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The effects of nutrient timing on training adaptations in resistance-trained females

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ABSTRACT

Objectives: The purpose of this study was to determine the effects of pre- vs. post-workout nutrition on strength, body composition, and metabolism in trained females over 6 weeks of high intensity resistance training (HIRT).
Design: Forty-three trained females (mean ± SD; age: 20.5 ± 2.2 yrs; height: 165.2 ± 5.7 cm; body mass: 66.5 ± 11.4 kg) were measured for strength, body composition, and metabolic variables before and after a HIRT intervention. Participants were randomized using a 2:2:1 matched block randomization scheme by baseline leg press strength into a group that consumed a 1:1.5 carbohydrate-protein supplement (16 g CHO/25 g PRO) pre-training (PRE), post-training (POST), or no supplement (CON).
Methods: Dual-energy X-ray absorptiometry was used to evaluate fat mass (FM), lean mass (LM), and percent fat (%fat). Strength was analyzed using a one repetition max on the leg and bench press (LP1RM and BP1RM, respectively). Participants completed HIRT twice per week for 6 weeks. At the first and last trainings, metabolic variables [resting energy expenditure (REE) and respiratory exchange ratio, RER] were measured.
Results: There were no significant differences between groups for any changes in body composition variables or LP1RM ($p = 0.170–0.959$). There were significant differences for BP1RM ($p = 0.007$), with PRE and POST experiencing greater increases than CON ($p = 0.010$ and 0.015, respectively). REE changes were not significant between groups ($p = 0.058–0.643$). PRE demonstrated greater fat oxidation (RER) at 30 min post-exercise ($p = 0.008–0.035$).
Conclusion: Peri-workout nutrition is potentially important for upper body strength and metabolism. PRE may be more effective for promoting fat utilization immediately post-workout.

1. Introduction

The timing of nutrients, rather than an individuals' daily consumption, has been proposed as an important consideration for exercise performance and recovery.[1,2] Previous research demonstrates the importance of nutrient timing in stimulating protein synthesis,[3] reducing muscle damage,[4] enhancing recovery,[3] and improving body composition.[5] Consumption of nutrients prior to a workout appears to have importance in regulating protein (PRO) synthesis during the workout,[2] and lengthening the anabolic window.[6] Some research indicates that ingestion of amino acids and carbohydrate (CHO) pre-workout may have a greater effect on muscle PRO synthesis and performance compared to post-workout.[7] Conversely, research on post-workout nutrition indicates that there may be a critical window of time for nutrient consumption to stimulate the greatest effects.[4]

Collectively, data suggest that nutrient availability is advantageous for stimulating training adaptations, promoting recovery, and enhancing performance, but one timing strategy may not be superior to the other. Additionally, the available results are largely garnered from males. Specifically, of 24 studies that met the criteria for a meta-analysis looking at the effects of PRO timing on muscular adaptations to training,[3] only two were completed in females. There are known physiological differences between males and females, particularly when undergoing resistance training (RT).[8–10] Nutrient metabolism is largely different, with females express-

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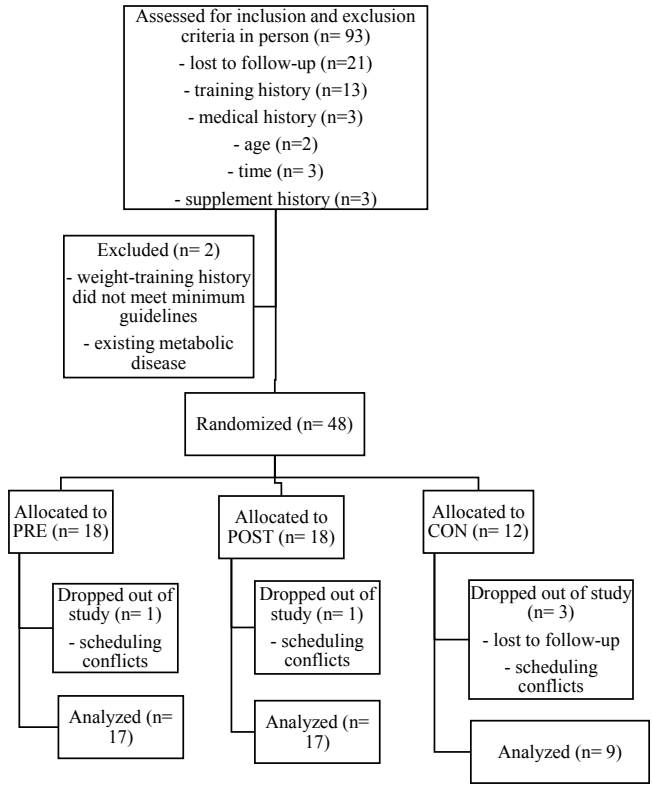

Fig. 1. CONSORT (Consolidated standards of reporting trials) diagram.

ing lower PRO and CHO oxidation,[8,9] and greater lipid oxidation.[8] Divergent metabolic characteristics are potentially due to differences in muscle morphology,[11] adiposity,[11] and hormones.[11,12] Recommendations provided to females derived from data in males should be met with caution, and research exploring nutrition strategies in females is warranted. The primary purpose of this study was to evaluate the effects of consuming PRO-CHO pre- or post-resistance training, or not at all, for six weeks on strength and body composition adaptations in trained females. The secondary purpose was to evaluate the effects on chronic metabolic adaptations following training. We hypothesized that there would be no significant differences between the nutrient groups in any adaptations, but both would demonstrate more advantageous results than the control.

2. Methods

Forty-eight resistance-trained females were enrolled in the study (Fig. 1). Five participants dropped out due to scheduling conflicts/lack of time. Forty-three healthy females (Mean ± SD; Age: 20.5 ± 2.2 yrs; Height: 165.2 ± 5.7 cm; Body Mass: 66.5 ± 11.4 kg) participated in this study. Women were included if they were 18–30 years old, had been resistance training a minimum of two times per week for six months, and were not currently using a PRO supplement before or after exercise. Participants were excluded if they were pregnant, injured, consumed creatine within the previous month, consuming branched chain amino acids more than once per day, or had a heart, lung, kidney, liver, or metabolic disease. The study protocol was approved by the Biomedical Institutional Review Board (#17-0950). Prior to enrolling in the study, written informed consent was obtained from all participants, as well as a health history questionnaire and urine pregnancy test.

Participants underwent similar baseline and post-testing of body composition and maximal strength. Participants were randomized using a block matched group design into either a pre-workout (PRE), post-workout (POST), or control (CON) group (2:2:1) allocated by baseline leg press strength. Following randomization, participants began the six-week training and supplement intervention. At the first and last training sessions, metabolic testing via indirect calorimetry was completed before and after the workout bout to determine resting energy expenditure (REE) and respiratory exchange ratio (RER). Saliva samples were also collected to measure salivary estradiol. The training intervention included six weeks of supervised progressive high intensity resistance training (HIRT), twice per week. Participants in the PRE and POST groups were instructed to consume the PRO-CHO supplement within fifteen minutes before (PRE) or after (POST) the HIRT bout, respectively. A protocol schematic demonstrating the outline of testing and training can be seen in Fig. 2.

Body composition was measured via dual energy X-ray absorptiometry (DEXA; GE Lunar iDXA, GE Medical Systems, Madison, WI, USA). Participants were asked to report to the laboratory a minimum eight hours fasted and having refrained from exercise the prior 24 h. Body composition measures included fat mass (FM), lean mass (LM), and body fat percentage (%fat). Prior to body composition testing, participants were instructed to remove all metal (jewelry, clothing, etc.). Participants laid supine and centered on the DEXA table. They were instructed to breathe normally but refrain from moving for the duration of the scan. The test-retest reliability for the DEXA from our lab for FM is as follows: intra-class correlation coefficient (ICC) = 0.998 and standard error of mean (SEM) = 0.462 kg. For LM, ICC = 0.998 and SEM = 0.806 kg. For %fat, ICC = 0.995 and SEM = 0.807%.[13] Prior research has demonstrated that the DEXA is a valid measure of body composition during all phases of the menstrual cycle phase.[14]

Maximal strength was tested using one repetition maximums (1RM) for leg press (LP1RM) and bench press (BP1RM), and multiple repetition maximums for accessory exercises. Prior to testing, to break the 8 h fast, participants consumed a standardized shake containing 190 kcal, 5 g of fat, 24 g of CHO, and 15 g of PRO (Special K, Kellogg's NA Co., MI). For the 1RM tests, participants completed a 5-min self-selected warm-up and were familiarized with the equipment. For LP1RM, the knee angle had to reach 90° for the repetition to be counted, if it did not, the repetition was considered a failed rep. For BP1RM, the bar was required to touch the individual's chest then come back to full lock out, while the upper back and glutes remained on the bench. Participants then completed two warm-up sets at 50% (8–10 repetitions) and 80% of their 1RM (4–6 repetitions) with approximately two minutes of rest in between. After two minutes of rest, they completed one rep at an estimated 1RM value. Up to four more attempts were used with increasing weight until a point of failure.

Repetitions to fatigue (RTF) tests were used to predict participants' 1RM on accessory exercises. These exercises included overhead shoulder press, biceps curl, overhead triceps extension, and alternating stationary lunge, all using dumbbells (PowerBlock, Inc., Owatonna, MN). Participants performed five repetitions with a light load to understand the form and warm-up. After 1–2 min of rest, the research staff added weight, aiming for 3–12 successful repetitions. If less than three, or greater than 12 repetitions were completed, the exercise was performed again at the end of testing with lighter or heavier weight, respectively. Participants were allowed two minutes of rest between exercises. The amount of weight used and the RTF were put in the following equation to predict 1RM[15]:

$$1\ RM = \frac{repweight}{0.522 + 0.419e^{-0.055*RTF}}$$

The projected 1RM value that was calculated from this equation was used to estimate 80% of max for the HIRT bouts.

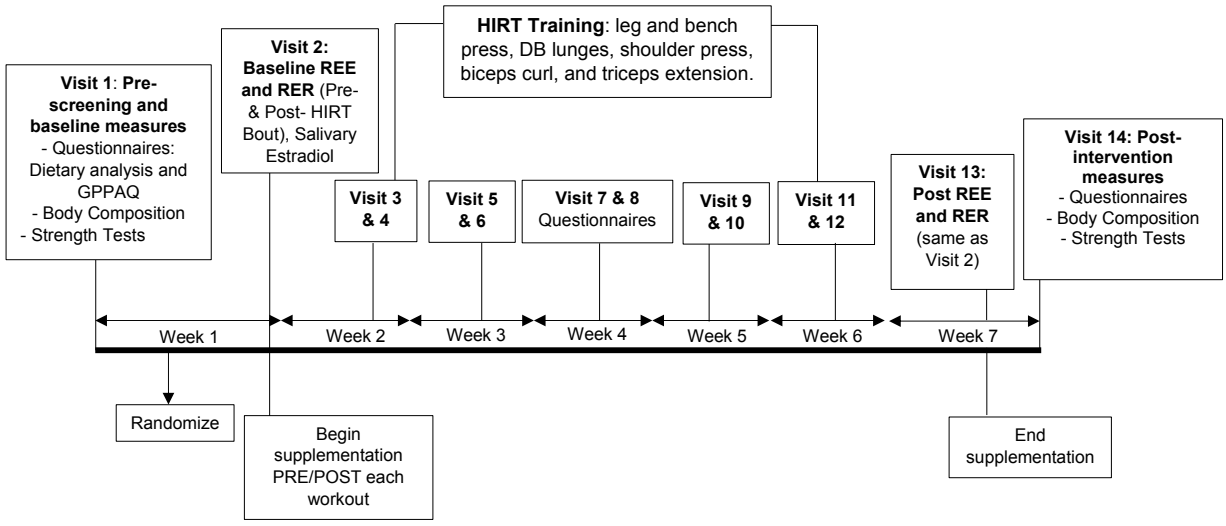

Fig. 2. A protocol schematic detailing the timeline of all testing and training visits.

During the first and last trainings, REE and RER were assessed for twenty-five minutes prior to the HIRT bout, and 90 min after exercise. As with body composition and strength testing, participants were required to fast a minimum of 8 h for metabolic testing visits. For the pre-exercise test, participants laid supine under a hood. The first five minutes were discarded and the remaining time averaged. For the post-exercise testing, participants remained seated, wearing nose clips and a mouthpiece that was connected by a tube to a metabolic cart. The last fifteen minutes of each time interval was averaged (i.e. 15–30, 45–60, and 75–90 min post-exercise). The metabolic cart and software (TrueOne 2400 Metabolic Measurement System, ParvoMedics, Inc., Sandy, UT, USA) measured oxygen uptake (VO_2) and the amount of carbon dioxide exhaled (VCO_2) to indirectly measure REE. A Polar heart rate monitor (Polar FT1, Polar USA, Port Washington, NY, USA) was worn during all metabolic testing. REE, expressed in kilocalories per day (kcal), was calculated using the following equation[16]:

$$REE = [(3.9 * (VO_2)) + (1.1 * (VCO_2))] * 1440\,\text{min}$$

RER was indirectly measured concurrently with REE. The following equation was used to calculate RER, measuring VO_2 and VCO_2 in L/min[17]:

$$RER = \frac{VCO_2}{VO_2}$$

At the beginning of metabolic testing, participants provided a saliva sample to determine estradiol-β-17 measurements using ELISA (Salivary 17β-Estradiol Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA). Participants were asked to refrain from brushing their teeth for 1 h. Participants rinsed their mouth with water 5–10 min before providing saliva to prevent any residue from entering the sample. About 2.5–5.0 mL of saliva was collected as passive drool transmitted directly into a cryotube using a plastic straw, then frozen at −80 °C. Menstrual function and contraception were not tracked, phase of menstrual cycle was indirectly accounted for via measurement of salivary estradiol.

Three-day dietary logs and the General Practice Physical Activity Questionnaire (GPPAQ) were given at baseline testing, half-way through training, and post-testing. A subset of food logs were analyzed using dietary analysis software (Food Processor; ESHA Research, Salem, OR). Participants were instructed to record two typical week days and one weekend day, including as much detail as possible (i.e. Time of consumption, amount, and brands). The GPPAQ measured physical activity done at work and home (including time spent completing actual workouts), and approximate walking speed during the day; GPPAQ scores were ranked as 1 = inactive, 2 = moderately inactive, 3 = moderately active, and 4 = active.

All participants were asked to report to the laboratory 1.5 h fasted for every training and remain 1.5 h fasted after. Participants were randomized to consume a liquid shake within 15 min prior to training (PRE), 15 min post training (POST), or neither (CON). Research assistants mixed the shake then timed participants; the beverage was finished within 10 min from the start of consumption. Participants were not blinded to treatment. The supplement that was used for the intervention contained 200 kcal, 3.5 g of fat, 16 g of carbohydrates, and 25 g of protein (Opti-fit Lean Protein Shake, Optimum Nutrition, Downers Grove, IL). This supplement was chosen for the ratio of protein to carbohydrates (1.5:1) and sufficient protein.

Participants completed twelve HIRT sessions, adapted from Paoli et al.[6] and previously described.[18] A leg press and free weights were used for: leg and bench press (York Barbell Co., York, PA, USA), dumbbell (DB) lunges, shoulder press, biceps curls, and overhead triceps extensions. Three sets of each were completed at 80% of 1RM with 20 to 30 s of rest between sets,[18] completing 6–10 repetitions in the first and second sets, then until muscular fatigue on the third set. The research staff spotted participants and made sure that proper form was used. Participants were allowed two minutes of rest between exercises.[6] The amount of weight used and repetitions completed were recorded for progression. If participants could perform 8 or more repetitions on all sets for two workouts in a row, leg press weight was increased by 10%, bench press by 5%, and accessory exercises by 2.5 lbs. Subjects had a minimum of 24–48 h of rest between sessions. Training sessions were completed at similar times of day (±2 h). Total training volume (load × reps for all visits) was determined by adding together cumulative volume across all training days.

The Shapiro–Wilk test was used to determine if all data were normally distributed. Training volume was evaluated via an ANOVA. Chronic changes in body composition and 1RM strength were evaluated using an analysis of covariance (ANCOVA) on the change values, covaried for baseline scores and training volume, with Bonferroni post hoc comparisons. Changes in metabolic data including change in pre-exercise, 30 min, 60 min, and 90 min post-exercise. RMR and RER were evaluated using separate ANCOVAs, with baseline values, estradiol, and training volume as covariates. If a time point was missing (n = 1), imputation was used and the

Table 1
Maximal strength change for lean mass (LM), body fat percentage (%fat), maximum leg press (LP1RM), bench press (BP1RM), and average training volume for each treatment group [Pre-workout (PRE), post-workout (POST), and control (CON) groups; Unadjusted mean ± SD].

Group	ΔLM (kg)	Δ%fat (%)	ΔLP1RM (kg)	ΔBP1RM (kg)	Average training volume (kgs)
PRE	0.96 ± 1.38	−1.06 ± 1.42	67.6 ± 30.5	5.6 ± 2.8*	82,447.2 ± 18,229.5
POST	0.64 ± 0.72	−0.70 ± 1.04	55.3 ± 23.5	4.8 ± 2.5*	63,354.1 ± 14,321.9+
CON	0.15 ± 1.35	−0.44 ± 0.91	62.9 ± 26.8	2.5 ± 1.8	86,491.9 ± 21,895.7

* Indicates a change significantly greater than CON ($p < 0.05$).
+ Indicates significantly lower than other groups ($p < 0.05$).

average change for the group was used for that individual. A subset of food logs (n = 12) were analyzed for differences over time using a one-way ANOVA. An alpha level was set at $p \leq 0.05$ and all analyses were performed using SPSS (Version 25.0, IBM Corp., Armonk, NY, USA).

3. Results

There were no significant between-group baseline differences for any variables ($p > 0.05$). Training volume (load × total reps, Table 1) was summed for all twelve training visits for each participant. There were significant between groups differences ($p = 0.002$, partial eta-squared (η^2) = 0.262). The POST group had a significantly lower average total volume than the PRE group (mean difference ± SD: −19,093.1 ± 12094.8 kg, $p = 0.009$), as well as the CON group (−23,137.9 ± 14536.2 kg, $p = 0.008$).

For FM, LM, and %fat there were no significant between-groups differences (Table 1; FM: $p = 0.959$; $\eta^2 = 0.002$; LM: $p = 0.17$; $\eta^2 = 0.112$; %fat: $p = 0.319$; $\eta^2 = 0.058$).

For LP1RM, there were no significant between groups differences ($p = 0.736$; $\eta^2 = 0.016$) (Table 1). For BP1RM, there were significant between groups differences ($p = 0.007$; $\eta^2 = 0.238$), with PRE experiencing an increase in strength (5.0 ± 1.1 kg) significantly greater than CON (2.2 ± 1.4 kg; $p = 0.010$), but not POST ($p = 1.000$). The change in POST (5.1 ± 1.1 kg) was significantly greater than CON (2.2 ± 1.5 kg; $p = 0.015$).

Estradiol at post training was significantly different between groups ($p = 0.022$), with POST values (0.86 ± 0.30 pg/mL) significantly lower than CON (1.32 ± 0.46 pg/mL; $p = 0.034$). There were no significant between groups differences in the change in REE from pre to post training (PREEX: $p = 0.058$; 30POST: $p = 0.643$; 60POST: $p = 0.222$; 90POST: $p = 0.282$) (Table 2). There was a significant change in PREEx RER ($p = 0.045$, $\eta^2 = 0.162$). Post hoc analyses revealed no significant differences between groups but the difference between CON and PRE approached significance ($p = 0.087$), with PRE demonstrating a larger decrease. There were significant between groups differences at 30POST ($p = 0.003$, $\eta^2 = 0.290$), with PRE demonstrating a larger decrease POST ($p = 0.035$) and CON ($p = 0.008$). There were no significant differences in RER changes at 60POST and 90POST ($p = 0.185$ and $p = 0.115$, respectively).

Table 2
Changes scores for resting energy expenditure (REE) and respiratory exchange ratio (RER) between visits 2 and visit 13. REE is expressed in kcal/day and RER is expressed as liters (L) VCO^2/L O^2.

Timepoint		PRE	POST	CON
ΔPREEx	REE	126.24 ± 103.35	97.06 ± 84.79	46.00 ± 141.22
	RER	−0.07 ± 0.07	−0.02 ± 0.06	−0.01 ± 0.05
Δ30POST	REE	89.53 ± 169.05	127.38 ± 127.83	120.25 ± 234.16
	RER	−0.06 ± 0.05[a]	−0.01 ± 0.04	−0.01 ± 0.04
Δ60POST	REE	110.65 ± 244.49	94.24 ± 188.01	69.63 ± 315.50
	RER	−0.04 ± 0.04	0.00 ± 0.06	−0.01 ± 0.05
Δ90POST	REE	131.65 ± 243.98	114.12 ± 196.73	60.00 ± 304.45
	RER	−0.03 ± 0.05	−0.01 ± 0.04	0.00 ± 0.05

[a] Denotes a significantly greater change compared to other groups.

There were no significant interaction or main effects for either time or group ($p = 0.242$–0.546). Three-day dietary logs demonstrated no change for kilocalories ($p = 0.085$; 1910 ± 609 kcal/day), CHO ($p = 0.060$; 3.0 ± 1.1 g/kg; 239 ± 92.6 g/day), PRO ($p = 0.654$; 1.1 ± 0.2 g/kg; 88.4 ± 9.9 g/day) or fat ($p = 0.094$; 0.85 ± 0.34 g/kg; 70.1 ± 27.4 g/day).

4. Discussion

This study, to our knowledge, is the first to evaluate the effectiveness of pre-versus post-exercise nutrition on body composition, strength, and metabolic adaptations in trained females. The results demonstrated no significant group differences in body composition or lower body strength. Upper body strength appeared to be more responsive to both PRE and POST nutrient timing, compared to CON. Chronic changes in REE were not significantly different between groups, but changes in RER demonstrated significance, with PRE experiencing greater increase in fat oxidation at 30POST relative to POST and CON.

Prior research has demonstrated that peri-workout nutrition is beneficial for stimulating protein synthesis,[7,19] ideally contributing to increased LM. Previous data has shown pre-workout nutrition improves LM and %fat, compared to consuming the same macronutrients in the morning and evening.[2] In contrast to these previous results, there were no significant differences between groups for body composition adaptations. The results are consistent with previous findings that consumption of protein pre- or post-workout does not alter its effects on protein synthesis or body composition.[6,19]

Beyond body composition, it is thought that peri-workout PRO and CHO may contribute to acute and chronic increases in performance. Schoenfeld et al.[6] and Candow et al.[20] evaluated the effects of pre- versus post-exercise PRO consumptions in trained young males and untrained older males, respectively. Similar to our results, both studies found that neither timing strategy was superior for increasing maximal strength. These demonstrate that the novelty of the training intervention was a greater stimulus than nutrient timing. The effectiveness of the training,[21] combined with sleep habits,[22] overall dietary habits,[23] and any outside training, may have contributed to the lack of between-group differences. It is unclear why BP1RM responded differently between groups; this may partially be due to the novelty of the barbell bench exercise for these women.

Three studies have addressed pre- versus post-workout nutrition, two have evaluated males,[6,20] and one used a mixed sample.[7] Prior research demonstrated that sexes differ in metabolic responses to exercise.[8] While RER may respond advantageously to post-absorptive exercise in males,[24] fasted exercise may blunt fat oxidation in females.[8] Similar to previous data in males,[25] our results indicate no differences between groups for REE changes. However, PRE demonstrated a greater reliance on fat at 30POST, agreeing with previously reported greater fax oxidation in post-prandial and trained females.[26,27] This may not have physiological significance as the increase in fat utilization did not necessarily lead to a decrease in FM over time. Further research is warranted to

determine whether adaptations in post-exercise substrate utilization translate to changes in body composition.

It has been shown that males and females respond similarly when beginning resistance training,[28] with females potentially experiencing greater increases in strength.[29] Even so, there are sex-based differences in physiological responses. One basis may be differences in PRO metabolism, with males having greater expression of positive PRO balance leading to greater muscle mass.[9] Furthermore, males and females differ in hormonal responses to training, particularly testosterone,[30] which may affect adaptations. Due to these differences, data in males cannot always be extrapolated to females for improved strength, body composition, and metabolism. The present study demonstrated that post-prandial exercise particularly benefited upper body strength and potentially chronic changes in substrate utilization post-exercise.

There were limitations in this study that need to be addressed. The menstrual cycle phase and use of birth control were not included, but variations were accounted for via measurement of estradiol during metabolic testing visits. Although participants were asked to not change their nutrition and training outside of the study, it was not strictly controlled for, and may have affected adaptations. Furthermore, nutritional habits such as caloric restriction were not accounted for when analyzing the results. The novelty and intense nature of the HIRT may have resulted in neural and strength adaptations across groups. In regards to the supplement, it may have been more beneficial to base PRO intake off of individual LM, as shown in previous research, but may reduce practicality.[31]

5. Conclusion

The present study demonstrates that neither pre- or post-workout protein-carbohydrate supplementation is superior to CON in stimulating body composition or lower body strength adaptations but may be more effective for upper body strength. The timing of nutrient consumption peri-workout is one of many factors that interact to initiate adaptations, including training stimuli, overall nutrition and PRO intake, hormonal milieu, and recovery. As discussed in recent literature,[6] the anabolic window for nutrient timing is likely not limited to PRE or POST, but the two interact. Further research is needed to better understand how female nutrition and training may differ from current recommendations deduced from male or mixed populations.

Practical implications

- While timing can be left to preference, it is best to ensure that PRO and CHO are consumed peri-workout.
- Post-prandial exercise may be more advantageous for female fat oxidation.
- It is likely that many factors interact, and adaptations cannot be deduced to only peri-workout habits.

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The results of the current study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results do not constitute endorsement by the American College of Sports Medicine.

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