

# NUTRIENT TIMING IN RESISTANCE-TRAINED WOMEN

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## **ABSTRACT**

Alexis Ann Pihoker: Nutrient Timing in Resistance-Trained Women  
(Under the direction of Abbie Smith-Ryan)

The purpose of this study was to evaluate the effects of different peri-workout nutrition strategies on strength, body composition, and metabolism in trained females. Young, trained females (n=43) completed body composition and strength testing before and after training. Participants were randomized into three groups using baseline leg press strength; pre-exercise supplementation of a protein-carbohydrate shake (PRE), post-exercise supplementation (POST), or a control group (CON). Following baseline testing, participants were trained twice per week for six weeks using high intensity resistance training. At the first and last training, metabolic testing was completed before and after exercise. There were significant main effects for time for LM ( $p=0.003$ ) and %fat ( $p<0.0005$ ). All three groups experienced significant increases in strength. There were no significant between groups differences for metabolic measurements ( $p>0.05$ ). These results demonstrate that peri-workout nutrition is potentially important for body composition, but either a PRE or POST strategy is effective.

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## **LIST OF ABBREVIATIONS**

BIS	Bioelectrical Impedance Spectroscopy
CON	Control Group
CHO	Carbohydrate
DEXA	Dual Energy X-ray Absorptiometry
FM	Fat Mass
HIRT	High Intensity Resistance Training
LM	Lean Mass
POST	Post-workout Supplement Group
PRE	Pre-workout Supplement Group
PRO	Protein
REE	Resting Energy Expenditure
RER	Respiratory Exchange Ratio
%fat	Body Fat Percentage
1RM	One Repetition Maximum

## CHAPTER I: INTRODUCTION

Nutrient timing has developed into the science of optimally timing the consumption of nutrients to produce the greatest physiological effect [1]. Previous research has demonstrated the importance of nutrient timing in stimulating protein synthesis [2], reducing muscle damage [3], enhancing recovery [2], and improving body composition [4]. Mechanisms supporting these adaptations include augmenting amino acid and glucose availability [5], altering energy expenditure, as well as modifying fuel utilization [6, 7]. Furthermore, data demonstrate that the timing of nutrients, rather than an individual's daily nutrient consumption may be more important for exercise performance and recovery [8, 9]. Protein is the primary nutrient responsible for stimulating anabolism [2], with carbohydrate intake enhancing this effect [3]. This combination of nutrients may be particularly useful for individuals participating in resistance exercise [10].

### Peri-Workout Nutrition:

Protein supplements are commonly consumed peri-workout to enhance exercise adaptations, with one of the most common being whey protein. Whey protein has a high concentration of leucine, which is known to regulate muscle protein synthesis [11]. Additionally, the digestion and absorption kinetics of whey protein are fast, allowing it to quickly stimulate a large blood amino acid peak [11]. Consumed peri-workout, studies have shown that whey protein can enhance increases in lean body mass [12], one repetition max strength [9], muscle glycogen content [9], and the cross-sectional area of muscle fibers [9]. The addition of carbohydrates to protein supplementation can amplify the effect on protein balance and glycogen re-synthesis [5], promoting both recovery and performance improvements in subsequent exercise bouts [3, 13]. The timing of supplementation can uniquely increase the muscle hypertrophic

response, as data have indicated that these adaptations are greater in those that consume protein around the exercise bout rather than at other time points throughout the day [9].

Consumption of nutrients prior to a workout appears to have importance in regulating protein synthesis during the workout [9], as well as lengthening the anabolic window post-workout [14]. Some research indicates that ingestion of amino acids and carbohydrates pre-workout may have a greater effect on muscle protein synthesis and performance than ingested post-workout [15]. Consuming protein prior to exercise increases muscle protein synthesis [3], while carbohydrate ingestion spares muscle glycogen and increases glucose availability, thus delaying fatigue [16, 17]. The addition of carbohydrates to protein, rather than carbohydrates alone, can lead to increased strength and body composition adaptations [3].

Research on post-workout nutrition indicates that there may be a critical window of time in which nutrients should be consumed after an exercise bout. Within 30 minutes post-exercise, the muscle appears to be more sensitive to available nutrients allowing for greater rebuilding and recovery. This is called the “anabolic window of opportunity”, and implies that post-workout nutrition is possibly the most critical and effective [8]. When consumed immediately post-workout, specifically during the first two hours, protein enhances amino acid kinetics and net protein balance between synthesis and degradation [3]. Consumption of carbohydrates post-exercise assists in the replenishment of intramuscular glycogen due to increased insulin sensitivity [3, 18]. As seen in pre-exercise supplementation, a combination of protein and carbohydrates may increase the availability of amino acids and protein synthesis even further than protein alone [3, 16].

A majority of the current data on nutrient timing is done in males, with very little research done in females. It is known that males and females differ in their physiological responses to exercise [19], even when adjusted for differences in fat and lean mass [20]. In regards to amino acids, oxidation in females is significantly lower than in males due to differences in musculature and predominant muscle fiber type [19-21]. Additionally, females tend to oxidize more lipids and less carbohydrates than males [19]. Possible explanations include muscle morphology[22], adiposity [22], and the sex hormones

estradiol and testosterone [22, 23]. Some data shows that male and female glycogen re-synthesis rates are similar post-exercise regardless of differences in substrate utilization during exercise [24]. Other evidence indicates that females may differ in their ability to maintain glucose homeostasis [22] and are not as sensitive to carbohydrate loading as males [19]. Known differences in metabolic responses to exercise imply that there may be a need for differences in nutrient timing recommendations.

While it is well recognized that peri-workout nutrition influences body composition and performance, more data are needed to determine whether pre- or post-workout nutrition is most important. Some research indicates that post-workout nutrition is most optimal [8], while other research implies that the general consumption of nutrients peri-workout is of the greatest importance, regardless of whether it is pre- or post-workout. Data suggest both pre- or post-exercise provision of nutrients produce similar adaptations during both aerobic training [17], and resistance training [14, 25]. With the existing debate over the most optimal nutrient timing method, as well as the lack of data from female populations, there is a need for research evaluating the effects of nutrient timing in trained females. Better evidence-based recommendations for this population could have important implications for performance, body composition, and metabolic adaptations.

**Purpose:**

1. The primary purpose of this study was to evaluate the effects of consuming a protein-carbohydrate (PRO-CHO) beverage pre- or post-workout during six weeks of high-intensity resistance training in resistance trained women, specifically evaluating body composition and maximal strength.
2. The secondary purpose of this study was to examine the acute and chronic alterations in substrate utilization (respiratory exchange ratio) and resting energy expenditure in response to consumption of a PRO-CHO supplement consumed before versus after a single bout of HIRT.

### **Research Questions:**

1. Does the timing of ingestion of a peri-workout PRO-CHO supplement blend combined with a high-intensity resistance training program affect chronic body composition and strength measurements?
  - a. Specifically, is there a significant decrease in body fat percentage and/or increase in total and segmental lean mass between groups?
  - b. Are one rep max bench and leg press measures improved between groups?
2. Does the timing of peri-workout nutrition acutely effect substrate utilization and resting energy expenditure in females?
  - a. Is there a difference between groups in which substrates are used before and/or after exercise, determined from respiratory exchange ratio?
  - b. Is there a significant difference in post-exercise resting energy expenditure between groups?
3. Does the timing of peri-workout PRO-CHO supplementation affect chronic adaptations in resting energy expenditure and substrate utilization?

### **Research Hypotheses:**

1. Strength adaptations will not differ between the pre-workout supplementation group (PRE) and post-workout supplementation group (POST), but will be greater than the CON group.
2. Body composition adaptations will not differ between PRE and POST, but LM will increase more in PRE and POST, than in CON.
3. Substrate utilization pre- and post-exercise will differ between groups. The PRE group will utilize less CHO after the workout bout than CON or POST.
4. Resting energy expenditure post-exercise will not be significantly different between PRE and POST, but will be lower in CON.

5. Groups will not differ in their chronic metabolic responses; the PRE and POST groups will have similar chronic adaptations in resting energy expenditure.

**Delimitations:**

1. Participants were resistance-trained females (resistance training two or more times per week for six months), ages 18-30 years old.
2. Participants were excluded if they are currently training to compete in a lifting competition, due to outside training intentionally influencing their strength and body composition.
3. The study consisted of 14 visits.
4. The duration of the supplement and exercise intervention was six weeks.
5. Body composition was analyzed using dual-energy X-ray absorptiometry (DEXA).
6. Participants were not consistently consumed a protein supplement immediately before or after their workouts (three or more times per week) for at least three months prior to the start of the study.
7. Participants were asked to maintain dietary intake throughout the duration of the study determined from baseline and bi-weekly dietary intake logs.
8. Participants were excluded if the physical activity questionnaire indicates a recent significant change in training frequency or intensity.
9. Participants were excluded if they currently use or plan to use another sports supplement (specifically, consumption of BCAAs more than once per day and/or consumption of creatine within 4 weeks prior to the start of the study).
10. Participants were excluded if the health history questionnaire indicates a recent history of musculoskeletal injury, musculoskeletal disorder, metabolic disorder, or eating disorder.
11. Participants were excluded if they have lost or gained  $\geq 5$  kg within the last two months.

**Limitations:**

1. Subjects were recruited at the University of North Carolina at Chapel Hill and gyms in the surrounding areas, therefore, recruitment of participants was not truly random.
2. The amount of protein supplement given to participants was not based on the subject's body composition [26, 27].
3. Individual diets (including macronutrient distribution) and outside exercise was not controlled for but was tracked using three-day dietary logs.
4. Results may have been influenced by an individual's current type of resistance training (ie. circuit versus traditional resistance training), and individual effort [28, 29].
5. Dietary intake around the participants' other workouts (outside of the intervention) was not controlled for but rather indicated in the dietary logs.
6. Indirect calorimetry, rather than direct calorimetry (the gold standard), was used to measure resting energy expenditure and respiratory exchange ratio.
7. Menstrual status was not an inclusion or exclusion criteria. Salivary estradiol was measured during metabolic testing to co-vary for estradiol levels and take out variability due to them in metabolic data.

**Assumptions:***Theoretical*

1. Subjects provided accurate answers to screening questions.
2. Subjects provided accurate dietary logs and physical activity questionnaires.
3. Subjects adhered to pre-visit guidelines, including fasting.
4. The one repetition maximum results reflected the subjects' true maximal values.
5. The multiple repetition maximum tests provided accurate predictions of true maximal values.
6. Subjects kept their dietary and training habits consistent throughout the duration of the study.
7. Subjects gave their full effort at each training visit, and effort did not differ between groups.

### *Statistical*

1. The sample of participants that was used in the study were from a population that is normally distributed.
2. Participants for the treatment groups were randomly assigned using matched pairs blocked design.
3. The variability in the study sample was approximately equal (homogeneity of variance assumption).

### **Definition of Terms:**

*High intensity resistance training*- a type of resistance training that is characterized by short rest periods, with participants using a ~80% load for a moderate volume (6-8 repetitions).

*One repetition maximum*- the maximum amount of weight that can be lifted for one repetition, while maintaining proper form.

*Peri-workout nutrition*- nutrients consumed during and/or around the workout (pre- or post-exercise).

*Repetitions to fatigue*- the maximum amount of repetitions that can be completed using a given amount of weight before an individual can no longer complete a full repetition.

*Resistance trained*- participating in resistance training at least 3 times per week for 6 months.

*Resting energy expenditure*- a measurement taken to approximate the amount of kilocalories the body would use at rest during a full twenty-four hour period.

*Substrate utilization*- the substrate (usually carbohydrate or fat) being used by the body to produce energy; analyzed by measuring respiratory exchange ratio, which is the amount of CO<sub>2</sub> produced divided by the amount of O<sub>2</sub> utilized [30].

### **Significance of Study:**

The aim of this study was to evaluate whether pre- or post-workout consumption of a PRO-CHO supplement produces more favorable body composition and strength adaptations with high-intensity



resistance exercise. While current research has revealed that there are benefits to peri-workout nutrition, the literature has mixed conclusions about the most optimal time to consume nutrients. Additionally, a majority of the data is in males, and only two known studies have specifically analyzed pre- versus post-workout nutrition [14, 15]. Further research comparing pre- versus post-exercise nutrient timing is needed, specifically in females, where there may be important applications for optimizing body composition, performance, and metabolic adaptations.

## CHAPTER II: LITERATURE REVIEW

Protein and carbohydrate supplements are commonly used by individuals to enhance training adaptations and performance. Resistance training provides the muscle with an anabolic environment, allowing the body to utilize nutrients in a super-compensatory manner relative to resting conditions [31]. Estimations of the length of this “anabolic window” vary from 45 minutes to 6 hours [14]. There is ample research demonstrating that the timing of nutrients peri-workout can favorably assist in acute recovery and performance, as well as chronic strength and body composition adaptations [3].

Additionally, nutrient timing and the type of nutrients consumed may alter resting energy expenditure and other metabolic adaptations [6, 32]. However, little data exists on whether pre-exercise nutrient consumption is more or less advantageous than post-exercise consumption. While most data is done in males, prior research has indicated sex-based differences in physiological responses to exercise [19]. This implies the need for potential sex-based differences in nutrient timing recommendations. The objectives of the current review are to analyze the literature on peri-workout nutrition and resistance training adaptations, as well as to describe sex differences in response to altered timing of nutrients and training.

### **Nutrient Timing:**

Nutrient timing is the purposeful timing of nutrient consumption, typically protein and carbohydrates, in and around the workout window. The goal of nutrient timing is to provide nutrients around exercise to maximize the response to exercise- both in recovery and adaptations. Previous data demonstrate that timing the consumption of carbohydrate and protein around the workout window allows the body to optimize glycogen replenishment and protein synthesis [3, 5], leading to beneficial physiological adaptations. While it is well established that protein and carbohydrate intake peri-workout will augment exercise adaptations, there is a lack of agreement between what period of time is of greater importance, pre-workout or post-workout nutrition. Recent data indicate that the timing of nutrient

consumption to optimize the anabolic response of muscle may be variable, and not as narrow of a window as it originally seemed [8, 14].

#### *Pre-exercise Nutrient Consumption*

Data have demonstrated a significant benefit of pre-exercise carbohydrate and protein consumption in preventing fatigue and muscle damage, as well as enhancing performance [3]. Tipton et al. [15] has previously reported an increase in amino acid uptake as a result of pre-workout essential amino acid and carbohydrate ingestion, potentially optimizing protein synthesis during and following a workout. Furthermore, it was reported that pre-exercise nutrition may increase the duration of the anabolic response of muscle, relative to post-workout nutrition [15]. Other literature has examined the use of pre-workout nutrition and its effects on both performance and peri-workout hormonal responses. It has been reported that consumption of a multi-nutrient supplement prior to a resistance exercise bout favorably alters the exercise-induced response of anabolic hormones, specifically testosterone and growth hormone [33]. Additionally, various strength and power measures acutely increased in response to pre-workout ingestion of the multi-nutrient supplement [33].

Research has shown that when resistance exercise is completed under fasted conditions, with no peri-workout nutrients, the net balance of protein is negative [34]. Pre-workout protein consumption has specific applications in the context of resistance training due to its ability to maximally stimulate protein synthesis post-exercise [3]. Consumption of whey protein, due to uniquely fast absorption rates, allows for increased amino acid availability [35]. Use of whey protein prior to an exercise bout may prolong an anabolic environment for muscle hypertrophy and growth [9], potentially diminishing the need for post-workout supplementation [8, 14]. Furthermore, pre-workout nutrition offers unique metabolic benefits beyond protein synthesis, particularly for women [36]. A recent study done by Wingfield et al. [6] demonstrated that ingestion of protein prior to a workout was superior to an iso-caloric amount of carbohydrates in stimulating resting energy expenditure post-workout [6]. A meta-analysis done by Henderson and Alderman [36] also indicated that pre-exercise nutrient consumption in women enhances fat oxidation, compared to fasting.

The addition of carbohydrates to pre-exercise supplementation may enhance the effects of protein on the rates of protein synthesis peri-workout [3], and substrate utilization during exercise [37]. Pre-exercise carbohydrate consumption has often been considered ideal for delaying a decrease in glycogen stores [3] by enhancing glucose availability [17]. Coyle et al. [37] demonstrated a positive impact of a high carbohydrate meal prior to prolonged exercise, resulting in an increased glucose utilization, and increased muscle glycogen prior to the workout [37]. During resistance exercise, individuals can experience a decrease in muscle glycogen content by up to 40% [38]. Consumption of carbohydrates prior to an exercise bout may attenuate this decrease [39], thereby preventing the onset of fatigue. While carbohydrates in isolation do not appear to benefit the metabolic environment as substantially as protein, the combined ingestion of nutrients prior to exercise may be ideal for increasing protein synthesis [15, 40], favorably altering metabolism [6], and enhancing the anabolic hormonal environment [41]

#### *Post-exercise Nutrient Consumption*

The period of time after an exercise bout is often referred to as the “anabolic window”, and has been shown to be an ideal time for nutrient consumption. This time period has been shown to be relevant immediately after exercise and extend up to an hour and a half post-workout [8]. During resistance exercise, the muscle experiences a loss of fuel stores and damage to tissues [8]. While the desirable response to training is muscle growth, this can only occur if nutrients are available [42]. As a result of the stimuli from exercise, the post-exercise period is characterized by an increase in the use of amino acids for anabolic activity [34]. A study done by Biolo et al. [43] established that while resistance training does induce increased rates of muscle protein turnover and protects against muscle catabolism, it does not directly stimulate protein synthesis. Rather, other components such as nutrient availability and timing, are likely required to create an anabolic environment. Post-workout protein consumption has been shown to offset protein breakdown and stimulate protein synthesis, causing a greater hypertrophic response and increased muscle growth [2, 8].

The specific type of nutrients available may be critical when establishing the most effective window and timing. A study done by Borsheim et al. [44] examined the response of net muscle protein

balance to essential amino acid intake after a bout of resistance exercise. While the rate of proteolysis remained unaffected, net protein balance increased, likely due to greater protein synthesis.

Correspondingly, Levenhagen et al. [32] examined the effect of a multi-nutrient supplement containing protein, carbohydrate, and a small amount of fat, immediately post-workout compared to three hours after exercise. Results revealed that while protein breakdown was not significantly different, immediate consumption stimulated greater glucose and amino acid uptake, with protein synthesis increasing by over three-fold. Data have also demonstrated that proteins with different rates of digestibility (i.e. whey versus casein protein) similarly stimulate protein synthesis to produce a positive net protein balance after resistance training [45]. Furthermore, research has established that the combination of protein and carbohydrate post-exercise positively effects glycogen re-synthesis, greater than carbohydrates in isolation [46, 47]. These results indicate that post-workout nutrition activates the physiological processes necessary for optimized training adaptations.

Post-workout protein and carbohydrate supplementation has consistently been shown to be beneficial towards promoting exercise-related body composition and strength adaptations [3]. Research done by Hartman et al. [48] using fat-free milk found that male weightlifters lost larger amounts of body fat and gained more lean mass when consuming the milk post-exercise than the group consuming an isocaloric carbohydrate beverage. Another study done by Burk et al. [49] analyzed the effects of consuming protein in the morning and again either before or five hours post-training. Those consuming the post-exercise dose of protein experienced a gain in fat-free mass and related increase in maximum leg strength. Research has also indicated that the use of protein during this the post-exercise period increases exercise adaptations more than carbohydrates consumed in isolation. Similarly, data have shown that combined whey and casein consumption illicit increases in body mass and lean mass more than a whey-amino acid mixture or carbohydrate placebo [50].

While a large amount of research supports the potential benefits of nutrient timing, some data exist suggesting total protein intake is a more important factor. Specifically, Hoffman et al. [51] demonstrated that chronic peri-workout protein timing does not differentially effect the peak power of

resistance-trained males, compared to isocaloric protein consumed evenly throughout the day. Tipton et al. [52] evaluated the effects of whey protein consumption immediately prior to a workout bout versus one hour post-workout on protein kinetics. The results indicated that both produced an elevated anabolic response, regardless of timing around the workout. Likewise, Schoenfeld et al. [14] confirmed that specific timing of peri-workout protein supplementation pre- or post-training does not cause significantly different effects in strength or lean mass in resistance-trained men. Research has confirmed the beneficial effects of pre-exercise carbohydrate ingestion on endurance performance and increasing muscle glycogen[53], yet data have not come to a clear conclusion on the most ideal timing of protein and carbohydrate consumption for resistance exercise, especially for females.

### **Sex-based Differences:**

While nutrition and exercise recommendations have remained sex-neutral, research has shown that males and females differ in physiological responses to exercise and metabolism of nutrients [21]. Previous data has demonstrated women to have lower relative muscle mass, and smaller cross-sectional area and type II fiber content compared to their male counterparts [21, 54, 55]. Research has also shown that women experience lower metabolic and protein oxidation rates despite controlling for differences in body composition [20, 56]. Preliminary data by Tipton et al. [21] further confirmed this, finding that post-exercise amino acid consumption resulted in similar protein kinetics as exercise done in the fasted state, indicating that post-exercise nutrients may not be as effective as pre-exercise nutrients in females. Additionally, at both rest and during exercise, females tend to metabolize a greater amount of lipids, as demonstrated by lower respiratory exchange ratio (RER) values [19, 57, 58]. Contrary to males, fasting prior to exercise has previously been shown to blunt fat oxidation to a greater degree in females [36], while pre-exercise ingestion of protein has increased both fat oxidation and post-exercise resting energy expenditure [6]. Data on chronic training adaptations in females indicate that, unlike males, carbohydrate oxidation is decreased as a mechanism to spare muscle glycogen, thus reducing the response females have to carbohydrate loading/supercompensation [57].

### *Hormonal Distinctions*

Substrate metabolism in females has been shown to be more sensitive to hormonal fluctuations [57, 59]. The tendency to spare glycogen and oxidize more lipid is potentially due to the female hormone 17- $\beta$ -estradiol [19, 60, 61], and the comparative absence of testosterone [62]. Elevated amounts of estrogen and progesterone in females may reduce catecholamine response to exercise and fasting, further increasing lipolytic activity and preserving glycogen [62]. In addition to decreased catecholamine activity, females also exhibit suppressed glucagon responses to low blood glucose levels [63], further indicating a greater reliance on lipolysis, and potentially lower carbohydrate needs. These notable sex-based differences may alter nutritional recommendations for females, highlighting the potential importance of nutrient timing around exercise.

### **Resistance Training:**

Research has shown that individuals differ in their physiological adaptations to resistance and endurance training. One of the primary adaptations that commonly develops through resistance training is an increase in muscle cross-sectional area due to hypertrophy [64]. While endurance training tends to result in increased size of slow-twitch fibers [65], resistance training commonly increases the size of fast-twitch fiber types [66]. A second widespread adaptation to resistance exercise is an increase in both muscular strength and endurance. A study done by Anderson et al. [67] demonstrated that high resistance/load and lower repetitions optimally results in the greatest strength gains. Research done in males has found that optimal repetition ranges for maximal strength tend to be lower to moderate [68]. Conversely, data in women indicate that they experience the greatest muscular adaptations in strength and lean body mass using higher volume [69]. Studies in women have verified that high resistance paired with lower volume does not produce statistically significant strength differences relative to lower loads with higher volume [70]. In addition to muscular adaptations, resistance training can alter metabolic parameters, specifically substrate utilization. After exercise training, both men and women decrease their reliance upon glycogen for energy, and increase utilization of fat [71]. In trained women specifically, resistance training can acutely increase both fat oxidation and oxygen consumption [72].

### *High Intensity Resistance Training*

Training that utilizes similar volume loads yet less rest, as seen in high intensity resistance training (HIRT) or circuit training, may be more time efficient while producing similar adaptations. Studies have shown that HIRT can produce alterations in muscle physiology after only five weeks, as shown by increases in muscle strength and cross-sectional area [73]. HIRT is characterized by heavy resistance (~80% 1RM), moderate repetitions (6-8 reps), and low rest (20-30 sec). A study done by Alcaraz et al. [74], revealed that heavy resistance circuit training produces similar strength and power adaptations as traditional resistance exercise, yet requiring less time per workout. Lawton et al. [75] confirmed this showing that continuous repetitions with little rest may produce larger increases in six repetition maximum strength than when using intra-set rest, and both methods produce similar increases in power. Furthermore, HIRT may create a more advantageous metabolic environment relative to both aerobic and traditional resistance training. Wingfield et al. [6] demonstrated that a bout of HIRT resulted in significantly lower RER values at 30 and 60 minutes post-workout, compared to aerobic endurance exercise in women, indicating greater lipid oxidation. Other research utilizing HIRT, found that it may result in higher resting energy expenditure and greater fat oxidation compared traditional resistance training [14].

### **Conclusion:**

Data strongly support the beneficial effects of consuming peri-workout nutrients to enhance training adaptations [76, 77]. Protein specifically has been shown to enhance muscular adaptations to exercise, including both strength and hypertrophy [2], with the addition of carbohydrates further maximizing this effect [3]. Even so, the literature has been inconclusive on which workout window creates the most optimal anabolic environment- pre- or post-exercise [14]. Resistance training combined with nutrient intake is a potent stimulus for protein synthesis [31]. Research has indicated that high-intensity resistance training may be a more effective, time-efficient exercise modality for muscular and metabolic adaptations to training [14, 73], particularly when paired with protein intake [6]. A majority of the current nutrient timing and resistance exercise data has been completed in males. While studies have demonstrated sex-based differences in metabolic and muscular responses to exercise [20, 21, 56, 70], the



authors are aware of only three that have analyzed female-specific responses to nutrient timing [4, 6, 78]. Further research is needed in both nutrient timing and female adaptations to give evidence-based nutrition recommendations, particularly in a population that is vulnerable to variable nutrition and training advice.

### CHAPTER III: METHODOLOGY

#### Participants:

Female participants (n=48) between the ages of 18 and 30 years were recruited. Regarding sample size, prior research has indicated an effect size (Cohen's *d*) of approximately 0.24 of peri-workout protein supplementation on muscle hypertrophy [2]. To determine sample size for the current study, we assumed an effect size of 0.24, a correlation of 0.6 among repeated measures, and 80% power at an alpha level set *a priori* at 0.05. Calculations indicated that the study would be sufficiently powered with a total sample size of N=38 (n=15 per treatment group, n=8 in the control group, for a 2:2:1 matched pairs blocked randomization approach). To account for a 15 percent dropout per group, we aimed to recruit 45 participants. Power was calculated using G\*Power Version 3.1.9.2.

Participants were required to be resistance-trained for a minimum six months, exercising at least three times per week. Participants were excluded if they had a history of musculoskeletal disease, recent injury, or recent changes in their training, diet, or body weight (lost or gained  $\geq 5$  kg within the last two months). Additionally, if an individual was consistently consuming protein peri-workout (3 or more times per week), consumed branched-chain amino acids (BCAAs) more than once per day, or had used creatine in the preceding 4 weeks, they were excluded. Supplementation and medical history were evaluated via a health history questionnaire. A three-day dietary log was completed immediately after visit 1, prior to beginning the intervention, to establish each participant's baseline diet. Individuals were instructed to maintain their typical dietary habits for the duration of the intervention. Two other dietary logs were completed during the study, one diet after visit 7 (the mid-point of the intervention), and one after the six week intervention. Diet logs were assessed for total calories, and grams of carbohydrate, protein, and fat to ensure constant dietary habits throughout the supplement intervention. Participants

completed a baseline physical activity questionnaire at visit 1, as well as at visits 7 and 14, to ensure constant training habits.

### **Experimental Design:**

In a matched-pairs randomized controlled intervention, participants were assigned to either a pre or post-exercise nutrition group. Prior to visit 1, individuals were verbally screened for eligibility. During visit 1, participants signed the informed consent document and completed a series of questionnaires for physical activity and health history. A three-day dietary log was sent home to be completed prior to the subsequent visit, as well as mid-intervention and post-intervention. Participants took a pregnancy test; upon confirmation of a negative result, they underwent body composition evaluation. After consumption of a standardized pre-testing shake, one repetition maximum (1RM) tests for both the leg press and bench press, and multiple repetition max tests for various accessory muscles was completed. Prior to visit 2, participants were randomized using a matched groups design based upon baseline 1RM leg press values, using Random Allocation Software (Isfahan, Iran) into either a pre- or post-supplementation group, or the control group. During visit 2, participants provided a salivary sample to measure levels of estradiol. Resting energy expenditure (REE) was measured 30 minutes prior to a training session and evaluated 90 minutes following. Respective supplement groups consumed their treatment no more than 15 minutes before or after a high-intensity resistance training (HIRT) bout. For visits 3-13, participants completed 30-minute bouts of progressive HIRT training, consuming their supplement during their assigned time. During visit 13, pre- and post-training REE, RER, and salivary estradiol were analyzed using the procedures described for visit 2. During visit 14, after 6 weeks of training, participants completed testing identical to visit 1 (Figure 1).

### **Preliminary Testing:**

#### *Body Composition*

Participants were at least eight hours fasted and refrained from exercise for at least 24 hours prior to all baseline and post-testing. Due to use of the dual energy x-ray absorptiometry (DEXA), which utilizes a very low dose of radiation, all participants had undergone a urine pregnancy test to exclude any

potential participants with a positive test result. All body composition measures, including fat mass (FM), lean mass (LM), and body fat percentage (%fat) were calculated using DEXA (GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA). For the DEXA scan, participants were asked to remove all metal from their bodies (including jewelry, glasses, and metal in clothing). Participant data, such as height (in inches), weight (in pounds), ethnicity, and birth date, were entered into the computer prior to the scan. Individuals lay supine in the center of the DEXA table with the guidance of the research staff. They were instructed to remain motionless during this procedure, which took approximately 7-13 minutes (depending on the size of the individual). Regions of interest lines were adjusted by the same research technician for all scans. The test-retest reliability for the DEXA from our lab for FM is as follows: intraclass correlation coefficient (ICC) = 0.998, standard error of mean (SEM) = 0.624 L, and minimum difference (MD) = 1.72 L. For %fat, ICC = 0.982, SEM = 0.960%, and MD = 2.60%. For FM, ICC = 0.995, SEM = 0.831kg, and MD = 2.30kg. Lastly, for LM, ICC = 0.996, SEM = 0.999kg, and MD = 2.75kg [79].

### *Maximal Strength Testing*

Maximal strength was tested using one repetition maximums (1RM) for the bench press and leg press, and multiple repetition maximums for accessory muscles. Prior to testing, participants consumed a standardized protein shake containing 190 kilocalories, 5 grams of fat, 24 grams of carbohydrates, and 15 grams of protein (Special K, Milk Chocolate Protein Shakes, Kelloggs NA Co, Battle Creek, MI). For the 1RM tests, participants did a five minute self-selected warm up. They were familiarized with the equipment to be used, as well as the proper technique for each lift. 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 repetition at an estimated 1RM value. They then completed up to three more single-repetition attempts with increasing weight and two minutes of rest between attempts until they reached a point of failure.

Multiple RM tests were used to predict participants' 1RM for four different accessory exercises. These exercises included an overhead shoulder press, a biceps curl, an overhead triceps extension, and an

alternating stationary lunge, all using dumbbells. The research staff determined what weight participants should begin with for each exercise, based on the training history of the individual. A warm up weight was done for five repetitions, then a working weight done for as many as possible, aiming for 3-12 repetitions completed before volitional failure was reached. If an individual did less than 3 or greater than 12 reps, the exercise was done again at the end of testing with an appropriate increase or decrease in weight to hit 3-12 reps. Participants were allowed one to two minutes of rest between each accessory exercise. The amount of weight used (rep weight) and the number of repetitions completed until fatigue (RTF) were put in the following equation to predict participants' 1RM [80]:

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

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

#### *Resting Energy Expenditure and Respiratory Exchange Ratio*

During visits 2 and 13, Resting Energy Expenditure (REE) and Respiratory Exchange Ratio (RER) data were collected immediately before and immediately after the 30 minute HIRT bout. REE was measured thirty minutes prior to the HIRT bout, before PRE supplementation, and for 90 minutes after exercise (after POST supplementation). The 90 minutes of measurement was divided into three thirty minute bouts to collect data at 30, 60, and 90 minutes post-exercise. The first five minutes of measurement were discarded and the remaining time was averaged. Participants remained seated, wearing a gas mask that was connected by a tube to a metabolic cart. 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. Oxygen uptake and  $VCO_2$  were measured in liters per minute (L/min). REE, expressed in kilocalories per day (kcal/day), was calculated using Equation 4 below [81]:

$$\text{Equation 4: } REE = [(3.9 * (VO_2)) + (1.1 * (VCO_2))] * 1440\ minutes$$

To analyze substrate utilization before and after the training bout, RER was indirectly measured concurrently with REE using the metabolic cart. The following equation (Equation 5) was used to calculate RER, measuring  $\text{VO}_2$  and  $\text{VCO}_2$  in L/min [82]:

$$\text{RER} = \frac{\text{VCO}_2}{\text{VO}_2}$$

The resulting data was expressed as a value between 0.7 and 1.1 L  $\text{CO}_2$ /L  $\text{O}_2$ . Values around 0.7 L  $\text{CO}_2$ /L  $\text{O}_2$  indicate fat utilization while values close to 1.0 L  $\text{CO}_2$ /L  $\text{O}_2$  indicate carbohydrate utilization. Values around 0.82 L  $\text{CO}_2$ /L  $\text{O}_2$  indicate mixed substrate utilization.

#### *Salivary Estradiol*

During visits 2 and 13, participants provided a 2.5-5.0 mL passive drool saliva sample to determine estradiol- $\beta$ -17 measurements using an ELISA assay (Salivary 17 $\beta$ -Estradiol Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA). Prior to each visit, participants were asked to refrain from dental work for a minimum of 48 hours, and from brushing their teeth for at least 1 hour. This was done to prevent the sample from containing blood. Participants rinsed their mouth with water 5 minutes 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 -20°C to maintain the integrity of the sample. This measurement was done to take estrogen variations into consideration when comparing RER and REE data, and was used as a covariate during data analysis.

#### *Questionnaires*

Throughout the intervention, participants were asked to complete a three-day dietary analysis and the General Practice Physical Activity Questionnaire (GPPAQ). Dietary logs and the GPPAQ were taken during visits 1, 7, and 14. The three-day dietary analysis, capturing two non-consecutive week days and one weekend day, was analyzed using a software (Food Processor; ESHA Research, Salem, OR). The GPPAQ measured the amount of physical activity done at work and home (including time spent completing actual workouts), and approximate walking speed during the day. These surveys were

completed to account for any changes in dietary or exercise habits that may have occurred during the six week intervention. At visit 1, participants also completed a Health History Questionnaire to make sure there was no history of metabolic or musculoskeletal disease, recent injury, or substantial changes in weight (have lost or gained  $\geq 5$  kg within the last two months). This questionnaire also determined if participants were actively consuming sports supplements (including peri-workout protein shakes, BCAAs, and creatine).

### **Intervention:**

#### *Supplement Intervention*

Prior to visit 2, participants were randomized into one of three groups using Random Allocation Software (Isfahan, Iran) by matching baseline 1RM leg press values. One group orally consumed the supplement 15 minutes prior to training (PRE), the second group consumed the supplement 15 minutes after training (POST), and the third group received no supplement (CON). Research assistants ensured that the beverage was consumed within 10 minutes. The supplement that was used for the intervention consisted of 52 grams of a protein powder (Opti-Fit Lean Protein Shake, Optimum Nutrition, Inc., Aurora, IL Figure 2). The beverage contained 200 kilocalories, with 3.5 grams of fat, 16 grams of carbohydrates, and 25 grams of protein. Participants and research staff were not blinded to the treatment groups.

#### *Training Intervention*

Throughout the duration of the training (visits 2 through 13), participants completed two 30 minute HIRT sessions per week over the course of six weeks, adapted from Paoli et al [14]. Participants reported to the lab a minimum of 90 minutes fasted prior to each training session. Free weights were used to perform six different exercises. The exercises were completed in the order of: leg press (York Barbell Co., York, PA, USA), , bench press, dumbbell (DB) lunges, DB shoulder press, DB biceps curls, and DB triceps extensions. Before beginning the training session, participants were allowed to warm-up as they desire for up to 5 minutes [14]. Three sets were done for each exercise at a 6RM to 8RM, with 20 to 30 seconds of rest between sets [6]. The research staff spotted participants when necessary and made sure

that proper form was being used. Participants were allowed 1.5-2 minutes of rest between exercises [14]. The amount of weight used and repetitions completed per set was recorded during each training session for progression. If participants could get a minimum of 8 reps on all three sets, the weight was increased by 10% on leg press, and 5% on the other exercises. Heart rate (using the Polar heart rate monitor) and rate of perceived exertion [83] were also recorded after completion of each set. HIRT bouts took approximately 30 minutes to complete. Subjects had 1 to 2 days of rest between training sessions.

### **Statistical Analyses:**

Chronic adaptations of strength and body composition were evaluated using a mixed factorial analysis of variance (ANOVA) [time (pre-intervention vs. post-intervention)  $\times$  treatment (PRE vs. POST vs. CON)]. If a significant interaction effect was identified, a Bonferroni post-hoc analysis was done to evaluate pairwise comparisons. For acute measurements of REE and RER, a  $4 \times 3$  mixed factorial analysis of covariance (ANCOVA) was completed [time (baseline vs. 30 minutes post-workout vs. 60 minutes vs. 90 minutes)  $\times$  treatment (PRE vs. POST vs. CON)], using baseline estrogen levels as the covariate. An additional ANCOVA was done to examine chronic adaptations of post-workout metabolic measurements [time (change scores for baseline vs. 30 minutes post-workout vs. 60 minutes vs. 90 minutes)  $\times$  treatment (PRE vs. POST vs. CON)], with estrogen levels as the covariate. A  $3 \times 3$  mixed factorial ANOVA was used to assess dietary variables and the physical activity questionnaire [time (base vs. visit 7 vs. post)  $\times$  treatment (PRE vs POST vs CON)]. All analyses were performed using SPSS (Version 21.0 Armonk, NY, USA).



## CHAPTER IV: MANUSCRIPT

### Introduction

Approximately two billion dollars are spent each year on sports nutrition supplements [84]. Pre- and post-workout supplements make up a large portion of the market, yet with little consensus as to which timing strategy is more effective. Previous research has demonstrated the importance of nutrient timing in stimulating protein synthesis [2], reducing muscle damage [3], enhancing recovery [2], and improving body composition [4]. Mechanisms supporting these adaptations include augmenting amino acid and glucose availability [5], altering energy expenditure, as well as modifying fuel utilization [6, 7]. Data also demonstrates that the timing of nutrients, rather than an individuals' daily nutrient consumption, may be more important for exercise performance and recovery [8, 9]. Two of the most common nutrients consumed as part of peri-workout supplements are protein (PRO) and carbohydrates (CHO).

Protein is the primary nutrient responsible for stimulating anabolism [2], with carbohydrate intake enhancing this effect [3]. Whey protein specifically has a high concentration of leucine, which is known to regulate muscle protein synthesis due to its fast digestion and absorption kinetics [11]. Consumed peri-workout, studies have shown that whey protein can enhance increases in lean body mass [12], one repetition max strength [9], muscle glycogen content [9], and the cross-sectional area of muscle fibers [9]. The addition of carbohydrates to protein supplementation can amplify the effect on protein balance and glycogen re-synthesis [5], promoting both recovery and performance improvements in subsequent exercise bouts [3, 13]. Consumption of nutrients prior to a workout appears to have importance in regulating protein synthesis during the workout [9], as well as lengthening the anabolic window post-workout [14]. Some research indicates that ingestion of amino acids and carbohydrates pre-workout may have a greater effect on muscle protein synthesis and performance than ingested post-workout [15]. Conversely, research on post-workout nutrition indicates that there may be a critical window of time in

which nutrients should be consumed after an exercise bout in order to stimulate the greatest effects of exercise. While it is well recognized that both pre and post-workout nutrition likely have a positive effect on body composition and performance, more data are needed to determine whether pre- or post-workout nutrition is more advantageous.

Resistance training is well known for increasing lean mass, strength, and muscle endurance. One type of resistance training that is characterized by higher volume and shorter rest periods is high intensity resistance training (HIRT). HIRT utilizes heavier resistance (~80% of a projected 1RM) with moderate volume (6-8 repetitions), and rest as short as 20-30 seconds. As demonstrated by Alcaraz et al. [74], heavy resistance circuit training may be equally as effective as more traditional strength training routines in increasing strength and power, yet requiring less time. Additionally, recent data have demonstrated that the use of HIRT in young women enhances lipid oxidation post-workout to a greater degree than a bout of aerobic exercise [6].

Numerous studies have evaluated the effects of various nutrient timing strategies. Collectively, data suggests 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 men. Specifically, of the 24 studies that met the criteria for a meta-analysis looking at the effects of PRO timing on muscular adaptations to training [2], only two were completed in women. There are known physiological differences between males and females; specifically when undergoing resistance training [19, 21, 55, 85]. Nutrient metabolism is largely different between males and females, with females expressing lower amino acid and carbohydrate oxidation [19-21], and greater lipid oxidation [19]. Divergent metabolic characteristics are potentially due to differences in muscle morphology [22], adiposity [22], and sex hormones-- estradiol and testosterone [22, 23]. Some data shows that male and female glycogen re-synthesis rates are similar post-exercise regardless of differences in substrate utilization during exercise [24]. Even so, other evidence indicates that females may differ in their ability to maintain glucose homeostasis [22], and are not as sensitive to carbohydrate loading as males [19]. Additionally, data demonstrates women have lower protein oxidation

post-exercise, compared to their male counterparts [19]. Recommendations provided to females that are derived from data in males should be met with caution. Research exploring optimal nutrient timing strategies in females is warranted. Therefore, the primary purpose of the present study was to evaluate the effects of consuming a PRO-CHO supplement either pre- or post-workout, or not at all, for 6 weeks on chronic strength and body composition adaptations in resistance-trained females. The secondary purpose of this study was to analyze the effects of nutrient timing on acute and chronic metabolic adaptations following a 6-week training and nutrition intervention. We hypothesized that there would be no significant differences between the PRE and POST groups in chronic strength, body composition, and metabolism adaptations, but in regards to acute metabolic changes, PRE will utilize less CHO post-exercise.

## **Methods**

### *Participants*

Forty-eight resistance-trained females were enrolled in the study (Figure 3). Five participants dropped out due to scheduling conflicts/lack of time. Forty-three women (Mean  $\pm$  SD; Age:  $20.5 \pm 2.2$  yrs; Height:  $165.2 \pm 5.7$  cm; Weight:  $66.5 \pm 11.4$  kg; Body Mass Index:  $24.3 \pm 3.7$  kg/m<sup>2</sup>) finished the study and were included in the final statistical analyses. Participants were included in the study if they were a female between the ages of 18 and 30 years, had been resistance training a minimum of two times per week for the prior six months, and were not currently using a peri-workout protein supplement. Participants were excluded if they were pregnant, had an injury, had consumed creatine or other supplements within the previous month that may have influenced study outcomes, were 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. Prior to enrolling in the study, written informed consent was obtained from all participants, as well as a health history questionnaire and a urine pregnancy test.

### *Experimental Design*

Once enrolled, a urine pregnancy test was conducted and baseline measurements of body composition from a multi-compartment approach, and maximal strength were assessed (Figure 1). Participants were then randomized using a block matched-paired design into either a pre-workout (PRE), post-workout (POST), or control (CON) group (2:2:1) allocated based on baseline leg press strength. Following randomization, participants began the six week training and supplement intervention. At the first and last training sessions (V2 and V13), 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), completed twice per week. Participants in the PRE and POST groups were instructed to consume the protein-carbohydrate supplement within fifteen minutes before (PRE) or after (POST) the HIRT bout, respectively. Workouts were comprised of six exercises; completing three sets at 80% of the predicted maximum weight, aiming for 6-8 repetitions per set. All participants were told to refrain from eating for 1.5 hours before and after the workout bouts. Body composition and maximal strength were tested again at the final visit (V14).

### *Experimental Protocol*

#### Body Composition

Body composition was measured via dual energy X-ray absorptiometry (DEXA; GE Lunar iDXA, GE Medical Systems Ultrasound and Primary Care Diagnostics, Madison, WI, USA). Participants were asked to report to the laboratory a minimum of 8 hours fasted and having refrained from exercise the prior 24 hours. 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.). Height and weight were collected. Participants laid supine and were centered on the DEXA table. They were instructed to breathe normally but refrain from moving for the duration of the scan (approximately 7 minutes). The test-retest reliability for the DEXA from our lab for FM is as

follows: intra-class correlation coefficient (ICC)= 0.98 and standard error of mean (SEM)= 0.85 kg. For LM, ICC= 0.99 and SEM= 1.97 kg. For %fat, ICC= 0.96 and SEM= 1.279% [86].

### Maximal Strength Testing

Maximal strength was tested using one repetition maximums (1RM) for the bench press and leg press, and six repetition maximums (6RM) for accessory muscles. Prior to testing, to break the 8 hour fast, participants consumed a standardized protein shake containing 190 kilocalories, 5 grams of fat, 24 grams of carbohydrates, and 15 grams of protein (Special K, Milk Chocolate Protein Shakes, Kellogg's NA Co, Battle Creek, MI). For the 1RM tests, participants completed a 5-minute self-selected warm-up, were familiarized with the equipment, and practiced proper technique for each lift. 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, and two minutes of rest between attempts, until they reached a point of failure.

Multiple RM tests were used to predict participants' 1RM on four different accessory exercises. These exercises included an overhead shoulder press, a biceps curl, an overhead triceps extension, and an alternating stationary lunge, all using dumbbells. The research staff determined what weight participants began with for each exercise, based on the prior training of the individual. Participants did five repetitions with a light load to understand the form and to warm-up. After 1-2 minutes 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 done again at the end of the testing with a lighter or heavier weight, respectively. Participants were allowed two minutes of rest between each exercise. The amount of weight used (rep weight) and the number of repetitions completed until fatigue (RTF) were put in the following equation to predict participants' 1RM :

$$1\text{ RM} = \frac{\text{rep weight}}{0.522 + 0.419e^{-0.055 \cdot \text{RTF}}}$$

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

#### Resting Energy Expenditure and Respiratory Exchange Ratio

During the first and last training visits, REE and RER were assessed immediately before and immediately after the 30 minute HIRT bout. REE was measured for twenty-five minutes prior to the HIRT bout, and for 90 minutes after exercise. The 90 minutes of measurement was divided into three thirty-minute bouts to collect data at 30, 60, and 90 minutes post-exercise. For the pre-exercise REE test, participants laid supine under a clear hood. The first five minutes were discarded and the remaining time averaged. For the post-exercise REE testing, participants remained seated, wearing a gas mask that was connected by a tube to a metabolic cart. The last fifteen minutes of each time interval was averaged (ie. 15-30 min, 45-60 min, 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. Oxygen uptake and  $VCO_2$  were measured in liters per minute (L/min). REE, expressed in kilocalories per day (kcal/day), was calculated using Equation 4 below [81]:

$$\text{Equation 4: } REE = [(3.9 * (VO_2)) + (1.1 * (VCO_2))] * 1440 \text{ minutes}$$

To analyze substrate utilization before and after the training bout, RER was indirectly measured concurrently with REE using the metabolic cart. The following equation (Equation 5) was used to calculate RER, measuring  $VO_2$  and  $VCO_2$  in L/min [82]:

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

The resulting data was expressed as a value between 0.7 and 1.1 L  $CO_2$ /L  $O_2$ . Values around 0.7 L  $CO_2$ /L  $O_2$  indicated fat utilization while values close to 1.0 L  $CO_2$ /L  $O_2$  indicated carbohydrate utilization. Values around 0.82 L  $CO_2$ /L  $O_2$  indicated mixed substrate utilization.

### Salivary Estradiol

Before and after the training intervention, participants provided a 2.5-5.0 mL passive drool saliva sample to determine estradiol- $\beta$ -17 measurements using an ELISA assay (Salivary 17 $\beta$ -Estradiol Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA). Prior to each visit, participants were asked to refrain from dental work for a minimum of 48 hours and from brushing their teeth for at least 1 hour, to prevent the sample from containing blood. Participants rinsed their mouth with water 5-10 minutes 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 to maintain the integrity of the sample. This measurement was done to take estrogen variations into consideration when comparing REE and RER data, and was used as a covariate during data analysis.

### Questionnaires

At three time points during the intervention, participants were asked to complete a three-day dietary intake log and the General Practice Physical Activity Questionnaire (GPPAQ). Food logs and the GPPAQ were given at baseline testing, half-way through training (visit 7), and post-testing. The three-day dietary analysis, capturing two non-consecutive week days and one weekend day, was analyzed using a software (Food Processor; ESHA Research, Salem, OR). The GPPAQ measured the amount of 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. These surveys were completed to account for any variation in dietary or exercise habits that may have occurred during the six-week intervention.

### Supplement Intervention

Prior to the first training session, participants were randomized into one of three groups using Random Allocation Software (Isfahan, Iran) by matching baseline 1RM leg press values, in a 2:2:1 block. Participants were randomized to consume a liquid shake within 15 minutes prior to training (PRE), within 15 minutes post training (POST), or no food two hours before or after (CON). Research assistants timed participants and made sure that the beverage was finished within 10 minutes from the start of

consumption. The supplement that was used for the intervention contained 200 kilocalories, with 3.5 grams of fat, 16 grams of carbohydrates, and 25 grams of protein (Opti-fit Lean Protein Shake, Optimum Nutrition, Downers Grove, IL). This supplement was chosen for the ratio of protein to carbohydrates (2:1), as well as a level of protein within the optimal anabolic range (which is 20-40g) [8]. Participants and research staff were not blinded to the treatment groups; participants were blinded to the supplement label.

### Training Intervention

Throughout the duration of the training (visits 2 through 13), participants completed twelve 30 minute HIRT sessions, adapted from Paoli et al [14] and previously described [6]. Participants reported to the lab a minimum of 1.5 hours fasted prior to each training session. Free weights were used to perform six different exercises. The exercises were completed in the order of: leg press and bench press (York Barbell Co., York, PA, USA), dumbbell (DB) lunges, DB shoulder press, DB biceps curls, and DB overhead triceps extensions. Before beginning the training session, participants were allowed to complete a self-selected 5-minute warm-up. Three sets of each exercise were completed at a 6RM to 8RM, with 20 to 30 seconds of rest between sets [6]. The research staff spotted participants when necessary and made sure that proper form was being used. Participants were allowed 2 minutes of rest between exercises [14]. The amount of weight used and repetitions completed per set were recorded during each training session for progression. If participants could perform 8 or more repetitions on all three 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. If necessary, assistance was given on the last few repetitions of accessory exercises in order to prevent instability. Heart rate (using the Polar heart rate monitor) and rate of perceived exertion [83] were also recorded after completion of each set. HIRT bouts took approximately 30 minutes to complete. Subjects had a minimum of 24-48 hours of rest between training sessions. Training volume (load  $\times$  reps) was determined by adding together cumulative volume across all training days.



### *Statistical Analyses*

The Shapiro-Wilk test was used to assess for normality of data. To examine baseline differences, a series of one-way ANOVAs were done to compare groups. For chronic body composition and strength adaptations, a series of  $3 \times 2$  repeated measures ANOVAs (group  $\times$  time) were completed. Metabolic data, including both acute (pre to post workout) and chronic changes (pre to post intervention), were examined using a series of  $3 \times 2$  repeated measures ANCOVAs (group  $\times$  time), with salivary estradiol as the covariate. Training volume was evaluated via ANOVA. Lastly, a series of  $3 \times 3$  repeated measures ANOVAs (group  $\times$  time) were used to evaluate changes in dietary and physical activity variables. If a significant interaction was found, separate Bonferroni-corrected paired samples t-tests were completed. 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).

### **Results**

All demographic data are presented in Table 1; all data were normally distributed. There were no significant between-group baseline differences for any variables ( $p > 0.05$ ).

#### *Body Composition*

For FM there was no significant interaction ( $p = 0.777$ ,  $\eta^2 = 0.013$ ), and no main effect for group ( $p = 0.300$ ,  $\eta^2 = 0.058$ ). There was a main effect for time ( $p = 0.019$ ,  $\eta^2 = 0.130$ ). Post hoc analyses demonstrated no significant changes in FM for PRE ( $p = 0.081$ ), POST ( $p = 0.098$ ), or CON ( $p = 0.328$ ) (Table 2). For LM, there was no significant interaction ( $p = 0.249$ ,  $\eta^2 = 0.067$ ), no main effect for group ( $p = 0.425$ ,  $\eta^2 = 0.042$ ), but there was a main effect for time ( $p = 0.003$ ,  $\eta^2 = 0.201$ ). Both PRE and POST groups demonstrated a significant increase in LM ( $p = 0.011$  and  $p = 0.002$ , respectively), with no change in CON ( $p = 0.745$ ). Similarly, for %fat, there was no significant interaction ( $p = 0.418$ ,  $\eta^2 = 0.043$ ), or main effect for group ( $p = 0.096$ ,  $\eta^2 = 0.111$ ). There was a significant main effect for time ( $p < 0.0005$ ,  $\eta^2 = 0.276$ ); significant decreases were observed for only the PRE and POST groups ( $p = 0.007$  and  $p = 0.014$ , respectively), with no change for CON ( $p = 0.181$ ).

### *Maximal Strength*

For LPmax, there was no significant interaction ( $p=0.422$ ,  $\eta=0.042$ ), and no main effect for group ( $p=0.199$ ,  $\eta=0.078$ ). There was a significant main effect for time ( $p<0.0005$ ,  $\eta=0.837$ ). Further post hoc analyses demonstrated significant changes for all groups over time ( $p<0.0005$  for all groups) (Table 3). For BPmax, there was a significant interaction effect ( $p=0.017$ ,  $\eta=0.184$ ). When decomposed, post hoc analyses demonstrated significant increases in strength for all three groups (PRE and POST:  $p<0.0005$ , CON:  $p=0.003$ ), with no significant between-group differences ( $p=0.083$ ,  $\eta=0.117$ ).

### *Metabolic Data*

Acute Effects: For REE, there was a significant interaction effect ( $p=0.005$ ,  $\eta^2=0.164$ ). There was a main effect for REE over time ( $p<0.0005$ ,  $\eta^2=0.367$ ). Pre-exercise REE (PREEx) was significantly lower than all other timepoints ( $p<0.0005$ ). Thirty minutes post-exercise REE (30POST) was significantly higher than PREEx ( $\Delta$ :  $275.4 \pm 48.8$  kcal,  $p<0.0005$ ), and lower than sixty minutes post-exercise (60POST,  $\Delta$ :  $-59.0 \pm 40.6$  kcal,  $p=0.037$ ), and ninety minutes post-exercise (90POST,  $\Delta$ :  $-83.0 \pm 53.4$  kcal,  $p=0.021$ ). There were no significant between group differences for REE ( $p=0.093$ ), or estradiol levels ( $p=0.660$ ). For RER, there was a significant interaction effect ( $p=0.028$ ,  $\eta^2=0.123$ ). There was a significant main effect for RER over time ( $p<0.0005$ ,  $\eta^2=0.241$ ), but no significant between groups differences ( $p=0.392$ ). Post hoc analyses revealed significant differences between every time point ( $p<0.0005$ ), except for 60POST and 90POST. PREEx was significantly higher than all other time points (30POST:  $\Delta 0.08 \pm 0.01$  a.u., 60POST:  $\Delta 0.05 \pm 0.02$  a.u., 90POST:  $\Delta 0.04 \pm 0.01$  a.u.). 30POST was significantly lower than all other timepoints (60POST:  $\Delta -0.03 \pm 0.01$  a.u., 90POST:  $\Delta -0.04 \pm 0.01$  a.u.). There was no significant difference between 60POST and 90POST ( $p=0.169$ ).

Chronic Effects: When chronic change scores for each metabolic time point were analyzed, there was no significant interaction for REE ( $p=0.875$ ), or main effects for group or time ( $p=0.569$  and  $p=0.616$ , respectively). While nonsignificant, the PRE and POST groups had greater mean increases in REE for each measured time point relative to CON (Table 4). There was no significant interaction for RER ( $p=0.219$ ), nor main effect for time ( $p=0.590$ ). There was a significant main effect for group

( $p=0.044$ ,  $\eta^2=0.156$ ). Pairwise comparisons revealed that the PRE group demonstrated a non-significant greater mean decrease in RER ( $\Delta$ :  $-0.051 \pm 0.024$  a.u.), compared to POST ( $p=0.087$ ;  $\Delta$ :  $-0.011 \pm 0.026$  a.u.) from pre to post training.

### *Training Volume*

Training volume (load  $\times$  total reps) was summed for all twelve training visits for each participant. There were significant between groups differences ( $p=0.002$ ,  $\eta^2=0.262$ ). The POST group had a significantly lower average total volume than the PRE group (Mean difference  $\pm$  SD:  $-19093.1 \pm 12094.8$  kg,  $p=0.009$ ), as well as the CON group ( $-23137.9 \pm 14536.2$  kg,  $p=0.008$ ). The average total volumes for the PRE and CON groups were not significantly different ( $-4044.8 \pm 14536.2$  kg,  $p=1.000$ ).

### *Questionnaires*

There was no significant interaction effect for group or GPPAQ score over the course of the intervention ( $p=0.242$ ). There were no significant main effects for either time or group ( $p=0.548$  and  $p=0.546$ , respectively).

## **Discussion**

The aim of the current study was to analyze the effects of nutrient timing, specifically protein and carbohydrates, on body composition, strength, and metabolism in resistance-trained females, over the course of six weeks of HIRT. The results of this study found no significant differences between consuming nutrients prior to exercise versus after an exercise bout when evaluating chronic strength and body composition adaptations. When analyzing group differences individually, both PRE and POST nutrient timing groups demonstrated significant increases in LM and %fat, while the control group demonstrated no change. All three groups experienced significant changes in both upper and lower body strength outcomes. Furthermore, there were no significant between group differences in acute and chronic metabolic changes. These findings are similar to those published by Schoenfeld et al. [14], reporting that the use of protein pre or post workout produced similar adaptations in FM and lower body strength in trained men. This study, to our knowledge, is the first nutrient timing study to be done in females evaluating which timing strategy is most optimal.

Prior research has demonstrated that peri-workout nutrient intake is beneficial for stimulating protein synthesis [15, 87]. The goal of timing nutrient consumption is to encourage growth, which would contribute to an increase in LM. Cribb et al. [9] reported that using PRO, CHO, and creatine monohydrate peri-workout was more advantageous for improved LM and %fat, compared to consuming the same macronutrients in the morning and evening in male bodybuilders. Similar to those results, participants in the present study demonstrated an increase in lean mass and decrease in %fat over time in both PRE and POST groups. The present results contradict what would be expected based on findings by Tipton et al. [15], which demonstrated that PRE ingestion of essential amino acids and CHO was superior to POST in stimulating acute muscle protein synthesis; yet LM was not a reported outcome. One likely reason for this contrast is that acute PRO synthesis may not necessarily translate to chronic body composition adaptations, such as increases in LM, due to other interacting factors. Furthermore, other studies have confirmed that while peri-workout PRO supplementation enhances increases in LM in young healthy subjects [88], there may be no difference between PRE and POST adaptations [14].

Beyond body composition adaptations, it is thought that peri-workout PRO and CHO may contribute to acute and chronic increases in performance. When evaluating the combined effects of PRO or placebo with 10 weeks of resistance training, Willoughby et al. [76] reported that both PRO and placebo groups significantly increased in leg and bench press strength with the PRO group having experienced greater adaptations than the placebo. While no other studies to our knowledge have examined different peri-workout training strategies in females, prior research has examined the effects of timing in various male populations. Schoenfeld et al. [14] and Candow et al. [89] evaluated the effects pre- versus post-exercise PRO consumptions in trained young men and untrained older men, respectively. Similar to our results, both studies found that neither timing strategy was superior for increasing maximal strength. Specifically, Schoenfeld et al. [14] found that the average squat 1RM increased by 3.8% in the PRE group and 5.5% in the POST group. The average bench 1RM increased by 1.6% in the PRE group and 3.4% in the POST group. In the present study the average LPmax increase was 32.2%, 35.3%, and 32.8% in the PRE, POST, and CON groups, respectively. The average BPmax increase was 14.5%,

14.6%, and 6.2% in the PRE, POST, and CON groups, respectively. These results demonstrate that the high intensity nature of the training intervention, despite using trained females, was more a greater stimulus than when nutrients were provided. Furthermore, as established in our control group results, Candow et al. [89] found no between group differences when comparing the supplement groups to the placebo group. Similar to body composition adaptations, this may be due to other factors beyond stimulation of acute PRO synthesis influencing strength and performance adaptations. Specifically, the training protocol used in the current study has been shown to be quite effective [90]; the progressive high-intensity nature of the protocol was likely novel, resulting in improvements in all three groups. This stimulus, combined with other factors such as sleep habits [91], PRO intake and overall dietary habits outside of the workout window [92], and other training that occurred outside of the study intervention, may have contributed to the lack of between-group differences.

The effects of peri-workout nutrient timing have been explored in a number of studies; minimal data exists in women. It is well established that many factors that can differentiate the ways in which males and females adapt to training, including the hormonal response to exercise [93], differences in PRO metabolism [21], and muscle characteristics [55]. Among the available nutrient timing evidence, three have specifically addressed pre- versus post-workout nutrition, two have evaluated the effects in male populations [14, 89], and one used a mixed sample [15]. Despite the little data in women, nutrition recommendations are made for women are based on data largely evaluated using men; leading to potentially sub-optimal nutrition strategies. Prior research has shown that males and females differ in their metabolic response to exercise [19]. Specifically, while data have demonstrated a potential beneficial effect on RER for males training in the fasted state [94], other studies have indicated that fasted exercise may actually blunt fat oxidation in females [19]. Additionally, prior literature states that there is no difference between fed and fasted energy expenditure response to exercise, but the type of macronutrient consumed may influence REE [6]. While nonsignificant, our results demonstrated that the PRE and POST consumption maintained a higher REE up to 90 minutes post-exercise, compared to CON. From PREEx to 90POST, REE was  $352 \pm 211$  kcal higher in the PRE group,  $460 \pm 154$  kcal higher in the

POST group, and  $265 \pm 215$  kcal higher in the CON group. This not only counters the data that has been previously collected in male populations, showing no difference between energy expenditure after exercise in the fed versus postprandial state [85], but there may be physiological value in the higher REE seen in the PRE and POST groups. Our chronic RER data tended to agree with previously studied sex based substrate metabolism differences, with postprandial and trained females shifting to greater fat oxidation [36, 95]. While nonsignificant, the PRE group demonstrated a greater reliance on fat compared to the POST group ( $\Delta$ :  $-0.051 \pm 0.024$  a.u., versus  $-0.011 \pm 0.026$  a.u.). Similar to REE, there is potential for this to have physiological importance including promoting weight reduction [96].

Further research is required to fully understand potential differences between the ways in which trained males and females respond to training stimuli and nutrient timing. It has been well established that males and females respond similarly when first beginning resistance training [64, 97], with females potentially experiencing greater increases in strength than males [98]. Even so, there comes a point in development where females do not experience the same adaptations as men. One basis for this is the differences in PRO metabolism between males and females, with males potentially having greater expression of positive PRO balance leading to greater muscle mass [21]. Furthermore, males and females differ in their hormonal responses to resistance training, particularly related to testosterone [93], which has a significant effect on both hypertrophic and strength adaptations. Lemmer et al. [99] examined acute changes in RMR in older men and women versus younger men and women, and found that while older and younger men experienced elevated RMR post-resistance training, younger and older women did not. Over time, differences in RMR response could potentially translate to body composition adaptations, or the lack thereof. Due to these differences, data in trained men cannot always be extrapolated to recommendations for trained women to increase strength, improve body composition, and optimize their metabolism. Beyond the need for specific training data; evidence for dietary strategies in women to maximize hormonal and hypertrophic responses is needed. The present study demonstrated that the use of peri-workout nutrients paired with a training stimulus for 6 weeks, is enough to assist in increasing LM by 1 kg and decreasing %fat by 1%, while also increasing upper and lower body strength in trained

women. Despite no significant differences between groups, consuming nutrients either before or after training is likely more effective than fasting around exercise.

There were limitations in this study that need to be addressed. Foremost, the nutrition and activity of participants outside of the study was not strictly controlled for; physical activity was tracked using the GPAQQ and demonstrated no differences between groups. Nutrient intake was tracked from diet logs. A subset of diet logs were evaluated with no significant changes in kilocalories ( $p=0.085$ ), PRO ( $p=0.654$ ), CHO ( $p=0.060$ ), or fat intake ( $p=0.694$ ), over the course of the intervention. Additionally, while training history was documented and six months of lifting was considered sufficient, the type of resistance training that participants had engaged in prior to enrolling in the study varied between sports conditioning, powerlifting, Olympic lifting, and recreational lifting. There was no washout period for the training participants were doing prior to the study, and no familiarization training phase to make sure that all participants were accustomed to the high intensity nature of the protocol. This could have potentially caused some increases in strength due to neural adaptations if participants were not accustomed to lifting at 80% or more of a projected max. Furthermore, we could only use verbal affirmation to make sure subjects came ninety minutes fasted to training and remained fasted for ninety minutes post-training. In regards to the supplement, all participants were given the same amount of PRO and CHO (25 grams and 17 grams, respectively). It may have been more beneficial to base PRO intake off of each participant's LM, as has been shown in previous research, but may reduce practicality [26, 27]. Furthermore, this study did not examine whether more CHO would benefit female performance adaptations, or if a 1:2 CHO:PRO supplementation ratio was enough. Recommendations based on prior data suggest consuming 2-4 grams CHO to 1 gram PRO peri-workout [3], but the majority of this research is in men and uses endurance training rather than resistance training. Due to the tendency for women to rely upon fat utilization to a greater extent than men [19], more research should be done on the optimal amount of CHO to consume peri-workout to optimize both performance and body composition.

## CHAPTER V: CONCLUSION

This study aimed to analyze whether pre- or post-exercise nutrition was more optimal in encouraging chronic body composition and strength adaptations, as well as acute and chronic metabolic changes. The results demonstrate that neither timing strategy is superior in stimulating body composition or strength adaptations, however, PRE nutrient timing may encourage advantageous metabolic adaptations over time, increasing caloric expenditure and enhancing fat oxidation, compared to POST. Additionally, while all three groups demonstrated significant increases in strength, the CON group did not experience significant changes in LM or %fat, while both the PRE and POST groups did. The timing of nutrient consumption peri-workout is one of many factors that interact to initiate adaptations, including the training stimuli, overall daily nutrition and PRO intake, hormonal milieu, and appropriate recovery. It is likely that the anabolic window for nutrient timing is not limited to pre- or post-training, but that the two interact. Therefore, while exact timing can be left to individual preference, it is best to ensure that PRO and CHO are consumed either before or after the training bout. In recent literature [14], the anabolic window was described as being more like an anabolic “door”, where if nutrients are consumed pre-workout, there is a larger time buffer post-workout for an individual to wait to eat. Similarly, if nutrients are not consumed pre-workout, it would be better to ensure they are consumed close to when the workout bout is concluded. Further research is needed in females to better understand how nutrition recommendations, including timing of nutrients and optimal macronutrient ratios, may differ from current recommendations that have only been studied in male or mixed populations. Specifically, data in trained females is needed to better understand why progress falls behind that of trained males after a certain point in development, as well as what factors can be altered to stimulate progression of strength, body composition adaptations, and recovery.



## TABLES

Table 1: Demographic data by treatment group (Mean  $\pm$  SD).

Group	Age (yrs)	Height (cm)	Baseline Weight (kg)	Baseline BMI (kg/m <sup>2</sup> )
PRE (n=17)	20.4 $\pm$ 2.2	164.7 $\pm$ 6.2	68.5 $\pm$ 10.7	25.2 $\pm$ 3.6
POST (n=17)	20.7 $\pm$ 2.3	165.7 $\pm$ 6.5	65.6 $\pm$ 11.8	23.8 $\pm$ 3.2
CON (n=9)	20.1 $\pm$ 2.1	165.0 $\pm$ 2.8	64.1 $\pm$ 12.7	23.6 $\pm$ 4.8

Table 2: Body composition changes for fat mass (FM), lean mass (LM), and percent body fat (%fat) by group (Mean  $\pm$  SD).

Group	$\Delta$ FM (kgs)	$\Delta$ LM (kgs)	$\Delta$ %fat
PRE	-0.5 $\pm$ 1.2	1.0 $\pm$ 1.4*	-1.1 $\pm$ 1.4*
POST	-0.4 $\pm$ 1.0	0.6 $\pm$ 0.7*	-0.7 $\pm$ 1.0*
CON	-0.2 $\pm$ 0.7	0.2 $\pm$ 1.4	-0.4 $\pm$ 1.0

\*indicates a significant change over time ( $p < 0.05$ ).

Table 3: Maximal strength change for maximum leg press (LPmax), bench press (BPmax), and average training volume for each treatment group (Mean  $\pm$  SD).

Group	$\Delta$ LPmax (kgs)	$\Delta$ BPmax (kgs)	Average Training Volume (kgs)
PRE	67.6 $\pm$ 30.5*	5.6 $\pm$ 2.8*	82447.2 $\pm$ 18229.5
POST	55.3 $\pm$ 23.5*	4.8 $\pm$ 2.5*	63354.1 $\pm$ 14321.9
CON	62.9 $\pm$ 26.8*	2.5 $\pm$ 1.8*	86491.9 $\pm$ 21895.7

\*indicates a significant change over time

Table 4: Average acute change in resting energy expenditure [REE (kcal/day)] for pre-exercise (PREEx), 30 minutes post exercise (30POST), 60 minutes post exercise (60POST) and 90 minutes post-exercise (90POST). (Mean  $\pm$  SD).

Group	$\Delta$ PREEx (kcal)	$\Delta$ 30POST (kcal)	$\Delta$ 60POST (kcal)	$\Delta$ 90POST (kcal)
PRE	125.8 $\pm$ 52.8	86.9 $\pm$ 84.4	99.7 $\pm$ 115.4	129.6 $\pm$ 114.8
POST	103.2 $\pm$ 57.8	135.0 $\pm$ 92.4	134.7 $\pm$ 126.4	137.5 $\pm$ 125.8
CON	30.1 $\pm$ 75.6	97.6 $\pm$ 121.2	37.0 $\pm$ 165.6	77.4 $\pm$ 164.6

#Data is presented as adjusted mean when covaried for estradiol levels.

## FIGURES

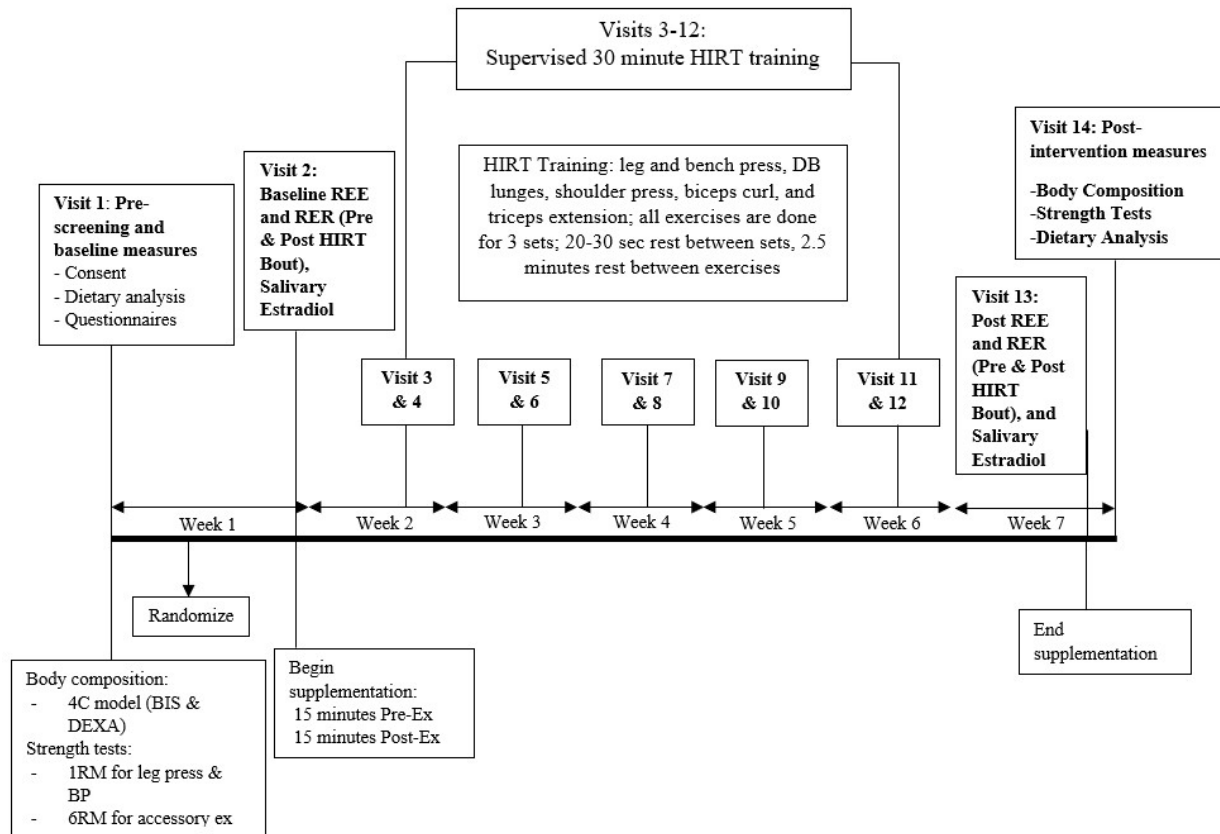


Figure 1: Experimental protocol schematic.

Serving Size 2 Scoops (52g)		
Servings Per Container 16		
<b>Amount Per Serving</b>		
Calories	200	
Calories from Fat	30	
	<b>% Daily Value **</b>	
Total Fat	3.5 g	5%
Saturated Fat	1.5 g	8%
Trans Fat	0 g	
Cholesterol	75 mg	25%
Sodium	190 mg	8%
Potassium	290 mg	8%
Total Carbohydrate	16 g	5%
Dietary Fiber	6 g	24%
Sugars	3 g	
Protein	25 g	50%
Vitamin A	20%	
Vitamin C	50%	
Calcium	40%	
Iron	30%	
Vitamin D	25%	
Vitamin E	20%	
Thiamin	20%	
Riboflavin	15%	
Niacin	20%	
Vitamin B6	20%	
Folic Acid	20%	
Vitamin B12	20%	
Biotin	20%	
Pantothenic Acid	20%	
Phosphorus	30%	
Iodine	15%	
Magnesium	30%	
Zinc	20%	
Selenium	20%	
Copper	20%	
Manganese	20%	
Chromium	20%	
Molybdenum	20%	

Figure 2: Supplement facts panel for treatment used in the PRE and POST supplementation groups.

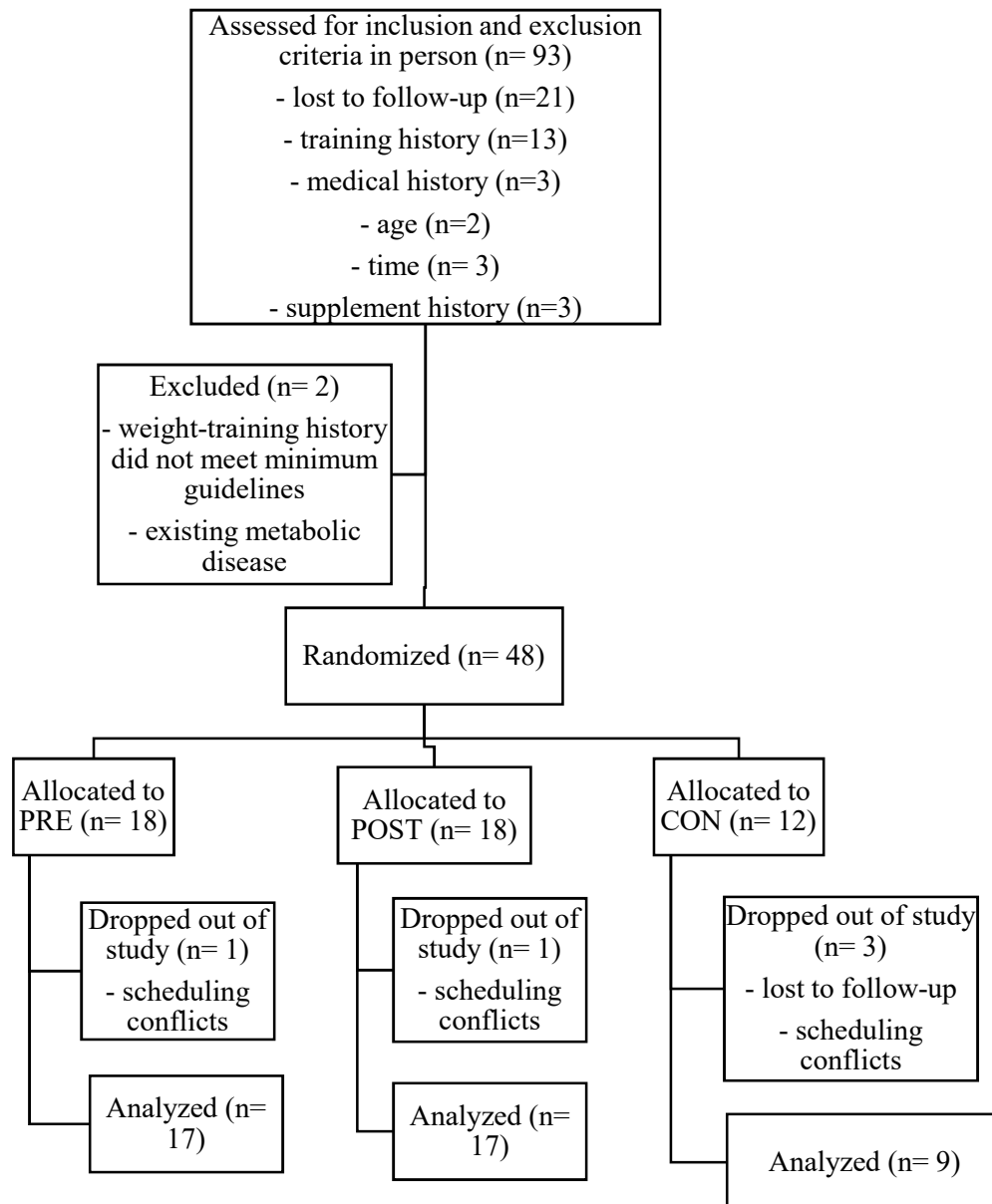


Figure 3: CONSORT (Consolidated Standards of Reporting Trials) diagram.

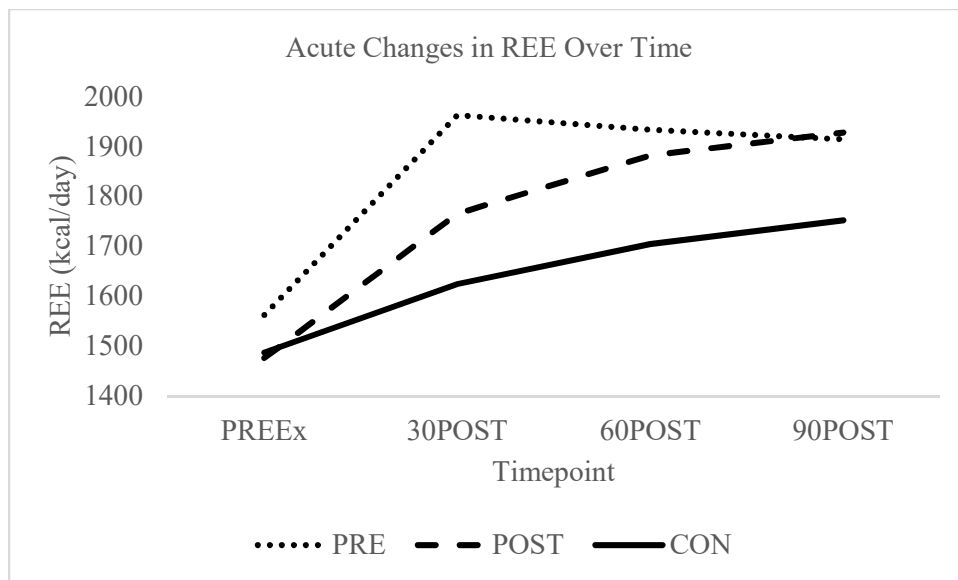


Figure 4: Acute changes in REE from pre-exercise to 90 minutes post-exercise (kcal/day).



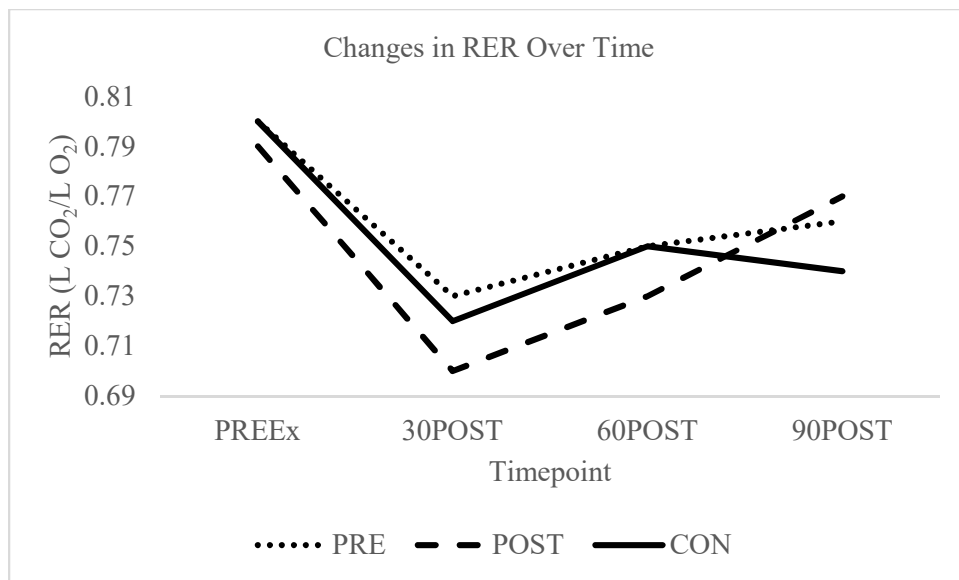


Figure 5: Acute changes in RER from pre-exercise to 90 minutes post-exercise (L CO<sub>2</sub>/L O<sub>2</sub>).

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