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*Original Research*

# Training Status Impacts Metabolic Response to A High-Protein Weight Loss Diet in Recreationally Resistance-Trained Females

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ABSTRACT

***International Journal of Exercise Science 16(2): 377-392, 2023.*** This study investigated the effects of a novel high-protein diet template on postprandial metabolism and body composition (e.g., waist and hip circumference, body fat (%), fat mass, fat-free mass) in recreationally resistance-trained females. Fifteen females adhered to an eight-week high-protein dietary intervention (~1.5-1.6 g·kg$^{-1}$·day$^{-1}$) administered via template format. Pre- and post-intervention visits included anthropometrics, measurement of body composition, and an acute high-fat meal challenge. The high-fat meal challenge (61% fat) consisted of fasting postprandial blood glucose, resting metabolic rate (RMR), fat and carbohydrate oxidation assessed at 60-, 120-, and 180-minutes. Participants were split into high (HTF; 5-6 days ·week$^{-1}$ of resistance training; $n$ = 8) and low-training frequency (LTF; 2-3 days ·week$^{-1}$ of resistance training; $n$ = 7) groups. All metabolism data were assessed as absolute (kcal or g) and relative (kcal or g·kg·FFM$^{-1}$·minutes$^{-1}$) to fat-free mass. Post-intervention, there was a significant reduction in HTF waist circumference ($p$ = 0.044), LTF body fat % ($p$ = 0.012), and LTF fat mass ($p$ = 0.014). Post-intervention, HTF females had significantly lower absolute RMR area under the curve (AUC) than LTF females ($p$ = 0.036). LTF females had higher absolute fat oxidation AUC compared to HTF females' pre-intervention ($p$ = 0.048) but a significant decrease in absolute ($p$ = 0.050) and relative ($p$ = 0.050) fat oxidation AUC post-intervention. LTF females had a significant increase in absolute ($p$ = 0.032) and relative ($p$ = 0.029) carbohydrate oxidation AUC pre- to post-intervention ($p$ = 0.032). For blood glucose, no significant differences between groups were detected ($p$ > 0.05). These findings suggest that a novel high-protein diet template elicits a metabolic shift favoring carbohydrate oxidation in females engaging in low-frequency resistance training but did not alter fat and carbohydrate metabolism in females engaging in HTF resistance training.

KEY WORDS: Metabolic flexibility; fat oxidation; high-protein diet; dietary template

## INTRODUCTION

Being overweight or obese is associated with metabolic irregularities that exacerbate chronic disease risk (36, 37). Decreased physical activity, low-quality energy-dense high-fat food consumption, and increased weight are associated with metabolic abnormalities, including improper substrate utilization (12, 23). The inability to oxidize available substrates in response to dietary intake is known as metabolic inflexibility (10, 15, 33, 40). A state of chronic nutrient

oversupply increasing weight results in metabolic inflexibility and is associated with dyslipidemia, insulin resistance, and hyperglycemia (14, 33). Chronic exposure to elevated levels of body fat induces changes at multiple levels, potentially originating at the substrate utilization/storage level, ultimately leading to tissue and organ pathology that increases the risk of chronic disease (33, 37).

Regular exercise training enhances metabolic flexibility (22) through improvements in insulin sensitivity (20) and skeletal muscle regulation of fat oxidation (e.g., mitochondrial biogenesis, content, and function) (11) and improves downstream benefits of reducing chronic disease risk (3). Seven days of aerobic exercise improves metabolic flexibility in lean and obese men compared to baseline (4). Furthermore, increased physical activity levels are associated with greater metabolic flexibility, towards fat oxidation, throughout the day (4, 6, 18, 25, 42). Interestingly, reports on the impact of fitness status on metabolic flexibility among females are limited. Bowden & McMurray found when given a high-fat meal, no differences in resting metabolic rate or respiratory exchange ratio were observed in high aerobically trained vs. low aerobically trained females (8). However, substrate partitioning of respiratory data was not calculated. Additionally, while the role that aerobic fitness status plays on fat oxidation and metabolic flexibility demonstrates a favorable response to exercise training (6), little is known about the influences of resistance training on metabolic flexibility, specifically in females. Resistance training is an important component of physical activity for females due to improved glucose regulation, increased muscle mass, bone density protection, and elevated resting metabolism (26, 27). However, it is unknown if resistance training elicits the same benefits as aerobic regarding improvements in metabolic flexibility.

Approaches to improve metabolic flexibility should utilize a combination of exercise and dietary changes that improve overall activity status and body composition (e.g., waist and hip circumference, body fat (%), fat mass, fat-free mass) (6, 40). High-protein diets combined with resistance exercise may be one strategy to reduce body fat without muscle loss or negatively influence metabolic flexibility (9). The Dietary Reference Intakes for dietary protein for all individuals is 0.8 g protein.$kg^{-1}$.$d^{-1}$, however, there is consistent evidence that suggests individuals engaging in resistance training require more protein (35). Individuals consuming a high-protein diet retain lean mass, maintain basal metabolic rate, decrease body weight, increase triglyceride modulation, and increase glucose regulation and insulin response to feeding (24, 26-28, 30, 38). A high-protein diet may also be especially necessary for resistance training individuals, where adequate protein intake can support the development or retention of skeletal muscle mass (35). Due to this, higher protein diets have been adopted in these populations and are defined as much as 30%-35% of total daily intake coming from protein (35). Despite this, finding feasible and consumer-available dietary interventions that consider retention of skeletal muscle mass in resistance-trained females is limited. Online commercially available dietary interventions have recently become available; however, their feasibility has yet to be studied. Furthermore, the impact of high-protein diets on metabolic flexibility in recreationally resistance-trained females is unknown. Therefore, this study aims to investigate the effects of a

high-protein diet template on metabolism and body composition measures in recreationally resistance-trained females.

## METHODS

A quasi-experimental design was used in which each participant served as their own control. Participants underwent an 8-week dietary intervention administered electronically in a template format and pre- and post-intervention testing sessions that included anthropometrics and resting and postprandial metabolism measurements. The study was approved by the University of Georgia Institutional Review Board (study no. 5229), with written informed consent being obtained before any experimental procedures. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database. This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (34).

*Participants*

Participants were recruited via word of mouth and flyers from the Athens, Georgia, area. Potential participants completed a screening questionnaire to determine study eligibility. The study's inclusion criteria included the following: 1) the participant must be a premenopausal woman, 2) resistance-trained (at least two days per week for the past six months) and did not exceed the physical activity guidelines for aerobic exercise (150 min·week$^{-1}$), 3) free from injury, 4) not taking medications that interfere with metabolism, 5) a body mass index between 18.5-34.9 kg·m$^2$, and 6) free of any history of cardiovascular, metabolic, musculoskeletal disease or illness. Exclusion criteria included pregnancy, trying to become pregnant or breastfeeding, or were pregnant < 10 months prior to the start of the intervention. Participants were allowed to take or use contraceptives if they reported no underlying hormonal issues and a regular menstrual cycle. Females not taking oral contraceptives were included if they reported having a regular monthly menstrual cycle for the 12 months prior to starting the study. If a potential participant reported supplement use, they were instructed to follow a 2-week washout phase before testing. No participants reported supplementation use; thus, no washout period was warranted.

Participants were split into two groups based on self-reported exercise training frequency and were asked to maintain this exercise training frequency throughout the study. High-training frequency (HTF) was defined as ≥ 5 days ·week$^{-1,}$ and low-training frequency (LTF) was defined as 2-3 days ·week$^{-1}$ of resistance training.

*Protocol*

Participants underwent identical pre-intervention (week zero) and post-intervention (week eight) testing sessions that included anthropometric measures and an acute high-fat meal challenge (as described below). Participants were asked to log their normal diet intake for five days prior to the intervention and to begin the dietary intervention within 2 days of pre-intervention testing. Post-intervention testing occurred within 3 days of ending the 8-week dietary intervention, and participants remained on the diet until its completion.

Prior to the testing session, participants were instructed to eat a recommended dinner consisting of 30% predicted resting energy expenditure (50% carbohydrate, 30% fat, and 20% protein) (32). Participants were given guidance on food selection to meet the prescribed energy content and macronutrient composition for this meal. Participants arrived at the laboratory the next morning after a 10-12 hour overnight fast. Participants were asked to abstain from physical activity and alcohol consumption for at least 24 hours before the testing session. Participants' height, weight, waist, and hip circumferences were assessed. Participant body fat (%), fat mass, fat-free, visceral adipose tissue, and bone mineral density were assessed via dual-energy X-ray absorptiometry (DEXA; Horizon® DXA System, Hologic, Inc., Marlborough, MA). After, participants rested in a supine position on a bed while their blood glucose (OneTouch® UltraMini®, LifeScan, Inc., Milpitas, CA) and resting metabolic rate (RMR) using indirect calorimetry were measured via metabolic cart (TrueOne 2400, Parvo Medics, Sandy, UT). Participants then underwent a high-fat meal challenge where they consumed a single high-fat meal (HFM) [1127 (132) kcals, 74.6 (4.7) g fat, 82.5 (5.2) g carbohydrate, and 27.1 (1.7) g protein]. The high-fat meal was individualized based on body surface area, ensuring each participant consumed the prescribed macronutrient profile (61% fat, 29% carbohydrate, 10% protein). The HFM consisted of heavy whipping cream (225 g), dry milk (69 g), and chocolate syrup (78g) per $m^2$ of body surface area (21). After finishing the test meal, participants were allowed to drink water ad libitum. Blood glucose and RMR were measured at 60, 120, and 180 minutes postprandial.

Each RMR (kcal·day$^{-1}$) measurement was recorded for 20 minutes. Subjects were instructed to remain motionless without sleeping while respiratory gases were collected. Twenty minutes of respiratory gases were collected, but only the final 15 minutes of data were used to calculate RMR using the Weir equation (44) and macronutrient oxidation using equations developed by Frayn (13): fat (g·minutes$^{-1}$) = (1.67*$VO_2$ (L·minutes$^{-1}$)) – (1.67*$VCO_2$ (L·minutes$^{-1}$)) and carbohydrate (g·minutes$^{-1}$) = (4.55*$VCO_2$ (L·minutes$^{-1}$)) – (3.21*$VO_2$ (L·minutes$^{-1}$)). The first 5 minutes of each test were discarded to ensure the analysis of steady-state metabolic data.

Participants tracked their dietary intake by hand in an Excel spreadsheet provided by the study team or with an electronic application on their phone so that total energy and macronutrient composition could be quantified. Participants were taught how to use the dietary template and were provided with digital and written materials with instructions. They were asked to maintain their resistance training routine throughout the intervention. During the 8-week intervention, study team members used weekly check-ins via email to help participants navigate the dietary template experience. These check-ins included collecting participants' weekly dietary logs, self-reported dietary adherence, and answering any questions they may have had pertaining to the diet, such as logging, tracking, measuring, or reporting food intake correctly. These check-in's also included participant self-report of weekly resistance training frequency (total days lifting). If participants did not reply to the weekly check-in, the team followed up within 48 hours. If participants failed to respond to the study team, they were removed from the study and did not complete post-testing. All dietary logs were assessed using the United States Department of Agriculture National Nutrient Database for Standard Reference (1) by a blinded study team

member quantified pre-intervention and weekly dietary logs. Total calories, protein, carbohydrate, fat, and snack calories were quantified each day and averaged for each week.

The commercially available dietary templates focused on a high-protein intake, increased food quality, and meal timing around exercise sessions. The dietary template was provided as an Excel (Microsoft Corporation, Redmond, WA) based document (Supplemental Material 1). Daily protein was set to be ~1.6-1.7 g·kg $BW^{-1}$·$day^{-1}$. The total dietary intake for the dietary template was ~30% protein, ~40% carbohydrate, and ~30% fat. Each template gave meal macronutrient splits for both 3 and 4 meals·$day^{-1}$; meals were split so that the total daily intake was the same regardless of meal frequency. Participants were encouraged to eat their first meal upon waking within 1.5 hours and the regularly across the day every 4-6 hours. Each meal was to be comprised of a lean protein source, vegetables, fruits and/or grains, and healthy fats. Participants were instructed to eat the desired grams of each macronutrient (e.g., 25 g of protein), not the total scale weight of food (e.g., 25 g of chicken) such as the total weight of the item did not matter if the desired macronutrient target was obtained. The dietary template also provided information about how to estimate serving size based on commonly used items, i.e., a deck of cards or the palm, if a scale was unavailable. Vegetable servings were listed in handfuls or cups (e.g., 1 small handful or about 1 cup) for each meal. Each template also included a set amount of non-macro-specific snack calories to be used towards any food at any time. Snacks were optional and could be skipped and saved upwards of three days to use on a larger meal or snack later in the week based on the participant's preference (e.g., 250 kcals * 3 days = 750 "free calories" on day three) to ensure calorie balance across the week.

All macronutrient categories on the template included a list of acceptable food items to select from when making meals that met the template's criteria. Protein sources were identified as any animal protein source with less than 35% of total calories from fat, soy, or any seafood. Carbohydrate source suggestions were primarily unrefined grains (e.g., quinoa, rice, whole grain breads) and fruits. Fat source suggestions were primarily unsaturated fat sources (e.g., nuts, seeds, unsaturated oils). The amount of protein, vegetables, carbohydrates, and fat for each meal and a daily snack was based on the participants starting weight and estimated caloric needs per the manufacturer's instructions. The participants were given freedom over their food choices for snacks and daily meals, leading to a 10-15% variation in total energy intake and fat/carb nutrient intake based on food preference.

*Statistical Analysis*

All statistical analyses were performed using SPSS v25.0 (SPSS, Chicago, IL). Assumptions of normality were verified for all outcome measures. Relative oxidation values were calculated by dividing the absolute value by kilogram (kg) of fat-free mass (FFM) of each participant. A two-way (dietary intervention *x* postprandial response *x* training frequency) repeated-measures ANOVA was conducted to assess the statistical significance of the effects of the diet and training frequency on fat and carbohydrate oxidation rates, resting energy expenditure, blood glucose, waist circumference (cm), hip circumference (cm), waist-to-hip ratio, body fat (%), fat mass (kg), fat-free mass (kg), VAT (g) and BMD ($g/cm^2$) measures. Tukey's HSD post hoc assessments

were used for any significant main effects. Total areas under the curves (AUC) were calculated using the trapezoid rule (31). Students paired *t*-tests were used to assess the statistical significance of the effects of diet on demographics and AUC calculations. Statistical significance was accepted at $P \leq 0.05$. Data presented as mean (SD). Participants who failed to complete both testing sessions were removed from the analysis.

## RESULTS

Thirty-one participants were recruited. Participants were removed from the study or analysis if they did not complete the study for personal reasons ($n$ = 10) or lack of dietary adherence ($n$ = 6). Therefore, 15 total participants completed the full intervention and were included in the final data analysis.

HTF females reported significantly more training (day·week$^{-1}$) compared to LTF females ($p$ < 0.001, Table 1). Among all females, there was a significant reduction in body fat (%), fat mass, waist circumference, and waist-to-hip ratio following the dietary intervention ($p$ = 0.032, $p$ = 0.017, $p$ = 0.033, $p$ = 0.009, and $p$ = 0.033, respectively, Table 1). Among HTF ($n$ = 8), there was a significant reduction in waist circumference following the dietary intervention ($p$ = 0.044, Table 1). Among LTF females ($n$ = 7), there was a significant reduction in body fat (%) and fat mass following the dietary intervention ($p$ = 0.012 and $p$ = 0.014, respectively, Table 1). Prior to the dietary intervention, HTF females had significantly lower body fat (%), fat mass, and hip circumference compared to LTF females ($p$ = 0.014, $p$ = 0.029, $p$ = 0.049, and $p$ = 0.024, respectively, Table 1). Following the dietary intervention, HTF had significantly lower body fat (%), and hip circumference but a higher waist-to-hip ratio compared to LTF females ($p$ = 0.008, $p$ = 0.046, $p$ = 0.017, and $p$ = 0.011, respectively, Table 1). There were no changes in fat-free mass in all females or in either group p pre- to post-intervention.

There was no difference between groups following the intervention in dietary adherence (Table 1). Following the dietary intervention at the completion of the study, only 11/15 females (7/8 HTF, 4/7 LTF) provided complete dietary records (Table 2). Furthermore, there was a significant decrease in carbohydrate and fat intake and an increase in protein intake among all females ($p$ = 0.002, $p$ = 0.018, and $p$ = 0.032, respectively, Table 2). Participants consumed significantly less protein than the template recommendations in all HTF and LTF females ($P$ < 0.001, $p$ = 0.017, and $p$ = 0.006, respectively, Table 2). HTF females had a significant decrease in carbohydrate intake and an increase in protein intake during the dietary intervention ($p$ = 0.012 and $p$ = 0.049, respectively, Table 2). Adherence to the diet was not significantly different between groups for total kcal, carbohydrate, protein, or fat ($p$ = 0.793, $p$ = 0.841, $p$ = 0.103, $p$ = 0.763, Table 2).

Among all participants, there was a significant time effect of the HFM for absolute (kcals·day$^{-1}$; ANOVA; *time,* $P$ < 0.001, $\eta_p^2$ = 0.929, Figure 1a) and relative RMR (kcals·kg FFM$^{-1}$; ANOVA; *time,* $P$ < 0.001, $\eta_p^2$ = 0.930, Figure 1c), RER (RER; ANOVA; *time,* $p$ = 0.002, $\eta_p^2$ = 0.723, Figure 2a), absolute (g·minutes$^{-1}$; ANOVA; *time,* $P$ < 0.001, $\eta_p^2$ = 0.826, Figure 3a) and relative fat oxidation (g·kg FFM$^{-1}$·minutes$^{-1}$; ANOVA; *time,* $P$ < 0.001, $\eta_p^2$ = 0.814, Figure 3c), absolute (g·minutes$^{-1}$;

ANOVA; *time*, $p = 0.006$, $\eta_p^2 = 0.660$, Figure 4a) and relative carbohydrate oxidation (g·kg FFM$^{-1}$·minutes$^{-1}$; ANOVA; *time*, $p = 0.003$, $\eta_p^2 = 0.701$, Figure 4c), and blood glucose (mg·dL$^{-1}$; ANOVA; *time*, $p = 0.001$, $\eta_p^2 = 0.840$, Figure 5a). Additionally, there was a significant time*group interaction effect during the HFM for blood glucose (mg·dL$^{-1}$; ANOVA; *time*group*, $p = 0.032$, $\eta_p^2 = 0.647$, Figure 5a). However, follow-up post-hoc analysis revealed no significant differences between groups ($P > 0.05$).

**Table 1.** Participant characteristics pre- vs. post-intervention.

| | *All (n = 15)* | | *HTF (n = 8)* | | *LTF (n = 7)* | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post |
| Age (y) | 22.27 (2.43) | | 23.38 (1.00) | | 21.00 (0.38) | |
| Training Frequency (day/week) | 3.73 (1.10) | | 4.63 (0.18)[b] | | 2.71 (0.18)[b] | |
| Dietary Adherence (Average day/week) | 5.70 (0.65) | | 6.00 (0.38)[†] | | 5.36 (0.75)[†] | |
| Dietary adherence (%) | 81.42 (0.09) | | 85.71 (0.05) | | 76.53 (0.10)[†] | |
| Weight (kg) | 71.23 (7.92) | 70.05 (7.83) | 69.02 (7.68) | 67.73 (7.57) | 73.81 (7.96) | 72.70 (7.79) |
| BMI (kg/m$^2$) | 26.10 (3.08) | 25.66 (3.06) | 25.09 (1.53) | 24.60 (1.25) | 27.27 (4.05) | 26.87 (4.10) |
| Waist Circumference (cm) | 78.83 (5.49)[a] | 76.82 (5.70)[a] | 78.31 (5.70)[a] | 76.39 (5.95)[a] | 79.43 (5.62) | 77.32 (5.82) |
| Hip Circumference (cm) | 103.67 (5.94) | 104.05 (7.08) | 100.56 (4.83)[b] | 99.19 (5.42)[c] | 107.21 (5.24)[b] | 107.46 (6.32)[c] |
| Waist-to-Hip Ratio | 0.76 (0.05)[a] | 0.75 (0.04)[a] | 0.78 (0.05) | 0.77 (0.04)[c] | 0.74 (0.04) | 0.72 (0.03)[c] |
| body fat (%) | 31.65 (5.82)[a] | 30.83 (5.30)[a] | 28.69 (4.09)[b] | 28.33 (4.02)[c] | 35.03 (5.88)[ab] | 33.69 (5.37)[ac] |
| Fat mass (kg) | 23.22 (6.40)[a] | 22.26 (5.83)[a] | 20.24 (4.18)[b] | 19.63 (3.92) | 26.63 (7.06)[ab] | 25.26 (6.46)[a] |
| Fat-Free mass (kg) | 49.35 (4.66) | 49.28 (4.57) | 50.06 (5.38) | 49.54 (5.50) | 48.53 (3.93) | 48.98 (3.64) |
| VAT (g) | 282.20 (104.35) | 270.20 (72.73) | 268.38 (117.30) | 263.50 (75.13) | 298.00 (93.84) | 277.86 (75.02) |
| BMD (g/cm$^2$) | 1.07 (0.09) | 1.09 (1.09) | 1.10 (0.04) | 1.12 (0.04) | 1.04 (0.12) | 1.06 (0.13) |

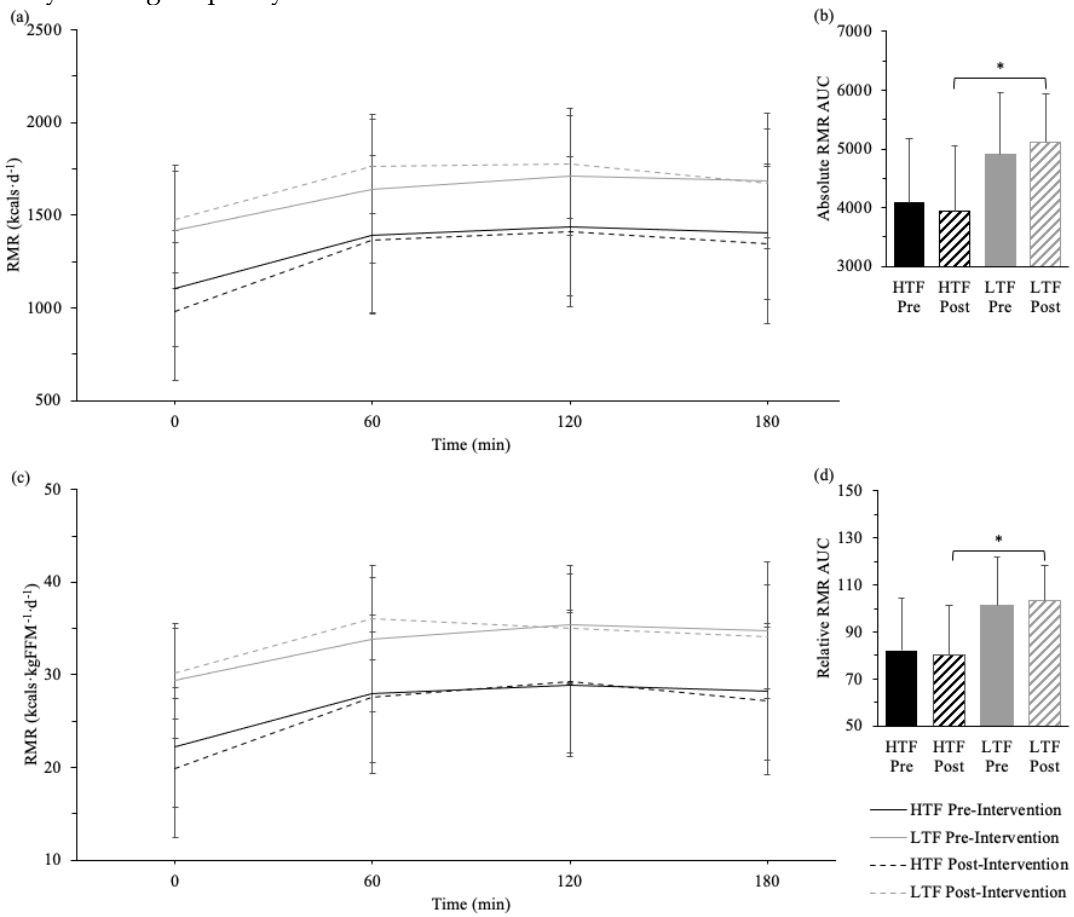
Note: HTF, high-training frequency; LTF, low-training frequency; y, years; day, days; week, week; kg, kilogram; g, grams; m, meter; cm, centimeter; mmHg; millimeters of mercury; BMI, body mass index; VAT, visceral adipose tissue. [a] $P \leq 0.05$ indicates intervention differences; [b] $P \leq 0.05$ indicates pre-intervention training status differences; [c] $P \leq 0.05$ indicates post-intervention training status differences; [†]$p = 0.051$.

**Table 2.** Participant dietary intake pre-intervention vs. average reported during the intervention and diet prescription**.**

| | *All (n = 11)* | | | *HTF (n = 7)* | | | *LTF (n = 4)* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre | Avg | Template | Pre | Avg | Template | Pre | Avg | Template |
| Total Energy (kcals) | 1870.27 (402.16) | 1702.02 (246.70) | 1715.36 (166.06) | 1896.87 (453.23) | 1716.92 (302.29) | 1693.43 (169.18) | 1823.71 (351.77) | 1675.93 (136.69) | 1753.75 (177.73) |
| Carbohydrate (g) | 183.19 (50.60)[a] | 130.24 (29.29)[a] | 128.18 (14.01) | 193.53 (53.21)[a] | 130.19 (31.98)[a] | 126.43 (14.64) | 165.11 (46.76) | 130.32 (28.55) | 131.25 (14.36) |
| Protein (g) | 102.16 (32.44)[a] | 126.88 (13.25)[ab] | 153.50 (12.69)[b] | 106.90 (27.02)[a] | 131.50 (11.12)[ab] | 151.57 (13.81)[b] | 93.86 (43.62) | 118.80 (14.19)[b] | 156.88 (11.45)[b] |
| Fat (g) | 63.66 (15.77)[a] | 54.74 (11.93)[a] | 65.40 (7.14) | 63.75 (15.53) | 53.12 (12.45) | 64.60 (6.82) | 63.50 (18.61) | 57.58 (12.13) | 66.81 (8.53) |

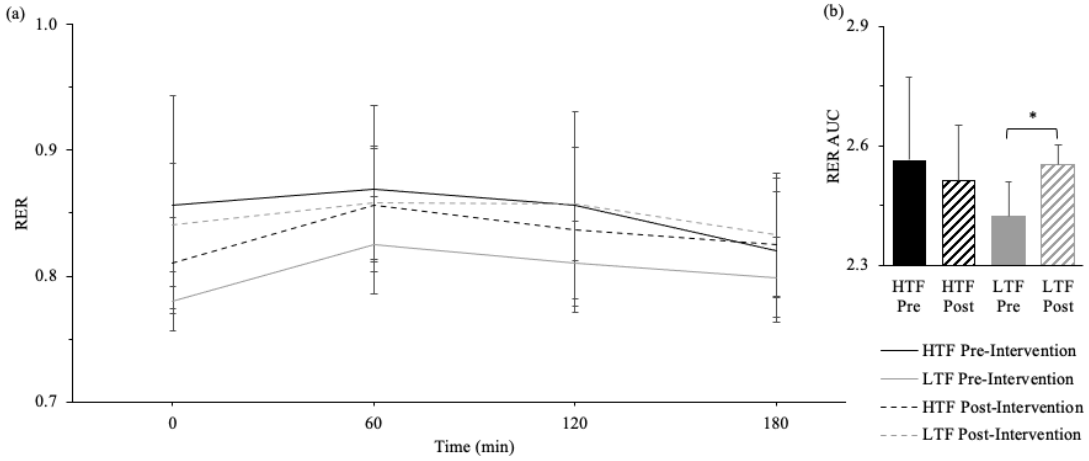
Note: HTF, high-training frequency; LTF, low-training frequency; g, grams; kcals, kilocalories; [a] $P \leq 0.05$ indicates intervention differences; [b] $P \leq 0.05$ indicates differences between template recommendations and post-intervention dietary intake.

**Figure 1.** Comparison of pre- and post-intervention baseline and postprandial resting metabolic rate response to a high-fat meal by training frequency.



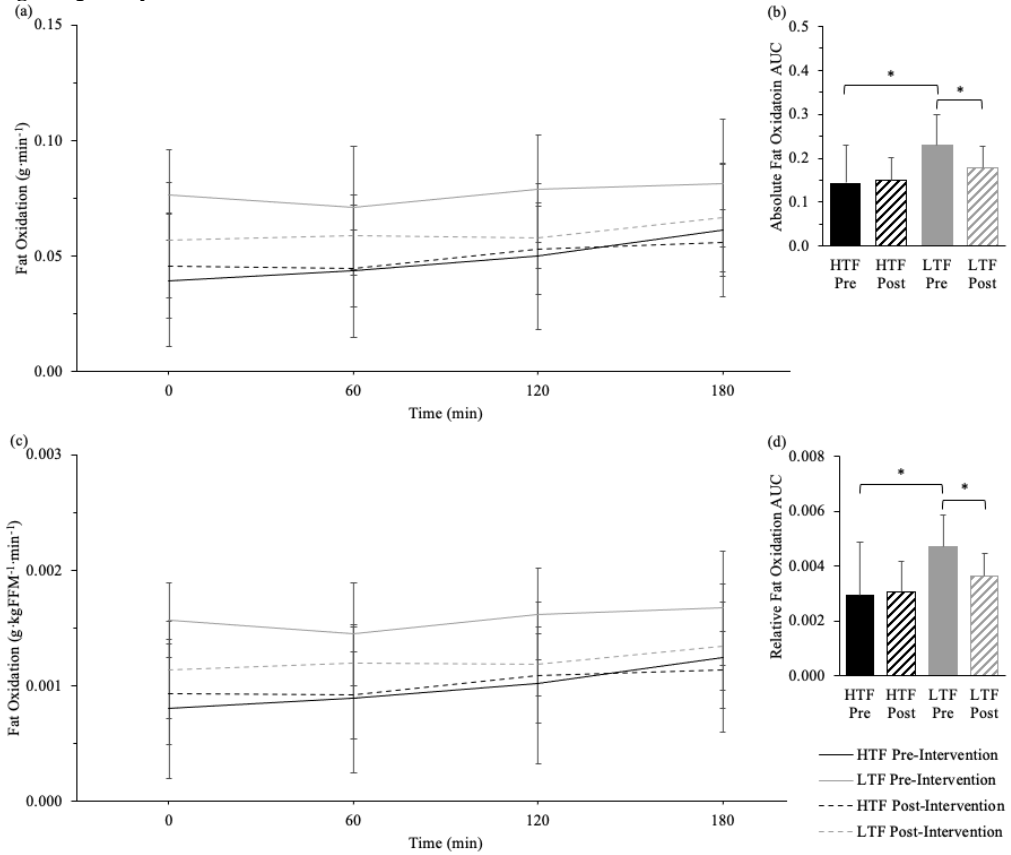

Note: (a) absolute resting metabolic rate (kcals·day$^{-1}$) at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time*, $P < 0.001$, $\eta_p^2 = 0.929$); (b) absolute resting metabolic rate AUC (HTF vs. LTF ANOVA, $p = 0.036$); (c) relative resting metabolic rate (kcals·kg FFM$^{-1}$·day$^{-1}$) at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time*, $P < 0.001$, $\eta_p^2 = 0.930$); (d) relative resting metabolic rate AUC (HTF vs. LTF ANOVA, $p = 0.031$). RMR, resting metabolic rate; kcals, kilocalories; min, minute; d, day; kg, kilogram; FFM, fat-free mass; AUC, area under the curve; HTF, high-training frequency, LTF, low-training frequency; Pre, pre-intervention; post, post-intervention.

**Figure 2.** Comparison of pre- and post-intervention baseline and postprandial respiratory exchange ratio response to a high-fat meal by training frequency.




Note: (a) respiratory exchange ratio at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time*, $p = 0.002$, $\eta_p^2 = 0.723$); (b) respiratory exchange ratio AUC (LTF pre- vs. post-intervention paired *t*-test, $p = 0.041$); RER, respiratory exchange ratio; AUC, area under the curve; HTF, high-training frequency, LTF, low-training frequency; Pre, pre-intervention; post, post-intervention.

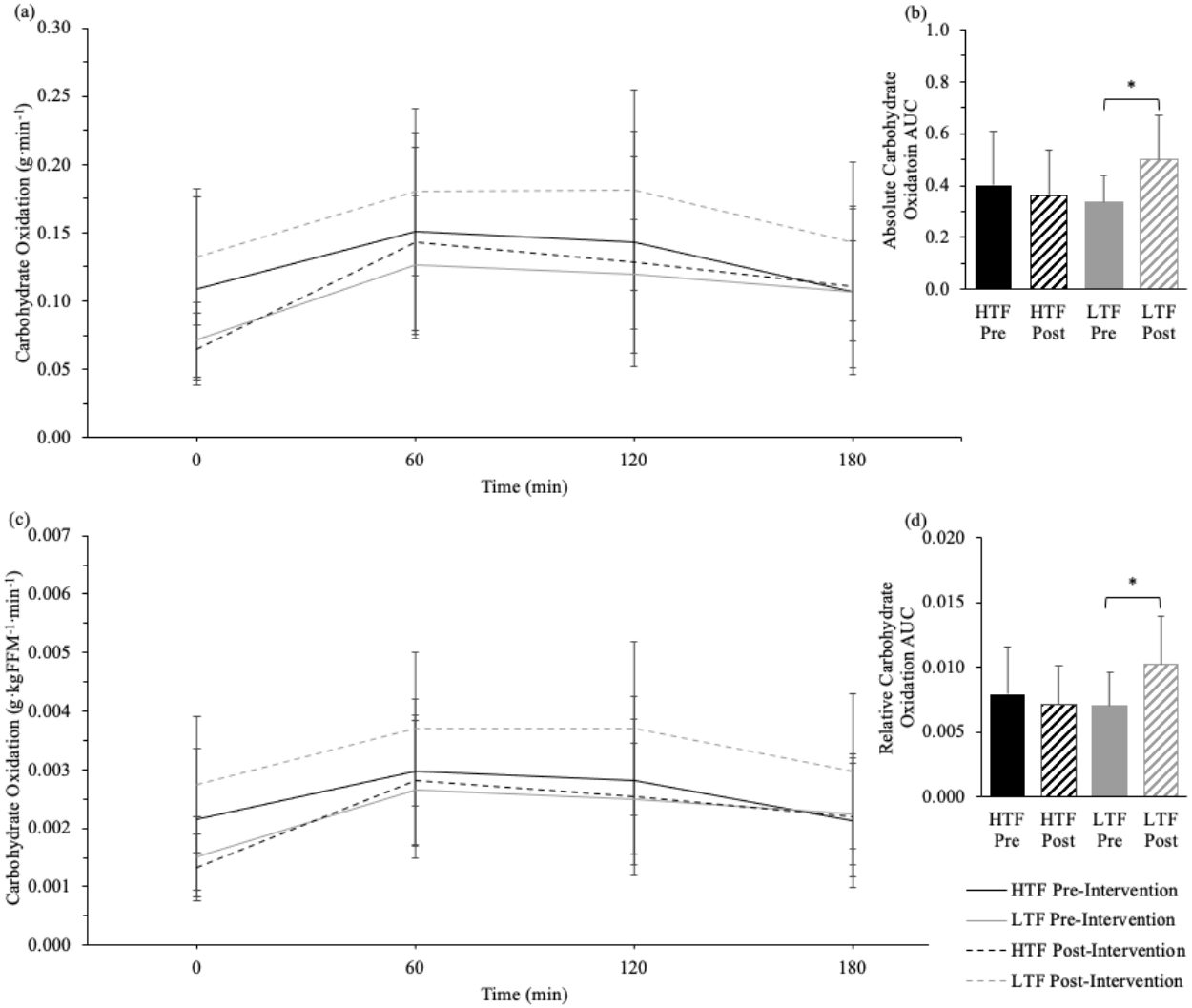
**Figure 3.** Comparison of pre- and post-intervention baseline and postprandial fat oxidation response to a high-fat meal by training frequency.




Note: (a) absolute fat oxidation (g·minutes$^{-1}$) at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time*, $P < 0.001$, $\eta_p^2 = 0.826$); (b) absolute fat oxidation AUC (HTF vs. LTF ANOVA, $p = 0.048$ and LTF pre- vs. post-

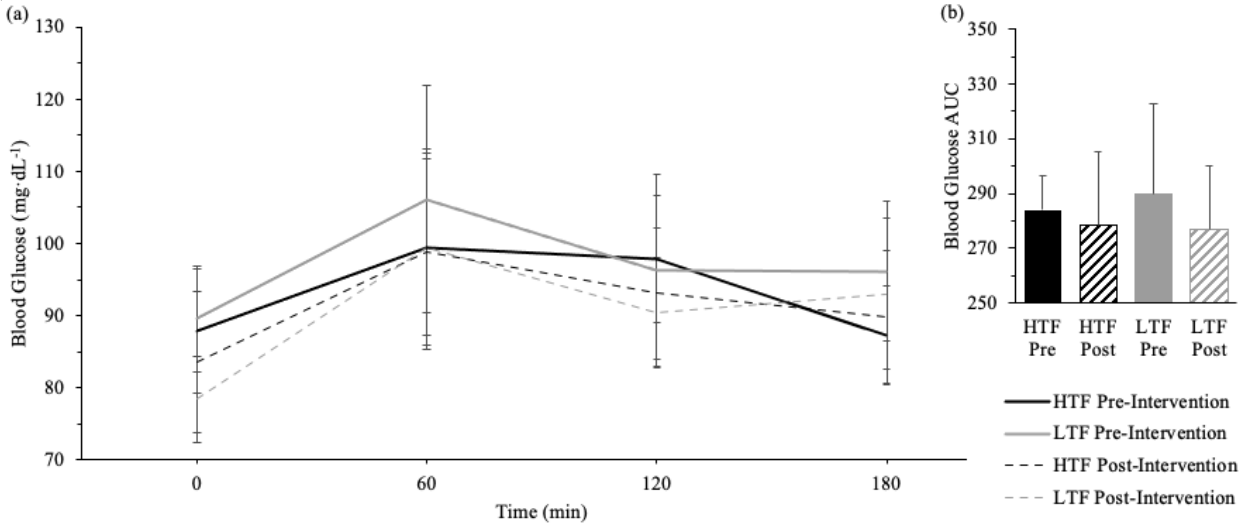
intervention paired *t*-test, $p = 0.050$); (c) relative fat oxidation (g·kg FFM$^{-1}$·minutes$^{-1}$) at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time,* $P < 0.001$, $\eta_p^2 = 0.814$); (d) relative fat oxidation AUC (HTF vs. LTF ANOVA, $p = 0.044$ and LTF pre- vs. post-intervention paired *t*-test, $p = 0.050$). min, minute; g, gram; kg, kilogram; FFM, fat-free mass; AUC, area under the curve; HTF, high-training frequency, LTF, low-training frequency; Pre, pre-intervention; post, post-intervention.

**Figure 4.** Comparison of pre- and post-intervention baseline and postprandial carbohydrate oxidation response to a high-fat meal by training frequency.




Note: (a) absolute carbohydrate oxidation (g·minutes$^{-1}$) at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time,* $p = 0.006$, $\eta_p^2 = 0.660$); (b) absolute carbohydrate oxidation AUC (LTF pre- vs. post-intervention paired *t*-test, $p = 0.032$); (c) relative carbohydrate oxidation (g·kg FFM$^{-1}$·minutes$^{-1}$) at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time,* $p = 0.003$, $\eta_p^2 = 0.701$); (d) relative carbohydrate oxidation AUC (LTF pre- vs. post-intervention paired *t*-test, $p = 0.029$). min, minute; g, gram; kg, kilogram; FFM, fat-free mass; AUC, area under the curve; HTF, high-training frequency, LTF, low-training frequency; Pre, pre-intervention; post, post-intervention.

**Figure 5.** Comparison of pre- and post-intervention baseline and postprandial blood glucose oxidation response to a high-fat meal by training frequency.




Note: (a) blood glucose (mg·dL$^{-1}$) at baseline, 60-, 120- and 180-minutes postprandial (ANOVA; *time*, $p = 0.001$, $\eta_p^2 = 0.840$ and ANOVA; *time*group*, $p = 0.032$, $\eta_p^2 = 0.647$); (b) blood glucose AUC. mg, milligram; dL, deciliter; AUC, area under the curve; HTF, high-training frequency, LTF, low-training frequency; Pre, pre-intervention; post, post-intervention.

## DISCUSSION

The current study investigated the effects of adhering to a novel high-protein diet template on metabolism and body composition (e.g., waist and hip circumference, body fat (%), fat mass, fat-free mass), measures in recreationally resistance-trained females. This study has two main findings. First, adhering to a novel high-protein diet template elicits a metabolic shift favoring carbohydrate oxidation in females engaging in LTF resistance training but does not alter fat and carbohydrate metabolism in females engaging in HTF resistance training. Second, a high-protein diet in resistance-trained females preserves lean mass while decreasing body fat (%) and fat mass in LTF resistance training females and waist circumference in HTF females. Our data suggest that resistance training frequency may modulate the metabolic response when adhering to a high-protein diet template and should be considered by practitioners when addressing individual diet and metabolic response to dietary intervention.

Impaired metabolic flexibility is associated with several components of metabolic syndrome, including low energy expenditure and increased insulin resistance (14, 19, 39). Diminished metabolic flexibility also predicts future weight gain (5, 45). High levels of daily physical activity and fitness status are associated with greater metabolic flexibility and energy expenditure (4, 6, 7). While studies on aerobic training in females have demonstrated engagement in aerobic training and elevated aerobic fitness yields greater metabolic flexibility vs. poor aerobic fitness (2, 8), the effects of resistance training frequency on metabolic flexibility in females are unclear. We found that resistance training status impacts substrate utilization among females undergoing a high-protein dietary intervention. Specifically, we found that those with a LTF experienced a decrease in fat oxidation and an increase in carbohydrate oxidation. However,

females with a HTF exhibited similar fat oxidation and carbohydrate metabolism following the intervention compared to baseline. These differences in substrate oxidation were found in LTF females without any within-group changes in energy expenditure, suggesting a shift in substrate preference. While previous data support our finding of poor metabolic flexibility in lower-trained females, they do not elucidate this distinctly opposite response in postprandial metabolism seen between our groups.

Lower-trained females exhibit a blunted peak in energy expenditure and greater carbohydrate oxidation following a high-carb meal but not a high-fat meal, compared to highly trained subjects (8, 43). Suggesting that the carbohydrate content of our meal (82.5 ± 5.2 g) may be a major metabolic driving factor in the postprandial meal response observed in our LTF females (8, 43). Interestingly, this increase in carbohydrate metabolism was not met with any significant changes in postprandial blood glucose response in LTF females compared to baseline or our HTF group. The possible mechanism for the noted differences in metabolic responses between training frequencies could also be attributed to differences in glycogen storage capacity and depletion (41). While both groups consumed the same diet, the HTF group would theoretically have had a greater weekly depletion of their intramuscular and liver glycogen stores due to increased exercise training vs. diet intake (41). This decrease in carbohydrate storage may have led to an increased reliance on fat oxidation (16). In the LTF females, this mismatch of carbohydrate depletion and dietary uptake could have led to an increase in carbohydrate availability to be readily oxidized. Due to the inverse relationship of carbohydrate and fat metabolism (16, 40, 41), this supports the almost entire switching of resting and postprandial substrate use from fat to carbohydrate in the LTF females pre- to post-intervention, but less pronounced changes observed in the HTF group.

Muscle tissue plays a vital role in substrate metabolism, and the preservation of lean-mass via a high-protein diet and resistance training may play an important role in substrate metabolism and health in this population (40). Females undergoing high-protein diets have improved cardiovascular health outcomes such as increased fat metabolism (30, 38). Furthermore, poor metabolic flexibility is associated with elevated body fat and improves with fat loss (40). Our LTF females significantly decreased body fat (%) and fat mass following the intervention. While metabolic flexibility did not improve in LTF females, this may be due to significant but non-clinically meaningful changes in body fat (%) and fat mass. It is also worth noting that muscle mass was neither altered by the intervention nor different between groups at either study time point. This suggests that muscle tissue alone may not fully explain the metabolic response to an acute high-fat meal. Our HTF group had significantly lower body fat (%) than our LTF females at both baseline and following the intervention, suggesting perhaps that improved body composition (e.g., lower body fat (%) and fat mass) alongside increased training frequency improves metabolic flexibility in resistance-trained females. Changes in body composition improving metabolic flexibility align with current data suggesting that elevated body weight and activity levels are strongly related to metabolic flexibility (2). While the dietary intervention was able to preserve muscle mass when combined with resistance training for HTF and LTF females, fat and carbohydrate metabolism remained non-significantly different compared to

baseline only in HTF females. Future work should investigate the impacts of resistance training frequency and volume alongside dietary interventions to understand how resistance training modulates the metabolic response in females.

These data suggest that resistance training status may result in carbohydrate sparing and increased fat oxidation at rest and during the postprandial period in HTF females compared to LTF (43). Since metabolic flexibility is a mechanism and adaptation to energy resources (40), we propose that the influx in carbohydrates in the LTF females is a physiological response to increased carbohydrate intake vs. use. These results indicate that a lower and more appropriate carbohydrate intake may be more favorable to match lower training frequencies when considering using dietary templates or high-protein diets to preserve or improve muscle mass and metabolic outcomes and long-term health (16, 41).

Since this study was done as a free-living diet intervention, and while weekly check-ins and dietary log collection were implemented to ensure participant adherence to the dietary template, variation in adherence beyond what was reported may have occurred. As demonstrated here, reported intake vs. estimated expenditure was different between our groups at baseline and during the intervention. Diet adherence was 81.42% for the entire study, with a 51% dropout rate (16/31 participants). However, our adherence is similar to other studies that report an overall dietary adherence of 75-90% adherence, with greater attrition rates in women (17). Additionally, the overall average adherence to dietary interventions is around 60.5% (29). These findings suggest that online dietary tools have similar success rates to standard dietary interventions. However, we have a major limitation of participants' inconsistent and incomplete dietary reporting. While this was suboptimal for control across our study, it is important for practitioners who use pre-made dietary templates to consider this. As observed here, these templates may be more effective in individuals who are more highly trained in preserving metabolism. Additionally, since the pre-intervention diet was not controlled, a 3-day pre-testing control diet may have yielded a more consistent baseline metabolic response.

Additional limitations to the current study are that resistance training programs were not standardized among participants, leading to a discrepancy in total exercise volume between groups. While this intentionally mimics the free-nature utilization of dietary templates, future work should consider standardizing the total weekly volume split into high and low-training volumes to further expand upon these findings. Lastly, the menstrual cycle phase was not standardized across all participants. Taken together, future work should expand upon our study duration to assess the long-term impacts of high-protein diets on metabolic flexibility in resistance-trained females and control for or include the menstrual cycle phase in testing protocols.

Our study is one of the first studies to assess metabolic flexibility in response to high-protein dietary interventions when combined with resistance training in females. In summary, the primary findings of our study suggest that a novel high-protein diet template elicits a metabolic shift favoring carbohydrate oxidation in females engaging in low-frequency resistance training

but does not alter fat and carbohydrate metabolism in females engaging in high-training frequency resistance training. These results align with the body of literature that supports training status effects on metabolic flexibility and substrate metabolism. Our findings suggest that training frequency modulates the metabolic response to dietary intake and that higher training frequency resistance training may elicit a more favorable postprandial metabolic response in females.

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