53, 56, 57). Average PEDro score for included studies was 'good', equaling 6.4 (SD = 0.45). Overall, fasting glucose significantly decreased in the intervention groups compared to the control groups [SMD = −0.34 (95% CI: −0.67, −0.02), $p < 0.05$]. Low heterogeneity (I^2= 11%) was identified across studies (**Figure 2**). Too few studies including only non-obese participants were identified to sub-analyze the healthy-weight population; sub-analyses with the 3 studies which included obese participants [N = 100 participants; (49, 55, 58)] revealed no significant effect of exercise/physical activity on fasting glucose [SMD = −0.33 (95% CI: −0.78, 0.11), p = 0.14; I^2= 0%].

Analyses of serum leptin included data from 5 studies and 155 subjects (49, 52–54, 61, 65). Average PEDro score for included studies was "good", equaling 6.2 (SD = 0.75). Results revealed a significant decrease in serum leptin in the intervention group compared to the control, with a large negative effect [SMD = −0.92 (95% CI: −1.28, −0.57), $p < 0.00001$] and no detectible heterogeneity (I^2= 0%) (**Figure 3**). Subgroup analyses were conducted on these results to remove Markofski, the only study which included a fit, physically active control (54, 61). This subgroup analysis effectively doubled as a sensitivity analysis, removing the only report with a PEDro score <6.0 (5.0). The modified results similarly suggested a large negative effect of exercise/physical activity on serum leptin [SMD = −0.91 (95% CI: −1.31, −0.51), $p < 0.0001$] with low heterogeneity (I^2= 16%). Additional subgroup analyses were conducted to remove Phillips (2012), as all participants were morbidly obese, and some cardiovascular co-morbidity without metabolic disease was indicated (65). Without Phillips (65), the large negative effect on leptin with low heterogeneity was retained both with and without inclusion of Markofski (54): [SMD = −0.81 (95% CI: −1.20, −0.42), $p < 0.00001$], (I^2= 0%) and [SMD = −0.75 (95% CI: −1.20, −0.31), $p < 0.001$], (I^2=0%) respectively. Sub-analyses of the 4 studies which included obese participants (N = 112 participants; (49, 52, 54, 61, 65) revealed a significant, large negative effect [SMD = −0.98 (95% CI: −1.39, −0.57), $p < 0.00001$] with low heterogeneity (I^2= 10%).

Visual examination of the random effects funnel plots for glucose and leptin (**Supplementary Figures 3**, **4**) suggested that study distribution was symmetrical for leptin, but some publication bias may exist for glucose. However, no publication bias was identified by the rank correlation test for funnel plot asymmetry for either group. Rosenthal's fail-safe N values estimate the number of non-significant studies required to make the results of analyses non-significant (42); the fail-safe N for glucose was $N = 6$ ($p < 0.01$) while fail safe N for leptin was N = 45 ($p < 0.0001$).

Although not specifically identified for meta-analysis, weight (kilogram) change score was extracted from 9 studies (**Table 1**).

TABLE 1 | Weight (kg) change score.

Article, Year	N: Control, Intervention	Control	Intervention
Butterworth et al. (46); Nieman et al. (55)	16, 14	2.6 ± 2.4	−0.4 ± 3.5
Fatourous et al. (49)	10, 40	0.0 ± 8.8	1.5 ± 9.6
Kim et al. (52)	10, 10	−0.7 ± 5.0	1.5 ± 4.0
Kohrt et al. (53)	16, 17	0.4 ± 1.7	−2.1 ± 2.2
Markofski et al. (54); Timmerman et al. (61)	15, 15	0.5 ± 9.6	−0.8 ± 19.4
Nishida et al. (56)	31, 31	−0.01 ± 1.1	−1.21 ± 1.3
Phillips et al. (65)	12, 11	−0.1 ± 13.1	−0.1 ± 8.7
Rodrigues-Krause et al. (57)	10, 20	0.8 ± 10.3	0.8 ± 11.1
Taaffe et al. (58)	11, 21	0.2 ± 8.3	0.2 ± 7.1

Note: Control and Intervention values = M ± SD; Estimates not possible for (47, 48, 50, 51).

No significant effect for this metric was identified. Sensitivity analyses revealed high heterogeneity between studies.

No mediation or meta-regression analyses were conducted due to the small number of included studies. This precluded investigation of the varying effects of weight (fat mass) change, age, and exercise intervention on appetite markers.

DISCUSSION

This systematic review with meta-analysis investigated the effects of exercise and physical activity on appetite regulation in older adults. The findings from this study suggest that exercise/physical activity, summed across modalities/intensities/durations and without concomitant dietary or lifestyle intervention, decreases fasting glucose and serum leptin in adults aged 60+. These changes are reflective of subjects' improved body composition and metabolism and may also correspond with increased satiety sensitivity in trained populations. This suggests that activity alone may provide a meaningful intervention to disease, in part, in this population. However, this study also identified a null effect of exercise and physical activity on adiponectin levels, dietary energy intake, and body weight associated with exercise training. These results are contradictory. Given the results in fasting glucose and serum leptin, increased fasting adiponectin levels were expected, as they are also suggestive of greater satiety sensitivity. However, no such effect on adiponectin was identified. Further, improvements in satiety sensitivity and appetite control should have induced greater changes in energy intake and body weight, but no significant effect on the exercising group over the control group was revealed. The absence of these anticipated changes to energy intake and weight have been similarly noted in previous reviews (13, 35).

Considering the significant effects of exercise on appetite, a large body of evidence supports that healthy levels of glucose and leptin are critical to maximizing fat-mass control, type-2 diabetes prevention, cardiovascular risk reduction, and improved quality of life (36, 66–69). In line with this study, existing research supports that improved exercise fidelity may help to independently reduce fasting glucose and leptin (13, 23). Although short-term reductions in these markers have been associated with compensatory eating by way of increased appetite and decreased activity, maintenance of these reductions has been shown to improve satiety sensitivity and increase energy expenditure (13, 23, 70). Increased satiation and activity may aid in risk reduction and weight maintenance, while the effects of body mass reductions may cyclically improve satiety sensitivity and fasting glucose (13, 23, 71). Of note, the relationships between exercise, physical activity, and appetite outcomes may be affected by the intensity or type of intervention, as well as participants' varying age, levels of fitness, and physiology (e.g., biological responsiveness) (18, 19, 23, 31). However, this review cannot make definitive conclusions about the mediating effects of these and confounding factors (e.g., weight, sex) although the absence of significant weight changes may suggest that the reductions in glucose and leptin are independent of changes in body composition.

The absence of a significant effect on adiponectin identified in this review was unexpected, as increased levels would align with effects on leptin and glucose, suggesting improved satiety (14, 72). Increases in adiponectin have been previously demonstrated in exercise interventions (54, 72, 73), although some evidence suggests there may be no mean effect (74–76). Adiponectin is negatively correlated with fat mass such that overweight/obesity reduces circulating levels (14). The absence of a mean effect may suggest that the insignificant weight changes identified correspond to negligible changes to fat mass and body composition, and that exercise and physical activity alone are insufficient to significantly increase circulating adiponectin levels in the absence of fat-mass reductions (14, 77). However, previous research has supported that exercise alone increases adiponectin in the absence of body composition changes (14, 72), including two of the three studies in this analysis (49, 54). Thus, it may be more likely that this null effect is a result of the small number of studies, and/or is attenuated by additional confounding factors, particularly the varying modalities/intensities/durations of the interventions.

The lack of significant weight change as a result of exercise may also support evidence that exercise and physical activity mitigate the muscle loss associated with reductions in fat-mass by promoting maintenance of fat-free mass (9, 78). Previous research suggests that increased physical activity may improve body composition with no significant effect on body mass by promoting skeletal muscle and reducing adiposity; this may ultimately reduce the risk of sarcopenia and other frailty-related diseases (25, 26, 36, 79). Further, it may be that older adults are more successful at buffering an exercise-induced hunger drive and that exercise or physical activity interventions would provide especially meaningful avenues for fat-mass loss and healthful living in aging populations.

Considering energy intake, identification of no mean effect is supported by recent research and reviews which have noted very little or no post-exercise change in older adults (13, 35, 40). This may be partially attributable to methodology, as some studies

specifically instruct participants to maintain their regular diets (47, 48, 65). Additionally, some mechanistic research suggests that the orexigenic effect of exercise is counterbalanced by increased satiety, which may help to reduce compensatory eating in these populations (22). Exercise-induced compensatory energy intake may be further limited within older populations due to the compounding effects of exercise and aging-induced anorexia (8, 12, 13, 80). Reduced gastric emptying and colonic motility, often associated with low activity levels in aging adults, can result in poor nutrition and reduced energy intake (12, 73, 74). However, activity-induced increases in motility may yet promote positive outcomes from improved eating drive and energy intake in some aging adults (81, 82).

Strengths and Limitations

Although some recent reviews and meta-analyses have probed questions related to exercise and appetite, they have differed significantly from this study in their aim and scope, having included a variety of interventions and measurements, participant ages, and population health statuses (35, 40, 83, 84). As such, to the best of the authors' knowledge, this is the only comprehensive review on the effect of exercise on appetite in healthy older adult populations. A notable strength of this study is that the reports identified in this review were of overall "good" quality (PEDro).

There are several limitations to this study, the primary being that, despite its importance, limited literature is focused on this topic in aging adults. This paucity of available empirical data limits the utility of meta-analyses but highlights the importance of continued investigation. It may be that the relationships between exercise/physical activity and appetite are unique or varied in aging populations, but this has yet to be identified. Further, studies focused on the relationships discussed may still be in progress, unpublished, or unavailable, increasing this review's potential for reporting and publication biases. While the small number of located reports suggests that the true effects of exercise and physical activity interventions on appetite may remain obscured, the identification of studies lacking an intervention effect supports that bias may not be particularly problematic.

Overall, the limited results of this study make confident assessment of bias difficult. Notably, publication bias is difficult to confidently assess in meta-analyses with a small number of included effect sizes (85–88). Methods for assessment are significantly reduced in power and reliability when the number of included studies drops below ten (85–88). Funnel plots are inherently subjective while fail-safe N assumes that all unreported effect sizes are equal to zero (85–88). As such, these results should be interpreted cautiously.

Additionally, because of a lack of available studies, heterogenous reports were compared. For example, all exercise/physical activity interventions were considered equal, despite there being a mixture of modalities, intensities, and durations. Only some studies controlled for diet or utilized managed diets. Similarly, certain studies identified in this review only accrued and reported on one sex, potentially skewing the results. Overall, the small number of studies limited the authors' ability to identify the direct and indirect effects of these confounding variables on the outcomes of interest. The spurious effects of both individual and study characteristics, including weight change, age, sex, and exercise interventions, on appetite markers remain unknown.

Finally, several studies lacked specificity and did not report data meaningful for meta-analysis. Of those which were applicable for quantitative review, many only provided pre-post values as opposed to change scores, and no studies reported correlation coefficients where they would have been appropriate. Change scores and r estimations were calculated, which may influence the validity of this study. Limited reporting is similarly reflected by poor performance in some aspects of the risk of bias assessment; although randomized controlled trials were mainly reported on, many favorable study aspects, including allocation concealment and blinding were unclear. Of note, identifying studies including only healthy adults within an older population presents specific challenges, and although this was an aim of the present study, it may be that some manuscripts simply did not report chronic/functional disease status in detail. This is one example of how emphasis on improved reporting methods and data transparency will continue to facilitate accurate aggregation of studies in future meta-analyses.

Recommendations for Future Research

Future research should aim to increase the body of evidence related to appetite, exercise, and physical activity in aging adults. Although some significant effects were identified within this meta-analysis, a limited number of relatively heterogenous reports were used to draw conclusions. Thus, results should be interpreted cautiously. Additional studies are required to better understand and accurately measure the reported effects. Further investigations should explore the extent, stability, and duration of exercise effects, as well as the feasibility of maintaining these with continued activity. When more data are available, it would be relevant to investigate the independent effects of exercise intensity (vigorous/moderate/light), with consideration of duration, modality, and maintenance of the intervention. Greater investigation of individual characteristics and confounding factors (e.g., age, weight, sex, race, health status, etc.) is also warranted. In future studies, this may be addressed via hierarchical models, mediation analyses (e.g., meta-analyses on indirect effects) or meta-regression. Studies should also seek to explore the differences between appetite regulation in disease-free adults at a normal body weight vs. overweight and underweight populations. It is relevant to further explore the impact of nutrition and diet on the relationships discussed, and to adequately control for any effects.

CONCLUSIONS

Overall, this study suggests the positive effect of exercise and physical activity interventions on some appetite markers but reveals the still-present gap in aging-focused exercise research. Still, the results support the increased prescription of exercise and physical activity for both prevention and treatment of disease across the lifespan. Implementation of an active lifestyle as

preventative and reactive medicine may aid in public health measures focused on lengthening disease-free years and reducing the burden of morbidity in later life.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JB, KK, and SH contributed to conception and design of the study. SH conducted the article search and selection process, supported by JB. KK assisted in reviewing final article selections. SH performed the statistical analysis and wrote the first draft of the manuscript. JB and KK contributed to sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

We would like to acknowledge Mollie George for assisting with some of the initial article search and extraction. The project described is supported by the National Institute of General Medical Sciences, U54 GM115458, which funds the Great Plains IDeA-CTR Network. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.734267/full#supplementary-material

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frontiers in Nutrition

ORIGINAL RESEARCH
published: 26 November 2021
doi: 10.3389/fnut.2021.737777

Examination of Athlete Triad Symptoms Among Endurance-Trained Male Athletes: A Field Study

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OPEN ACCESS

Edited by:
Stacy T. Sims, Auckland University of Technology, New Zealand

Reviewed by:
Katherine Black, University of Otago, New Zealand
Anna Katarina Melin, Linnaeus University, Sweden

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Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *07 July 2021*
Accepted: *14 October 2021*
Published: *26 November 2021*

Citation:
Moore EM, Drenowatz C, Stodden DF, Pritchett K, Brodrick TC, Williams BT, Goins JM and Torres-McGehee TM (2021) Examination of Athlete Triad Symptoms Among Endurance-Trained Male Athletes: A Field Study. Front. Nutr. 8:737777. doi: 10.3389/fnut.2021.737777

Background: Studies examining the physiological consequences associated with deficits in energy availability (EA) for male athletes are sparse.

Purpose: To examine male athlete triad components; low energy availability (LEA) with or without an eating disorder risk (ED), reproductive hormone [testosterone (T)], and bone mineral density (BMD) in endurance-trained male athletes during different training periods.

Methods: A cross-sectional design with 14 participants (age: 26.4 ± 4.2 years; weight: 70.6 ± 6.4 kg; height: 179.5 ± 4.3 cm; BMI: 21.9 ± 1.8 kg/m2) were recruited from the local community. Two separate training weeks [low (LV) and high (HV) training volumes] were used to collect the following: 7-day dietary and exercise logs, and blood concentration of T. Anthropometric measurements was taken prior to data collection. A one-time BMD measure (after the training weeks) and VO_{2max}-HR regressions were utilized to calculate EEE.

Results: Overall, EA presented as 27.6 ± 10.7 kcal/kgFFM·d-1 with 35% ($n = 5$) of participants demonstrating increased risk for ED. Examining male triad components, 64.3% presented with LEA (≤ 30 kcal/kgFFM·d-1) while participants presented with T (1780.6 ± 1672.6 ng/dl) and BMD (1.31 ±.09 g/cm^2) within normal reference ranges. No differences were found across the 2 training weeks for EI, with slight differences for EA and EEE. Twenty-five participants (89.3%) under-ingested CHO across both weeks, with no differences between weeks.

Conclusion: Majority of endurance-trained male athletes presented with one compromised component of the triad (LEA with or without ED risk); however, long-term negative effects on T and BMD were not demonstrated. Over 60% of the participants presented with an EA ≤ 30 kcal/kgFFM·d-1, along with almost 90% not meeting CHO

needs. These results suggest male endurance-trained athletes may be at risk to negative health outcomes similar to mechanistic behaviors related to EA with or without ED in female athletes.

Keywords: low energy availability, bone mineral density, testosterone, reproductive dysfunction, macronutrients, male endurance athletes, male athlete triad in endurance athletes

INTRODUCTION

Examination of low energy availability, defined as <30 kcal/kg^{-1}FFM·d^{-1} and the associated physiological ramifications is no longer only examined in females in sports research. Recent literature has demonstrated similar physiological consequences [decreased reproductive hormones and bone mineral density (BMD)] in males, predominately in endurance athletes (i.e., runners, cyclists and triathletes) (1–5) due to LEA (1–3, 5, 6). Due to high exercise energy expenditure (EEE) demands of endurance sports, male endurance athletes are at an increased risk of LEA, decreased testosterone (T), and low BMD (6) due to the nature of their sports, which includes massive EEE and inadequate energy intake (EI) compensation. Recent research has demonstrated negative physiological responses with LEA and hormonal changes in males (7). Two potential manifestations of decreased T in males include an acute response due to LEA and/or excessive training loads or a chronic response labeled exercise-hypogonadal male condition (EHMC) (8). Understanding the hormonal responses to LEA in male athletes is crucial for overall health and successful performances (i.e., illnesses/injuries).

A recently established working model for male athletes has demonstrated similar syndrome characteristics as demonstrated in the female athlete triad (Triad) (2). The triad is composed of three components: LEA with or without an eating disorder (ED), hypothalamic reproductive dysfunction, and compromised BMD (9). Symptoms for males include metabolic and endocrine changes (decreased leptin, insulin-like growth factor-1 GF-1, and increased cortisol) due to decreased EA, decreased T, libido and sperm quality, decreased BMD and bone markers, and increased risks of bone stress injuries (2). Clinically relevant biomarkers [reproductive hormones (luteinizing hormone, estrogen, and follicular stimulating hormone), bone mineral density, and bone hormone markers] have been used to assess LEA and the health of female athletes regarding physiological consequences that occur at and below an EA of 30 kcal/kg^{-1}FFM·d^{-1} and exist for females (9). These biomarkers have not been established for the physically active male, leading the female athlete triad coalition and the International Olympic Committee (IOC) to identify males as an under-researched population (4, 9). Due to the recognized physiological differences between males and females, necessity to establish independent clinical guidelines for males in regard to LEA and their collective metabolic and physiological impacts has been stressed (10). Parallels between triad symptoms in males and females and have been determined, and a better understanding of nutritional deficits is needed for males, including a definition of LEA for males, confirmation whether disordered eating (DE)/ED risk contributes to chronic LEA, and the relationship of EA to metabolic and physiological changes (1).

Our study sought to address this need by examining male triad symptoms (EA with or without ED risk, T, and BMD) among endurance trained male athletes. We hypothesized that endurance trained athletes would display at least one compromised component of the male athlete triad. A secondary purpose was to examine differences in energy needs (e.g., EI, EEE, and EA,) and hormonal changes (T) across 2 separate training volume weeks [high volume (HV) training week and low volume (LV) recovery week]. We hypothesized male endurance-trained athletes would display significant difference in energy needs between the 2 training weeks.

MATERIALS AND METHODS

Experimental Design and Participants

This study implemented a within-subject cross-sectional design examining recreational male endurance athletes (distance runners, triathletes, and obstacle racers), their energy needs (EA, EEE, and EI), T, and BMD. Fourteen male participants (age: 26.4 ± 4.2 years; weight: 70.6 ± 6.4 kg; height: 179.5 ± 4.3 cm) were recruited from a local community in the Southeastern region of the United States. Inclusion criteria were: (a) actively training and competing ≥10 h/week for at least 3 months (11, 12) within a competitive season, (b) body fat percentage (BFP) ≤12%, (11, 12) (c) maintained weight stability (±3 kg in past 6 months) (13), and d) had a VO_{2max} considered excellent for age-specific range. The VO_{2max} treadmill test using the method from Beashel et al. (14) targeted for endurance runners was administered prior to data collection. This test utilized a continuous speed (7 mph) with graded % incline (14). Exclusion criteria included past or present diagnosis of clinical ED, history of cardiovascular disease, thyroid, pituitary or other metabolic disease or orthopedic impairment that interferes with moderate to vigorous exercise. Institutional Review Board approval was obtained, and all the participants provided written consent.

Instruments/Protocols

Anthropometric Measurements and DXA

Basic demographic information and anthropometric measurements were collected according to ACSM standardized procedures (15). Body fat was assessed using Tanita scale (Tanita SC-331S Body Composition Scale, Tanita Co., Tokyo, Japan) for an inclusion criterion and Dual-Energy X-Ray Absorptiometry (DXA) (GE Lunar Prodigy densitometer) for data analysis and to measure BMD (g/cm^2) of the total body (16).

Energy Assessment

Multiple measures were assessed (EI and EEE), measured [resting metabolic rate (RMR)], and calculated [EA and energy balance (EB)] in this study. Indirect calorimetry *via* MedGem Analyzer (*MicrolifeMedGem*; HealthTech, Golden, CO) protocol, utilizing a mouthpiece and nose plug, was used to measure RMR at the beginning of data collection. Even though the MedGem is not the gold standard, it is a clinically validated RMR measurement device (17). To assess EI, the participants recorded their food and fluid intake (estimated) for 7 consecutive days during 2 separate weeks. Visual aids assisted subjects in entering portion sizes prior to recording into the ESHA food processor (ESHA food processor 8.0, Salem, OR). Dietary records were analyzed for total kilocalories (kcals), macronutrient and micronutrient consumption using a dietary analysis software program. Goldberg ratio was calculated for both the HV week (1.52) and LV weeks (1.49) to examine validity of EI reports (18). Individual VO_{2max} –HR regression slopes were used to calculate EEE *via* exercise logs and HR monitors (11). Energy outcomes were measured *via* energy balance (EB) and EA. Energy balance, defined as the total daily energy expenditure (TDEE), was measured with Bodymedia Sense Wear Armbands, and EI remaining at an equal level {EB = [EI (kcals/day) = TDEE kcal/day)]}. Energy availability, defined as the amount of dietary energy remaining after exercise, expressed as kcal/kg/free fat mass [EA = (EI–EEE) kcal/kgFFM·d^{-1}], was both examined (9).

Eating Disorder Risk

Risk of an ED was evaluated using the Eating Disorder Inventory-3 (EDI-3) and Symptom Checklist (EDI-3 SC). The EDI-3 is a screening tool designed specifically for Allied Health professionals to identify individuals at risk for ED while the EDI-3 SC provides information about the frequency of ED pathogenic behaviors (19). The EDI-3 is a self-reported survey (validated for males and females aged 13–53) that identifies subjects with DE patterns (19). Reliability is high with coefficient and median values for specific composites: ED risk ($r = 0.98$, median =0.95) and General Psychological Maladjustment ($r = 0.97$, median = 0.93) (19). To be determined "at risk" for ED, the participants must be identified as "typical clinical" or "elevated clinical" for at least 1 EDI composite score, and/or meet the criteria for risk of pathogenic behavior (19).

Blood Sampling and Storage

Blood samples were collected for both weeks between 0,530 and 0,730 h during the 8th day of the weekly protocol. The participants were instructed to refrain from exercise for 24 h and consumption of food 12 h prior to blood draws. All OSHA guidelines were followed to minimizing any exposure risks to the participants. Using 21G (19 mm) BD vacutainer needles and tubes (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ), samples were taken from the antecubital vein, centrifuged (Eppendor Centrifuge 5702F) for 15 min at 4°C and pipetted into 2-ml polyethylene tubes for storage in a

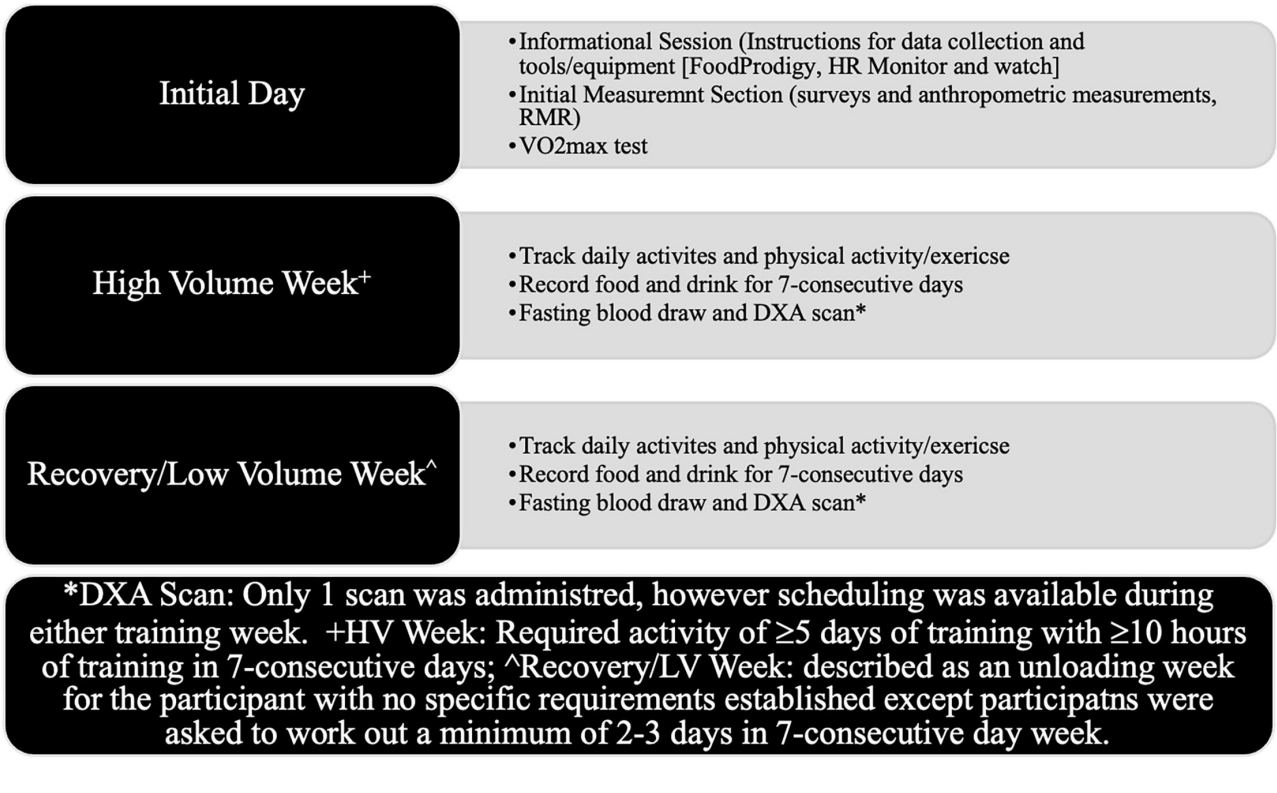

FIGURE 1 | Data collection protocol.

TABLE 1 | Basic demographics and an inclusion criterion for endurance trained male athletes ($n = 14$).

	ALL	
Basic demographics	**M**	**SD**
Age (years)	26.4	4.2
Height (cm)	179.5	4.3
Weight (kg)	70.6	6.4
BMI (kg/m^2)	21.9	1.8
Ethnicity	**N**	**%**
African American	2	14.3
Caucasian	11	78.6
Middle Eastern/Kurd	1	7.6
Education level		
High School Diploma/GED	1	7.1
Attained some level of college	4	28.6
Bachelor's Degree	3	35.7
Master's Degree	5	35.7
Clinical Doctorate	1	7.1
Inclusion criterion	**M**	**SD**
VO_{2max} (ml/kg/min)	62.3	6.9
Free Fat Mass (kg)	65.7	5.4
Tanita BFP (%)	7.1	2.2
DXA BFP (%)	13.6	3.5

Values are presented in mean ± standard deviation.

TABLE 2 | Energy needs assessment and male athlete triad components for endurance-trained male athletes ($n = 14$).

Energy needs assessment	**M**	**SD**	***p*-value**	
Resting Metabolic Rate (kcals)	1,799	549		
Exercise Energy Expenditure (kcals)	865	566	$p = 0.13$	
HV EEE (kcals)	1,048	805		
LV EEE (kcals)	682	326		
Energy Intake (kcals)	2,658	887		
HV EI (kcals)	2,687	878		
LV EI (kcals)	2,629	927	$p = 0.18$	
			$p = 0.31$	
Energy Availability (kcal/kgFFM·d)	27.6	12.1	$p = 0.13$	
HV EA (kcal/kgFFM·d)	25.2	12.9		
LV EA (kcal/kgFFM·d)	29.9	11.1		
Testosterone (ng/dL)	1,780	1,672	$p = 0.28$	
HV T (ng/dL)	1339	836		
LV T (ng/dL)	1455	889		
Bone Mineral Density (g/mc^2)	1.3	0.9	$p = 0.34$	
Overall Distance (miles)	49.1	77.9	$p = 0.08$	
HV Distance (miles)	63.4	100.6		
LV Distance (miles)	34.9	45.4		
Overall Training Time (hours)	5.6	4.3	$p = 0.06$	
HV Training Time (hours)	7.1	5.3		
LV Training Time (hours)	4.1	2.5		
Low energy availability risk	**HV%**	**N**	**LV%**	**N**
LEA <2 days per week–VO_2	35.7	5	50	7
LEA 3–4 days per week–VO_2	35.7	5	21.4	3

Values are presented in mean ± standard deviation. LEA, low energy availability; EEE, exercise energy expenditure, calculated by the average number of days of exercise/week across individuals.

−80°C freezer for 1 month prior to analysis. Blood samples were assessed using enzyme-linked immunosorbent assay (ELISA) kits to measure testosterone levels. Sensitivities of ELISAs are high, 1–10 ug/liter range with correlation coefficients ranging between 0.95 and 0.99 (20), specific T ranges 1–18 ng/ml.

Training Categorization

Differences in energy needs and T were assessed during two training weeks with different intensities. High-volume training week (HV) consisted of >5 days of training with >10 h of training in a 7-consecutive-day week and a low-volume training week (LV) described as an unloading week for the participant with no specific requirements established except the participants were asked to work out a minimum of 2–3 days. Specific protocols for data collection can be found in **Figure 1**.

Statistical Analysis

Specific cut point measures were defined for multiple variables, including EA where LEA defined as <30 kcal/kg FFM·d^{-1}, while EB was defined as EB [EI = TDEE], negative EB [EI < TDEE] or positive EB [EI > TDEE] (9). Testosterone cutoffs were based on previously established normative data specific for males, T = 270–1070 ng/dl (21). Testosterone was identified as low, within normal limits, or high based on normative data. A Z-score >-0.9 was considered within the normal range (16, 22).

IBM SPSS statistical software (version 26; SPSS Inc., Armonk, NY) and an *alpha* ≤0.05 were used for all analyses. Based upon power analysis *a priori* and based upon means of previous literature from Koehler et al. (13) and Loucks et al. (23), an effect size between 1 and 3 yielded a sample size of 6–10 subjects. Using the Wilcoxon-signed rank test, 14 subjects were allowed for full saturation. Descriptive statistics were calculated for all dependent variables. Frequencies and proportions with 95% confidence intervals (CI) were calculated for all categorical variables. Crosstabulations and chi-square analysis were used to examine "at risk" variables, while macro/micronutrient profiles were compared to ACSM recommendations. A 2-(week)-X7 (days) ANOVA and paired *t*-tests assessed differences between the training weeks for TDEE, EB, EA, EI, EEE, and macro/micronutrients. Pearson's correlations and regressions were used to examine relationships between T, LEA, and other continuous variables.

RESULTS

Eighteen participants began the study, three were eliminated for lack of compliance with study procedures, and one dropped out due to fear of needles, yielding a total of 14 participants. Demographic information, energy needs assessment, inclusion criterion, and macro/micronutrient intakes are reported in **Tables 1–5**.

TABLE 3 | Eating disorder characteristics among endurance trained male athletes ($n = 14$).

	EDI classification							
	Raw score		Low clinical		Typical clinical		Very typical	
	Mean	SD	*n*	%	*n*	%	*n*	%
Eating Disorders Risk Scale								
Drive for thinness (DT)	1.6	2.4	14	100	–	–	–	–
Bulimia (B)	0.9	1.9	13	92.9	**1**	**7.1**	–	–
Body dissatisfaction (BD)	1.6	2.4	14	100	–	–	–	–
Eating disorder risk composite (EDRC)	82.3	5.8	14	100	–	–	–	–
Psychological scale								
Low self-esteem (LSE)	2.2	3.8	13	92.9	**1**	**7.1**	–	–
Personal alienation (PA)	1.9	2.3	14	100	–	–	–	–
Interpersonal Insecurity (II)	4.9	4.4	11	78.6	**3**	**21.4**	–	–
Interpersonal alienation (IA)	3.4	3.2	10	71.4	**4**	**28.6**	–	–
Interceptive deficits (ID)	0.8	1.1	14	100	–	–	–	–
Emotional dysregulation (ED)	0.6	0.8	14	100	–	–	–	–
Perfectionism (P)	10.1	4.3	6	42.9	**7**	**50**	**1**	**7.1**
Asceticism (A)	5.4	3.8	11	78.6	**3**	**21.4**	–	–
Maturity fears (MF)	7.1	5.8	6	42.9	**5**	**35.7**	**3**	**21.4**
Composite								
Ineffectiveness composite (IC)	63.1	9.6	13	92.9	**1**	**7.1**	–	–
Interpersonal problems composite (IPC)	76.3	11.5	11	78.6	**3**	**21.4**	–	–
Affective problems composite (APC)	66.6	2.1	14	100	–	–	–	–
Over control composite (OC)	80.6	12.4	10	71.4	**4**	**28.6**	–	–
General psychological maladjustment (GPMC)	331.1	30.5	14	100	–	–	–	–

Data are presented in frequency (n) and percent (%). Bold values signify findings of significant values of note.

TABLE 4 | Eating disorder pathogenic behaviors among recreational endurance-trained male athletes ($n = 14$).

	All data	
Exercise to control weight	**N**	**%**
0% of time	8	57.1
<25% of time	**4**	**28.6**
25–50% of time	**2**	**14.3**
More than 75% of time	0	0
100% of time	0	0

Data are presented in frequency (n) and percent (%). Bold values signify findings of significant values of note.

Male Athlete Triad

Overall, no participants met the criteria for all three male triad components, and the participants did not present at risk for either low T levels or compromised bone health. Results demonstrated 64.3% ($n = 9$) of the participants presented with LEA over the 2 training weeks, and, of those, 60% ($n = 3$) presented as LEA with an ED risk. Overall, 35.7% ($n = 5$) of the participants presented at risk for EDI-3 composite scales (see **Table 3**). One participant did demonstrate a typical clinical score for Bulimia (7.1%). One specific composite, maturity fears, presented with the highest scores between all the sub-scales with 35.7% ($n = 5$), demonstrating typical clinical scores and 21.4% ($n = 3$) with elevated clinical scores.

Energy Needs

No differences were found across the 2 training weeks for EI (see **Table 4**). Slight differences were elicited for EA across the training weeks (HV: 25.2 ± 12.9 kcal/kg FFM·d^{-1} vs. LV: 29.9 ± 11.1 kcal/kg FFM·d^{-1}, $p = 0.13$. This decrease was not due to a change in EI but rather to a difference noted in EEE with the LV week eliciting lower EEE kcals (HV: 1,048.5 ± 805.6 kcals/week vs. LV: 682.3 ± 326.5 kcals/week, $p = 0.13$) (see **Table 4**). Carbohydrate intake was low with 89.3% ($n = 25$; HV: 92.9%, $n = 13$, LV: 85.7%, $n = 12$) of participants consuming <5 g/kg of CHO across both training weeks. Due to missing data cells (determined by the days the participants exercised), a paired samples *t*-test was calculated to compare the mean of the training weeks. No significant differences were elicited between the weeks ($t_{(13)} = 1.7$; $p = 0.10$). Average TDEE of the participants for both weeks was 2,993 ± 160 kcal, which resulted in an overall negative EB of −39 ± 201 kcal. There was no significant main effect found between the training weeks and TDEE: $F_{(1,11)} = 4.02$ ($p = 0.07$) or EB: $F_{(1,13)} = 4.40$, ($p = 0.06$).

Testosterone

Mean T levels between the 2 training weeks can be found in **Table 2**. A negative correlation was found between overall T levels to EI: ($r_{(26)} = -0.47$, $p = 0.02$), RMR: ($r_{(26)} = -0.64$,

TABLE 5 | Daily energy, macro and micronutrient intake in the high- and low-volume training weeks ($n = 14$).

	Overall ($n = 14$)		High volume		Low volume		
	M	SD	M	SD	M	SD	*p*-value
Energy Intake (kcals)	2,658	887	2,687	878	2,629	927	0.18
CHO (g/kg)	4.9	1.7	4.6	0.5	4.7	0.5	0.7
PRO (g/kg)	1.7	0.6	1.7	0.2	1.7	0.1	0.28
Fats (% of kcals)	32.3	5	31.6	1.7	31.5	1.4	0.95
Vitamin B_6 (mg)	2.4	1.2	1.2	0.9	2.7	1.2	0.09
Vitamin B_{12} (mcg)	6.3	3.6	6.4	3.3	6.3	3.9	0.91
Calcium (mg)	1206.2	653	1222.5	621.9	1189.9	705.9	0.9
Iron (mg)	23.9	10.5	24.7	11.1	23.1	10.3	0.69

Means and standard deviation are reported for both training weeks. p-value equal to or <0.05 will indicate a significant difference.

$p < 0.001$), while a positive correlation for overall T levels to DXA_BFP [$r_{(26)} = 0.83$, $p < 0.001$]. Two outliers, determined as more than two standard deviations, were removed prior to regression analysis. The regression equation for T and EA was not significant [$F_{(1,23)} = 3.2$, $p = 0.89$].

DISCUSSION

This study aimed to examine all three components of the male athlete triad observed in free-living subjects. Our overall results supported our hypothesis that recreational endurance-trained male athletes would exhibit >1 component of the male triad. We found 64.3% ($n = 9$) exhibited LEA (with or without an ED risk); however, our participants did not demonstrate decreased T or BMD. Examination of LEA with or without ED may be prudent to understand mechanistic behaviors that affect T and BMD in males and assisting in defining a set point for LEA.

Energy Availability

Within this study, 2 separate training weeks (HV and LV) were used to examine energy needs (i.e., EA with or without ED, EI, EEE, TDEE, and EB). Our results yielded an average EA of 27.6 kcal/kg FFM·d^{-1}, similar to the results reported by Hooper et al. (7), which examined nine long-distance runners who presented with exercise-hypogonadal male condition eliciting EA levels of (27.2 ± 12.7 kcal/kg FFM·d^{-1}). Lane et al. (24) also found similar levels in cyclists (26.9 ± 17.4 kcal/kg FFM·d^{-1}) and in non-elite endurance athletes (28.7 ± 13.4 kcal/kg FFM·d^{-1}) (25).

The results from the present study indicated high prevalence of LEA compared with three studies resulting with prevalence rates at 23 (26), 42 (27), and 54% (28) of male cross-country runners. Within our participants, we found an increase in EEE during the HV training week, while EI remained stable between the weeks. This was demonstrated by Woods et al. (29), which exhibited no significant changes in EI related to different training blocks in cyclists. Viner et al. (30) found small difference in cyclists between preseason (18.8 ± 12.2 kcal/kg FFM·d^{-1}), competitive season (19.5 ± 8.5 kcal/kg FFM·d^{-1}), and off season (21.7 ± 9.2 kcal/kg FFM·d^{-1}), EA levels and EI during preseason (29.3 ± 6.8 kcal/kgbm·d^{-1}) competition season (34.7 ± 6. kcal/kgbm·d^{-1}) and off-season (31.8 ± 7.5 kcal/kgbm·d^{-1}). This is concerning as LEA is the catalyst for negative physiological functions associated with the triad (9). Koehler et al. (13) and Loucks et al. (23) have demonstrated LEA is elicited with or without exercise, which emphasizes the importance of proper nutritional practices throughout training protocols. Currently, there is no clear LEA cutoff for males. In this study, we found an EA level ranging between ∼25 and 29 kcal/kg FFM·d^{-1} during the 2 training weeks, which did not demonstrate negative T levels or BMD. This provides support to the belief that males may sustain lower EA levels and maintain functioning metabolic and endocrine systems. More research targeted between 15 and 25 may be warranted, as Koehler et al. (13) found metabolic changes (Leptin and IGF-I) at 15 kcal/kg FFM·d^{-1}.

In the present study, EI was not statistically different between the training weeks, suggesting this group of recreational male endurance-trained athletes are not changing their EI in relation to the demands of training volumes. While EI derived from the food frequency questionnaires demonstrated similar intakes (2,623 ± 796 kcals) in male ultramarathon runners (7), EI was lower when compared to mountain runners (3,199 ± 701 kcal/day) prerace-day diet (31) and non-elite endurance athletes (3,073 ± 777 kcals/day) (25). The absence of fueling changes between training weeks could be contributed to a singular or combination of mechanisms, including a lack of nutritional knowledge related to fueling needs of different training volumes, possible pathogenic eating, and feeding behaviors, or, perhaps, metabolic signals are being interrupted. One possible area contributing to LEA includes carbohydrate (CHO) intake. Levels were lower than the recommended intake for endurance athletes ranging from 5–10 g/kg, depending on activity levels. This is congruent with other studies that examined cyclists, runners, and triathletes ranging from 3.9 ± 1.2 g/kg (30) to 4.8 ± 1.5 g/kg/day (26). This suggests that recreational endurance athletes are under fueling or restricting CHO aids in LEA and potentially demonstrating pathogenic eating behaviors.

The participants in this study demonstrated either similar or lower values of EEE (∼870 kcals) compared to data from other studies with males, including army rangers (∼4,000 kcal/day) (12), race days for professional jockeys (∼3,952 kcal) (32), cyclists, runners, and triathletes [1,047 ± 718 kcals/day (30) [914.1 ± 143.5 kcals] (7), [760 ± 404 kcals/day] (26), 1,296 ±

466.7 kcals/day] (25). This decrease in EEE kcals may be due to the sample characteristics of the different studies. Military operation studies required significant EEE demands placed on soldiers compared to self-selecting workouts of the participants of this study. Variations in calculations and definitions of EEE could be a contributing factor to differences found within EEE to other male studies (33) as well as athletic elite status (i.e., professional vs. recreational athletes).

With or Without an Eating Disorder

Overall, 35.7% presented with an increased ED risk with 7.1% specific to Bulimia subscale. This percentage is elevated compared with previous, albeit limited research on male athletes (34–36). Research has estimated 10% of elite male athletes present with anorexia nervosa and 25% present with bulimia nervosa, (36) while another 1% of males represent "other specified feeding or eating disorders" (37). Norwegian researchers found athletes (13.5%) were two times as high to be diagnosed with a clinical and/or subclinical ED as non-athletes (4.6%) (35). Sundgot-Borgen et al. (35) also demonstrated 9% of endurance athletes presented with diagnosed ED. Athletes with increased rates of pathogenic behaviors, along with the underrepresented prevalence and rates in male athletes, are of paramount concern due to effects of pathogenic behaviors on fueling and EA (6, 35).

The EDI-3 has specific composite and sub-scale scores, which examine specific behavioral traits similar to those diagnosed with EDs, with "typical clinical" and "elevated clinical" scores, indicating increased risk factors in EDs. Two specific psychological risks identified in the over-control composite, which demonstrated 57.1%, were classified as typical/elevated clinical for perfectionism and maturity fears. The raw scores for perfectionism (10.1 $\pm$ 4.3) were similar to scores of female athletes, including equestrian: 13.2 $\pm$ 5.8, volleyball: 10.8 $\pm$ 4.3, softball: 15.1 $\pm$ 5.3, beach volleyball: 9.9 $\pm$ 4.8, and soccer: 12.7 $\pm$ 4.7 (38). The over-control composite reflects the significant need to avoid disappointing others as well as being the best. The sub-scale perfectionism is important to note, as this sub-scale is a distinguishing feature of EDs as well as strivings in other areas such as athletics (19). This sub-scale demonstrated in this study was over 50% of our participants demonstrating typical/elevated scores.

Pathogenic eating behaviors presented included using exercise for weight control. Research suggests 37% of male athletes (age, 18–22) exercise 2 or more hours per day for the purposeful intention to burn calories (39). With 42.9% of the participants using exercise to lose weight up to 50% of the time, this presents concern as to the motives of males exercising, whether for health benefits (i.e., cardiovascular fitness) or due to risks of ED behaviors. Torres-McGehee et al. (38) found 38% of female athletes, and performance artists also engaged in additional exercise to control their weight. Similar to the female literature, elite male athletes participating in sports that require leanness (endurance athletes) show increases in ED risk (37). However, to date, the literature focused on EDs is limited, and, currently, the ability to compare male athletes to other male control groups is currently not available (37). More research is needed for male athletes regarding pathogenic eating and feeding behaviors.

Energy Balance

Endurance-trained male athletes demonstrated negative EB (−289.4 kcal) in the HV training week, which elicits concern as this is indicative of poor fueling to meet the demands of TDEE. Prolonged negative EB and LEA lead to decreased physiological processes (i.e., lower RMR); (9) however, the disparity among EB and EA is that an athlete can have LEA but maintain their EB. This is due to the suppression of various physiological processes due to the lack of EA (9). Strubbs et al. (40) provided an example of the contrast between EB and EA, using eight lean men with suppressed caloric EI and an increased EEE, resulting in a constant EA of 30 kcal/kg FFM·d^{-1}. Additionally, they found that negative EB decreased toward zero at a rate of 90 kcal·d^{-1} due to the decreased physiological processes and estimated 3 weeks for the participants to elicit an EB of zero while stilling remaining in a LEA state (40). Thus, confirming EI intake was inappropriate for not just exercise but also the daily living of subjects.

Testosterone

Both weeks demonstrated values within or greater than the "normal" range of 270–1,070 ng/dL. Regression results demonstrated a poor predictor of T levels. With the average for both weeks demonstrating high levels of T, this was not congruent with the literature examining endurance runners (3, 5, 7, 41). Testosterone is considered a more robust hormone, with delays in response to external stimuli (i.e., decreased body fat and increased mileage) (42). One theory is the "volume threshold" where decreases in T are elicited when participants are trained at $\geq$100-km·week^{-1} (43). This threshold was congruent with our participants during the HV week for distance but did not demonstrate decreases in T. Our results are congruent with Koehler et al. (13), who examined six cyclists and did not find a decrease in T when EA was acutely reduced to 15 kcal/kg FFM·d^{-1} for 4 days. Of note, all the participants participated in weightlifting programs along with their endurance training. This was not a requirement for inclusion but was tracked for EEE/TDEE of training. While literature has demonstrated acute increases in androgen responses in males during weight lifting, chronic adaptations have not been expressed (44). Long-term weight lifting of the participants may be attributed to the lack of decreases in T. Also, variables may not have been stressed enough to elicit decreases in T, including EA ($\sim$27 kcal/kg FFM·d^{-1}) may not be low enough, body fat percentages ($\sim$13.5%) may need to be less, EEE ($\sim$670 kcals) may need to be increased, and consistent increased mileage ($\sim$101 km) increased.

Bone Mineral Density

Zero participants demonstrated low BMD; however, 29% (n = 4) participants ranged from −0.4 to 0.9, including an African-American participant with a BMD at −0.4, which was surprising due to African-Americans present with more dense bone in comparison to Caucasians (45). Two participants were well-over a Z-score of 2; these were obstacle course runners and, therefore, did large amounts of weight lifting along with endurance running. Many participants were active in a weight lifting regimen, which may be related to the adequate BMD levels.

Finally, due to mechanical limitations, segmental DXA scans were not available, and, in the future, specific sites, including spine and femur, should be examined.

Limitations and Future Research

There were limitations identified in this study. First, while we did meet power, our sample size was still low ($n = 14$). Second, while we used a crossover design, using a separate control group for future studies should be considered. Third, EI collection was a self-reported measurement and, with all self-reported measurements, could have errors, underreporting of non-nutritious foods and over reporting of nutritious foods (46); however, research has demonstrated that, despite food intake restrictions, reported intake accuracy was superior using a 7-consecutive-day diet record compared to a 3-day recall (47). More accuracy may be obtained using a ventilation hood for RMR data. Examination of EEE was calculated using VO_{2max}-HR regression due to the field nature of the study; double-labeled water is still the gold standard for accurate EEE, whereas measuring EEE by indirect calorimetry during exercise may decrease underestimation of EEE for field studies (48). Regarding potential errors in calculations of EI and EEE, Burke et al. (49) recommend development of a standardized measurement format for future work in triad and relative energy deficiency. In achieving due to the large normative range of the hormone T, establishing a "normative range" specific to male athletes is needed regarding identifying long-term testosterone responses to various training variables (i.e., running and strength training). Future research should examine reproductive hormones more specifically in relation to LH and FSH to examine the pulsatile nature of the response of the hormone, and possibly examining a sperm count to assess the output measures of LH and T in males. Future research should begin to look at and address subclinical T levels (∼50–75%) and establish a consistent unit of measure for T across studies for consistency.

CONCLUSION

Our study demonstrated 64.3% recreational endurance-trained males presented with one compromised component of the triad (LEA with or without ED), with participants demonstrating the use of pathogenic behaviors. Further research is needed to examine specific nutritional behaviors of male endurance athletes, including the restriction of CHO intake and pathogenic behaviors related to EDs, EA with or without EDs, and the corresponding physiological health markers, including metabolic hormones (Leptin, Insulin, Cortisol) and bone markers (procollagen type 1 N-terminal propeptide, C-terminal telopeptide type 1 collagen) in male athletes. A more accurate range of T levels also needs to be established in relation to male athletes *vs.* the general population. While females present physiological issues at an EA level of 30 kcal/kg $FFM \cdot d^{-1}$, this study and others have demonstrated set points of males may be lower. Thus, more research on the mechanistic nature of the triad (decreased EA with or without ED, reproductive hormones, and BMD) needs to be assessed and established in the male athlete population. Currently, there is no set cutoff point for LEA in the male population or a clear understanding of physiological consequences for males regarding triad symptoms. More research is needed for the overall metabolic and nutritional health and performance for these athletes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of South Carolina Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

This study was designed by EM, TT-M, CD, DS, and JG. Data were collected and analyzed by EM, TT-M, TB, BW, and KP. Data interpretation and manuscript preparation were undertaken by EM, TT-M, TB, BW, CD, KP, DS, and JG. All authors approved the final version of the paper. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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