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Dietary macronutrient distribution influences post-exercise substrate utilization in women: A cross-sectional evaluation of metabolic flexibility

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Abstract

AIM—Metabolic flexibility is the ability to alter substrate utilization in response to substrate availability, which may influence health and performance. The current study evaluated the effects of habitual macronutrient distribution on energy expenditure (EE) and metabolic flexibility in physically active women.

METHODS—Participants (n=20) completed a 3-day food log and a standardized bout of high-intensity interval training to determine EE and respiratory exchange ratio (RER). EE and RER were measured via indirect calorimetry at rest (PRE) and immediately (IP), 30 minutes (30min), and 60 minutes post-exercise (60min). To evaluate metabolic flexibility, RER changes were calculated from PRE to IP, IP to 30min, and IP to 60min. For each macronutrient, participants were categorized into high- and low-intake groups using a median split.

RESULTS—No significant correlations were observed between macronutrient distribution and EE when covaried for lean mass (all $p \geq 0.232$), and ANCOVAs revealed no significant group × time interactions (all $p \geq 0.241$). Fat intake was not associated with ΔRER (all $p \geq 0.477$). Correlations between PRO intake and ΔRER approached significance ($r = 0.373–0.411$; $p = 0.079–0.115$), as did inverse associations between CHO and ΔRER ($r = -0.404 – -0.409$; $p = 0.084–0.087$). Lower RER values were observed in the low-CHO group at 30min and 60min ($p = 0.030$) compared to high-CHO. Higher RER values were observed in the high-PRO group at IP ($p = 0.042$) compared to low-PRO. Estradiol was not correlated with RER at any time point, or different between diet groups (all $p > 0.401$).

CONCLUSION—Results suggest that high PRO and low CHO intakes are associated with greater metabolic flexibility in women.

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Keywords

Respiratory exchange ratio; excess post-exercise oxygen consumption; high-intensity interval training; fat oxidation; respiratory quotient

Introduction

Metabolic flexibility refers to the ability to alter substrate utilization in response to changes in substrate availability,[1, 2] which may have important implications for metabolic health. Poor metabolic flexibility is characterized by increased carbohydrate (CHO) utilization in the basal condition, but reduced CHO utilization in situations of high postprandial CHO availability, insulin stimulation, and moderate-intensity exercise.[2–4] A lack of metabolic flexibility has been associated with obesity, type 2 diabetes, cardiovascular disease, and metabolic syndrome.[5] Previous research has suggested that inflexibility of substrate utilization may impair fat oxidation at rest,[6] increase weight gain with overfeeding,[6] and contribute to the development of insulin resistance.[1] Metabolic flexibility may also have important implications for athletic performance by enhancing fat oxidation at rest, thus sparing glycogen for periods of high-intensity activity, and by facilitating CHO utilization during high intensity exercise.[3] Metabolic flexibility has previously been related to increased insulin sensitivity and mitochondrial oxidative capacity,[4, 7, 8] but may also be influenced by dietary macronutrient intake and distribution.[6, 9]

Respiratory exchange ratio (RER) describes the expiratory ratio of carbon dioxide (CO_2) to oxygen (O_2), which closely approximates the respiratory quotient (RQ) at the cellular level.[10] This measurement can be used to assess substrate utilization and metabolic flexibility; an RER value of 0.7 is representative of lipid oxidation, while an RER value of 1.0 or above represents carbohydrate (CHO) oxidation. Poor metabolic flexibility is characterized by the inability to switch between fat and CHO oxidation; as such, individuals with poor metabolic flexibility would be expected to demonstrate a smaller rise in RER following intense exercise or a high-CHO meal, and a smaller drop in RER when fasting. Previous research has used fasting RQ, 24-hour RQ, and acute RQ changes in the assessment of metabolic flexibility.[3, 7, 8] A lack of metabolic flexibility inhibits the acute rise in RER during exercise,[3] which may indicate potential impairment of glycolytic ATP production during intense exercise.

Researchers have previously used high CHO intakes or glucose infusions to evaluate the ability to upregulate CHO oxidation in response to increased CHO availability. In such circumstances, a more pronounced shift from lipid to CHO oxidation is indicative of greater metabolic flexibility in the presence of high CHO availability. It has been suggested that acute high-fat feeding may not sufficiently challenge the ability to upregulate fat oxidation,[1] as the metabolic challenge of fat oxidation is low in resting conditions due to low overall energy expenditure. High-intensity exercise is associated with a greater magnitude of excess post-exercise oxygen consumption (EPOC),[11] in which metabolic rate and substrate oxidation remain elevated following the cessation of exercise. Evaluating changes in RER following high-intensity interval training (HIIT) provides a novel way to evaluate metabolic

flexibility, as CHO is the predominant substrate utilized during high-intensity exercise,[12] and the transition from CHO to fat oxidation can be observed during a period of elevated energy expenditure following the bout. It was hypothesized that CHO intake would be inversely associated with metabolic flexibility. The purpose of the current study was to evaluate the influence of dietary macronutrient distribution on energy expenditure and substrate utilization in women following a bout of HIIT, using RER changes to estimate substrate utilization.

Methods

Experimental Design

The current study consisted of a cross-sectional evaluation of post-exercise metabolism in twenty physically active females. Participants completed a 3-day food log, body composition assessment via dual-energy X-ray absorptiometry (DXA), and a single standardized bout of HIIT. Energy expenditure and RER were measured at rest (PRE), immediately post-exercise (IP), 30 minutes after (30min), and 60 minutes after (60min) the exercise bout. To evaluate metabolic flexibility, calculations were performed to quantify the rise in RER from PRE to IP (ΔRER_{IP}), and the drop in RER from IP to 30min (ΔRER_{30}) and IP to 60min (ΔRER_{60}). To standardize metabolic responses, participants were instructed to abstain from food intake for at least three hours, abstain from caffeine intake for at least five hours, and abstain from strenuous exercise for at least 24 hours prior to exercise.

Participants

Twenty eumenorrheic females completed the current study (Mean ± SD; Age: 24.6 ± 3.9 yrs; Height: 164.4 ± 6.6 cm; Weight: 62.7 ± 6.6; Body fat: 28.2 ± 4.8%). Upon arrival to the laboratory, height and weight were measured using a stadiometer (Perspective Enterprises, Portage, MI, USA) and digital scale (Model 2101KL, Health o Meter, McCook, IL, USA). Height and weight were measured in light, athletic clothing with the shoes removed prior to measurement. Participants were considered physically active, as defined by 1–5 total hours per week of structured aerobic and/or resistance exercise. Questionnaires were provided to verify health status, menstrual status, and physical activity history. Participants were excluded if they were pregnant, possessed any health risks or injuries that would contraindicate exercise, or had been diagnosed with any disease of the heart, lungs, kidneys, or liver. The University's Institutional Review Board approved the protocol used in the current study, and all participants provided written informed consent prior to participation.

Dietary Intake

To estimate habitual dietary intake, participants completed a 3-day food record, which has been validated as an acceptable dietary assessment tool.[13] Participants were provided detailed instructions for estimating portion sizes and accurately recording food intake, and were instructed to include as much detail as possible, including brand of food, an estimate of serving size, any sauces or condiments used, and the method of cooking or preparation. Participants were asked to record intake on two weekdays and one weekend day that adequately represented their normal dietary habits. All diet records were analyzed by a trained laboratory technician to determine daily intakes of CHO, fat, protein (PRO), and

total calories using a nutrition software program (The Food Processor, Version 10.12.0, ESHA Research, Salem, OR, USA). Macronutrient intakes as a percentage of total caloric intake were used for statistical analyses. Average dietary intakes are listed in Table 1.

Body Composition Assessment

To account for the effects of lean mass (LM) on EE, body composition was determined via DXA (Hologic Discovery W, Bedford, MA, USA), using the device's default software (Apex Software Version 3.3). The absorptiometer uses rectilinear fan beam acquisition to provide a three-compartment assessment of body composition, including fat mass (FM), LM, and bone mineral content (BMC). Prior to the scan, participants removed all metal objects, then laid supine in the center of the platform with their hands positioned palms-down near their sides. For the duration of the scan, participants were instructed to breathe normally, but to limit movement as much as possible. All scans were performed by the same certified DXA technician. Before testing, the absorptiometer was calibrated according to the manufacturer's recommendations. Previous results from this laboratory have reported DXA test-retest reliability with an intraclass correlation coefficient (ICC) = 0.99 and standard error of measurement (SEM) = 1.07 kg for LM.

Exercise Protocol

Participants were given five minutes to complete a self-selected warmup, followed by a bout of high-intensity interval training completed on a motorized treadmill (T2100, GE Healthcare, Pollards Wood, UK). The exercise bout consisted of ten, 60-second runs at 85–95% of heart rate reserve, interspersed with 60-second periods of passive rest. To calculate the desired heart rate range, participants arrived to the laboratory in an overnight fasted state, where resting heart rate (RHR) was measured using a heart rate monitor strap (Polar FT1, Polar USA, Port Washington, NY, USA). Resting heart rate and the age-predicted maximal heart hate (MHR) were used to calculate the exercise heart rate range using the Karvonen equation [Target heart rate = ((MHR − RHR)(% intensity)) + RHR].[14] Throughout the test, treadmill speed was adjusted to maintain the desired heart rate during each work interval. Participants completely rested (i.e. standing; no movement) during the 60-second rest bouts. Metabolic flexibility was investigated in the context of HIIT, which subjects performed as part of a larger study investigating pre-exercise supplementation using multiple exercise modalities.[15]

Metabolic Measurements

Indirect calorimetry was used to determine EE and RER. Participants were seated in an upright position and connected to a metabolic cart (TrueOne 2400, ParvoMedics, Inc., Sandy, UT, USA) using a mouthpiece and hose. Nose clips were worn to ensure that all expired respiratory gases were collected. Participants were connected to the metabolic cart for fifteen continuous minutes prior to exercise (PRE), fifteen minutes immediately post-exercise (IP), and from minutes 20–35 (30min) and 45–60 (60min) following exercise. Measurements of oxygen uptake and carbon dioxide production were then used to calculate EE[16] and RER[10] using the following equations:

$$EE\left(\frac{kcal}{day}\right)=[(3.9*(VO_2(L*min^{-1})))+(1.1*(VCO_2(L*min^{-1})))]*1440min$$

$$RER=\frac{VCO_2(L*min^{-1})}{VO_2(L*min^{-1})}$$

Average values for EE and RER were calculated for the final 10 minutes of each time point and used for statistical analyses. To evaluate metabolic flexibility, calculations were performed to quantify changes in RER between time points. To calculate the rise in RER from rest to immediately post-exercise, the RER at PRE was subtracted from IP (IP – PRE; ΔRER_{IP}). To calculate the drop in RER from IP to 30min and 60min, RER values at 30min and 60min were subtracted from RER at IP to calculate ΔRER_{30} (IP – 30min) and ΔRER_{60} (IP – 60min), respectively. For metabolic flexibility calculations, all RER values were subtracted from IP to yield a positive change score value. To control for the effects of acute food intake on metabolism, participants arrived to the laboratory at least three hours fasted and received a standardized pre-exercise protein supplement. This beverage was low in CHO and fat to minimize elevations in resting RER and gastrointestinal discomfort during exercise.

Salivary Estradiol Measurement

To account for potential effects of estradiol on substrate utilization, a 2.5–5.0 mL saliva sample was obtained at rest prior to the exercise bout. Prior to providing saliva samples, participants were instructed to abstain from brushing teeth for at least 45 minutes and obtaining dental work for at least 48 hours. Upon arrival at the laboratory, participants rinsed their mouths thoroughly to remove any residue or particulate matter than may have adulterated the sample, rested for ten minutes, then provided the sample. Saliva samples were immediately placed on ice and maintained at 4°C, then transported to a freezer as soon as possible to be stored at −20°C. Salivary 17-β-estradiol concentrations were determined using a commercially available ELISA assay (Salivary 17β-Estradiol Enzyme Immunoassay Kit, Salimetrics, LLC, State College, PA, USA). Intra- and inter-assay coefficients of variation were 8.66–18.64% and 3.86%, respectively.

Statistical Analyses

A series of linear regressions were performed to examine relationships between macronutrient intake (CHO, FAT, PRO; expressed as a percentage of total energy intake) and EE at each time point (PRE, IP, 30min, 60min), with lean mass as a covariate. To evaluate metabolic flexibility, a series of linear regressions were performed to examine relationships between macronutrient intake (CHO, FAT, PRO) and RER changes at each time point (ΔRER_{IP}, ΔRER_{30}, ΔRER_{60}), using salivary estradiol as a covariate. A Pearson correlation test was used to evaluate the relationship between salivary estradiol values and RER at each time point (PRE, IP, 30min, 60min).

For each macronutrient (CHO, FAT, PRO), participants were categorized into high intake and low intake groups using a median split, with the 10 highest intakes being considered the high intake group. Body composition and energy intake are presented for each group in Table 1, and independent samples t-tests were used to compare high- and low-intake groups for each variable. Mixed model ANCOVAs were used to compare EE (covaried for LM) and RER (covaried for estradiol levels) at each time point for high and low intake groups for each macronutrient. In the event of a significant interaction, the model was decomposed using Bonferroni *post hoc* comparisons to determine the source of interaction. Independent samples t-tests were used to determine if salivary estradiol concentrations were different between high- and low-intake groups for each macronutrient (CHO, FAT, PRO). All statistical analyses were performed using SPSS software (Version 21.0; IBM, Armonk, NY, USA); statistical significance was set *a priori* at $p \leq 0.05$.

Results

Body Composition and Dietary Intake

Baseline values for body composition and dietary intake are presented in Table 1. Body mass was significantly lower in the high-CHO group compared to low-CHO (p=0.040), with no other between-group differences in anthropometric measurements (Table 1). Significant differences in dietary macronutrient distribution were observed between groups stratified by CHO, fat, and PRO intake (Table 1).

Energy Expenditure

Raw values for EE are presented in Table 2. When covaried for LM, linear regression did not indicate significant correlations between CHO and EE at PRE (r= −0.027, p=0.911), IP (r= −0.240, p=0.232), 30min (r= −0.057, p=0.810), or 60min (r= −0.045, p=0.848). Linear regression, when covaried for LM, did not indicate significant correlations between FAT and EE at PRE (r=0.018, p=0.940), IP (r=0.105, p=0.614), 30min (r= −0.101, p=0.674), or 60min (r= −0.054, p=0.822). When covaried for LM, linear regression did not identify significant correlations between PRO and EE at PRE (r=0.109, p=0.640), IP (r=0.107, p=0.595), 30min (r=0.040, p=0.862), or 60min (r=0.062, p=0.786).

Mixed model ANCOVAs, covaried for LM, did not reveal significant group (high intake vs. low intake) × time (PRE, IP, 30min, 60min) interactions for energy expenditure when grouped by CHO (p=0.592), FAT (p=0.776), or PRO (p=0.241) intake. A main effect of time was observed, with all post-exercise EE values significantly greater than PRE (p<0.01).

Respiratory Exchange Ratio

When covaried for estradiol concentrations, linear regression did not demonstrate a significant correlation between CHO and RER at PRE (r=0.096, p=0.693), and correlations were not statistically significant for ΔRER_{IP} (r= −0.409, p=0.084), ΔRER_{30} (r= −0.408, p=0.084), or ΔRER_{60} (r= −0.404, p=0.087). Linear regression using estradiol as a covariate did not indicate significant correlations between FAT and RER at PRE (r= −0.030, p=0.902), ΔRER_{IP} (r=0.135, p=0.581), ΔRER_{30} (r=0.174, p=0.477), or ΔRER_{60} (r=0.109, p=0.656). There was also no significant correlation between PRO and RER, when covaried for

estradiol, at PRE (r=0.176, p=0.463), ΔRER_{IP} (r=0.406, p=0.085), ΔRER_{30} (r=0.373, p=0.115), or ΔRER_{60} (r=0.411, p=0.079). Salivary estradiol was not significantly correlated with RER at any time point (all $p > 0.401$), and estradiol was not different between groups stratified by CHO, FAT, or PRO intake (all $p > 0.534$).

A mixed model ANCOVA, covaried for estradiol concentrations, revealed a significant group × time interaction for RER values when stratified by CHO intake (p=0.045). *Post hoc* comparisons revealed significantly higher RER values in the high-CHO group at 30min (0.752 vs 0.701; p=0.030) and 60min (0.764 vs 0.731; p=0.030; Figure 1). When stratified by FAT intake, a significant main effect for time was observed (p<0.001), but no group × time interaction was observed (p=0.289). When stratified by PRO intake, a significant group × time interaction was observed (p=0.049). *Post hoc* comparisons revealed significantly higher RER values in the high-PRO group at IP (1.019 vs 0.928; p=0.042; Figure 2).

Discussion

Dietary strategies that influence energy expenditure and substrate utilization may have important implications for athletes and obese individuals. Results of the current study suggest that habitual macronutrient distribution does not influence energy expenditure at rest or following exercise, but indicate that macronutrient distribution may influence substrate utilization and metabolic flexibility in women. Protein intake appeared to have a positive effect on metabolic flexibility. Moderate correlations between protein intake and RER changes approached the level of significance, and the high-PRO group achieved a significantly higher RER than the low-PRO group immediately post-exercise, despite having similar RER values at all other time points. The relationship observed may indicate that high-PRO diets allow for a more efficient transition to CHO utilization during intense exercise, without increasing CHO oxidation at rest or during recovery. Carbohydrate intake appeared to have a negative effect on metabolic flexibility. Moderate inverse correlations between CHO intake and RER changes approached the level of significance, and the low-CHO group achieved a higher RER at IP and significantly lower RER at 30min and 60min than the high-CHO group, despite similar RER values at rest. These findings suggest that high-CHO diets may delay the transition to fat oxidation during recovery from exercise, but may reduce CHO utilization during exercise performed in the absence of exogenous CHO. In contrast, the relative proportion of fat in the diet did not appear to influence resting RER or metabolic flexibility.

In the present study habitual macronutrient distribution, as estimated from 3-day food logs, did not have a significant influence on energy expenditure at rest or following exercise. Previous research has indicated that high-PRO diets increase 24-hour energy expenditure in comparison to low-PRO diets,[17–19] likely due to effects on lean body mass accretion and acute postprandial increases in thermogenesis.[20] In the current study, resting energy expenditure was measured after at least three hours of fasting, and groups stratified by macronutrient intake were relatively homogenous in terms of lean and total body mass (Table 1), which are primary determinants of metabolic rate.[21] Macronutrient distribution did not appear to influence resting energy expenditure, and this finding is consistent with those of Luscombe et al.,[22] which demonstrated that fasted energy expenditure was not

different between energy-matched diets of high (30% of energy) and low (15% of energy) protein content after 12 weeks. This observation is also consistent with previous research showing that basal metabolic rate is not significantly influenced by variation in PRO intake the day before measurement, as fasted energy expenditure was similar when subjects had consumed isocaloric diets consisting of 0.9, 1.2, or 1.5 g/kg of PRO the day prior.[23] It is likely that the positive effects of high-PRO diets on total daily energy expenditure are less apparent when energy expenditure is measured in the fasted state, as acute postprandial increases in thermogenesis are not present in this context. Similarly, significant differences in post-exercise EE values were not observed, as pre-exercise nutrition was standardized with equal protein content and macronutrient distribution between all groups.

Results of the current study suggest an inverse relationship between habitual CHO intake and metabolic flexibility in response to exercise. Previous studies have shown that CHO restriction increases fat oxidation with a concomitant reduction in CHO oxidation,[24] and augments enzymes necessary for fat metabolism while reducing glycolytic enzyme activity.[25] It is possible that individuals in the low-CHO group in the present study expressed a greater capacity for mitochondrial fat oxidation,[4, 8, 26, 27] thereby allowing a more rapid adjustment from CHO oxidation to lipid oxidation during recovery from exercise, although this was not directly measured. This finding may have implications for the management of obesity, as longitudinal studies have related 24-hour and nonsleeping RQ values to increased likelihood of fat gain.[28, 29] Some previous interventions with low-CHO diets have reported favorable outcomes for weight loss and body composition compared to low-fat diets,[30, 31] but others have failed to demonstrate the superiority of low-CHO diets for improving body composition.[32] Future research should seek to determine if the observed relationship is more pronounced in obese and/or sedentary populations, as insulin resistance has been associated with metabolic inflexibility.[1]

The current study found a trend for an inverse association between CHO intake and ΔRER_{IP}. Previous studies have evaluated the effects of CHO restriction on endurance performance, as evidence indicates that aerobic exercise performed in a glycogen-depleted state can enhance mitochondrial adaptations to training[26, 27, 33] and that low-CHO diets preferentially promote fat oxidation and spare glycogen.[24, 34, 35] Despite beneficial effects on fat oxidation, such trials have not consistently demonstrated performance improvements,[34–36] and very low-CHO diets (≤20% of energy) have been shown to inhibit CHO oxidation and power output during high-intensity exercise.[37] In contrast, RER data from the current study imply slightly greater CHO utilization during exercise in individuals consuming a lower percentage of dietary CHO. This discrepancy may relate to the degree of CHO restriction, as the low-CHO group in the current study averaged 40.9 ± 8.2% of energy from CHO. Further, evidence has indicated that individuals with impaired glucose tolerance display an inhibited ability to transition to CHO utilization during submaximal aerobic exercise performed in a fasted state.[3] The inverse relationship observed between CHO intake and CHO oxidation at IP may therefore be related to the effects of low-CHO, high-PRO diets on glycemic control.[38, 39] More studies are needed to determine if CHO availability can be strategically manipulated to enhance exercise adaptations without detrimental effects on CHO oxidation and high-intensity exercise performance.

Previous studies have document sex-based differences in substrate utilization during exercise, with females demonstrating lower RER and CHO utilization compared to males.[40, 41] Estradiol is thought to play an important role in this sex-based difference, and research in animal models has indicated that estradiol administration increases lipid oxidation while decreasing CHO oxidation.[42, 43] Conversely, other studies have previously found no sex-based RER differences during exercise,[44, 45] and the current study found no correlation between salivary estradiol and RER at any time point. This lack of a relationship could potentially be due to the use of a small, relatively homogeneous sample with relatively low variability in estradiol concentrations. However, the current study featured a higher exercise intensity than many of the studies indicating sex-based RER differences during submaximal exercise, and evidence suggests that maximal RER is similar between males and females.[46] In addition, Carter et al.[44] proposed that estradiol may influence tissue-specific respiratory quotient and substrate oxidation in a manner that is not reflected via whole-body RER. More research is warranted to determine the effects of estradiol on RER and substrate utilization at various exercise intensities.

In the current study, results indicated trends for a positive association between PRO intake and metabolic flexibility. Previous research has demonstrated that high-PRO, low-CHO diets increase the rate of hepatic gluconeogenesis,[19] primarily by influencing substrate availability and metabolic enzyme expression.[47–49] Multiple studies have indicated that habitual consumption of higher dietary PRO and lower CHO may result in adaptive responses that serve to maintain endogenous CHO availability and stabilize glycemic control.[39, 47, 50] Significantly higher RER values at IP in the high-PRO group may be attributable to an enhanced ability to maintain the production and utilization of endogenous CHO. While low-CHO, high-fat diets have been shown to suppress CHO oxidation and reduce RER during prolonged exercise following CHO intake,[34, 35, 37] it is possible that discrepant findings in the current study are explained by exercise taking place in the context of low exogenous CHO availability. Exercise occurred after a minimum of three hours fasting and following the provision of a pre-exercise beverage with minimal CHO content; as such, CHO oxidation was likely limited by the capacity for endogenous glucose production.

Limitations of the current study must be noted. While dietary logs have previously been validated as a tool to estimate energy intake[13] and pre-exercise nutrition was standardized, it would be preferable to strictly control dietary intake. Further, metabolic measurements taken immediately following exercise were used to infer substrate utilization during exercise, as the mask and apparatus used for indirect calorimetry worn during exercise would cause undue discomfort given the modality and intensity of exercise. Finally, exercise intensity and duration were standardized to compare metabolic responses, so future research is needed to determine if differences in metabolic flexibility translate to improved performance or training adaptations. While the mechanisms underlying differences in RER cannot be conclusively determined in the current study, future studies should employ more direct measurements of substrate utilization, such as muscle biopsy sampling and stable isotope tracer methodologies, to further investigate these findings.

Conclusions

The current study employed a novel method of evaluating metabolic flexibility in the post-exercise recovery period. Results indicated that diets with higher PRO and lower CHO content are associated with greater metabolic flexibility in women, which may have important implications for clinical and athletic populations. Fat intake was not associated with improvements or decrements in metabolic flexibility, and dietary macronutrient distribution did not appear to influence energy expenditure at any time point. Future research should seek to determine if high-PRO, low-CHO dietary interventions increase metabolic flexibility, and if this translates to improvements in clinical biomarkers or exercise performance.

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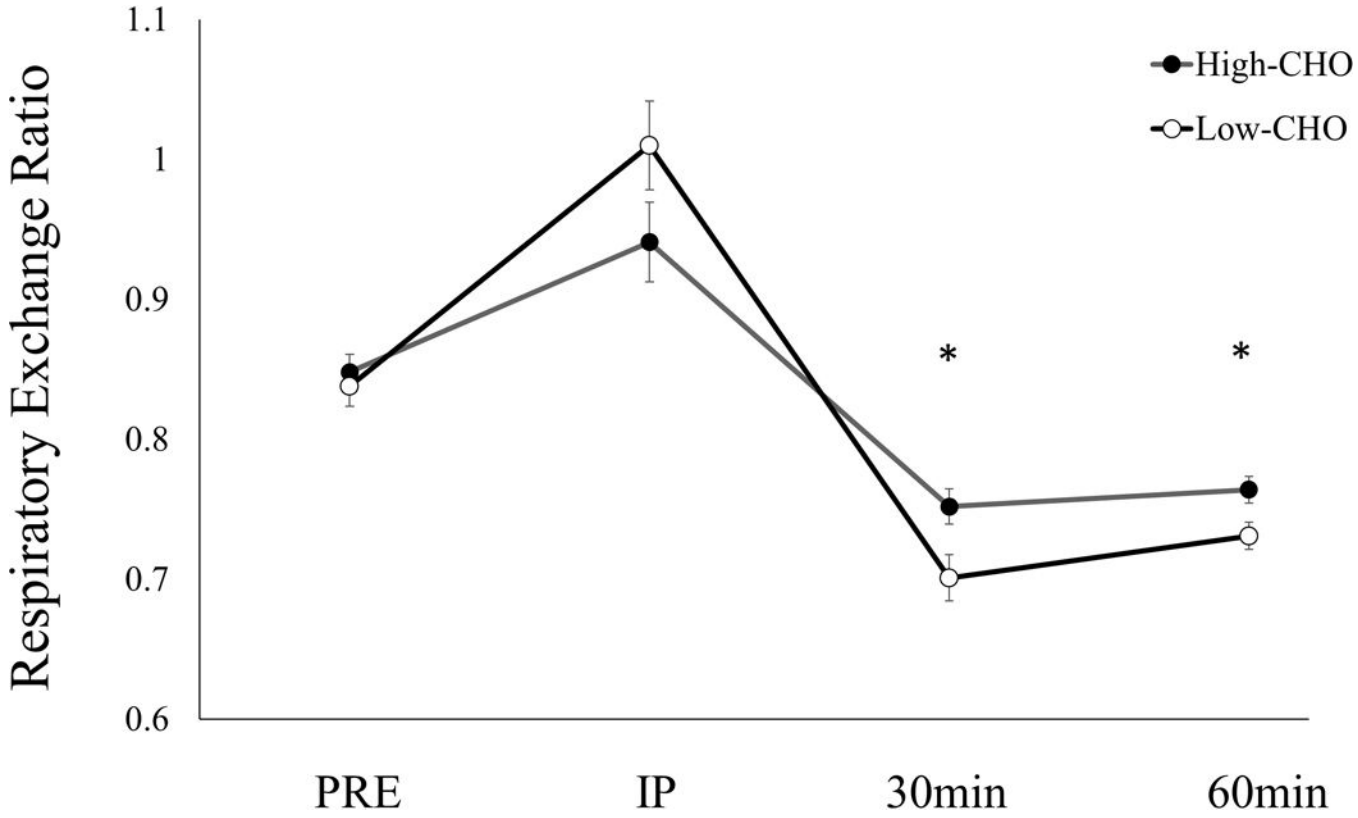

Figure 1.

Changes in RER for High-CHO and Low-CHO groups at PRE, IP, 30min, and 60min. Values are Mean ± SEM. *Significant difference between groups; $p<0.05$

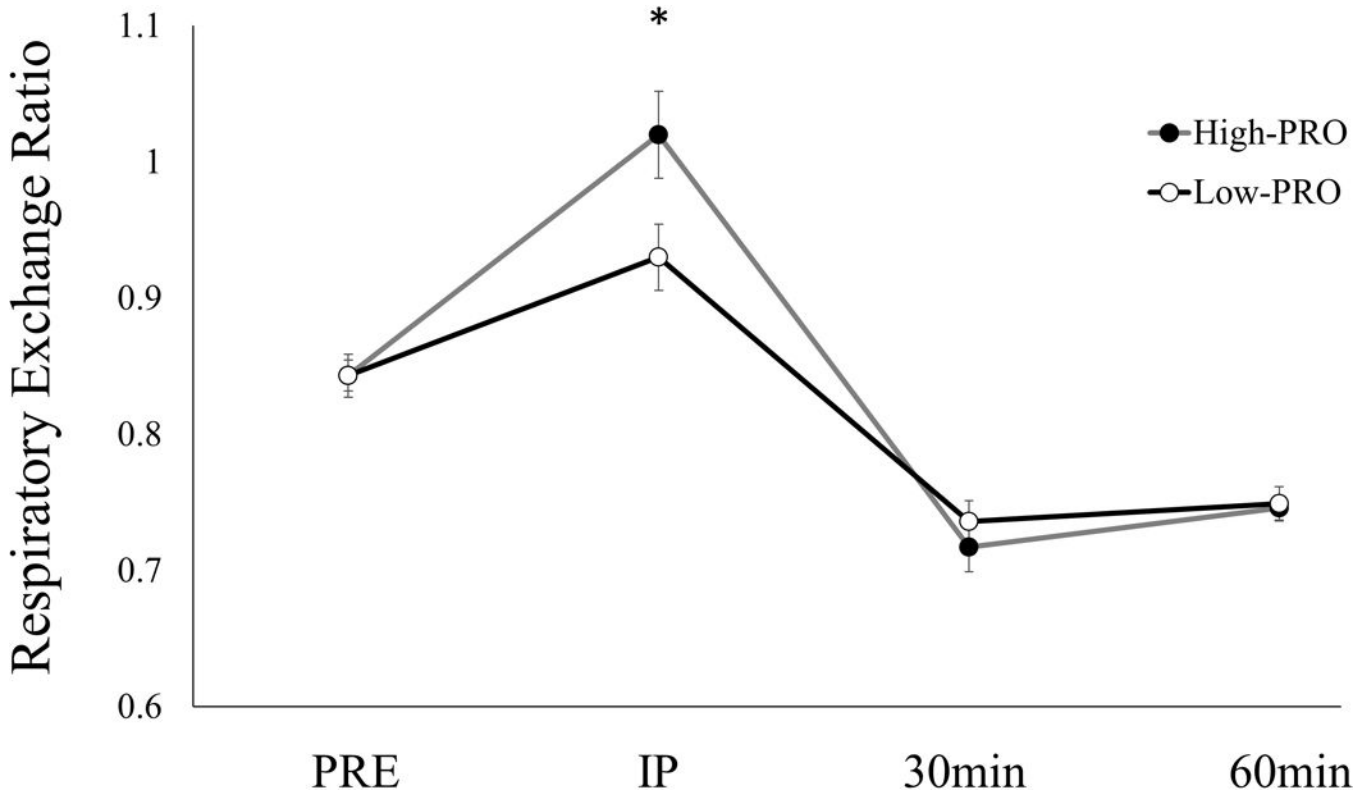

Figure 2.
Changes in RER for High-PRO and Low-PRO groups at PRE, IP, 30min, and 60min. Values are Mean ± SEM. *Significant difference between groups; $p<0.05$

Table 1

Body composition and energy intake by group. Data are presented as mean ± SD.

Characteristic	Group					
	Low-CHO (n=10)	**High-CHO (n=10)**	**Low-Fat (n=10)**	**High-Fat (n=10)**	**Low-PRO (n=10)**	**High-PRO (n=10)**
Age (yrs)	25.0 ± 4.1	24.2 ± 3.9	24.7 ± 3.6	24.5 ± 4.4	24.5 ± 3.7	24.7 ± 4.3
Height (cm)	166.1 ± 6.2	162.8 ± 6.8	162.4 ± 6.1	166.5 ± 6.7	164.1 ± 7.8	164.8 ± 5.5
Weight (kg)	65.7 ± 4.9	59.7 ± 7.0 *	60.1 ± 7.2	65.3 ± 5.0	61.4 ± 7.6	64.0 ± 5.5
Body fat (%)	28.6 ± 5.1	27.7 ± 4.7	27.7 ± 4.7	28.7 ± 5.0	27.8 ± 4.9	28.6 ± 4.9
Estradiol (pg/mL)	2.8 ± 1.2	2.9 ± 2.0	3.0 ± 1.9	2.7 ± 1.3	2.6 ± 1.8	3.1 ± 1.5
Energy Intake (kcal)	2065.1 ± 743.9	2092.3 ± 649.7	1905.8 ± 568.5	2251.7 ± 765.7	2281.4 ± 594.7	1876.0 ± 728.7
CHO (%)	40.9 ± 8.2	56.7 ± 5.8	56.3 ± 6.3	41.3 ± 8.7 *	55.2 ± 7.3	42.4 ± 9.8 *
Fat (%)	39.1 ± 8.0	29.3 ± 5.8 *	28.0 ± 5.1	40.5 ± 6.2 *	30.6 ± 7.1	37.8 ± 8.5
PRO (%)	18.9 ± 5.9	14.6 ± 3.3 *	14.5 ± 3.4	19.0 ± 5.8 *	13.3 ± 1.7	20.2 ± 5.1 *

Descriptive characteristics for groups stratified based on carbohydrate (CHO) intake (% of energy intake) and protein (PRO) intake.

* Significantly different from the low-intake group; $p<0.05$

Table 2

Raw values for energy expenditure (in kcal/day) by group before and after exercise

Group	Time			
	PRE	IP	30min	60min
Low-CHO	1590.1 ± 226.6	3042.3 ± 548.4 *	1801.0 ± 242.0 *	1777.1 ± 219.1 *
High-CHO	1502.7 ± 369.1	2613.0 ± 594.3 *	1698.3 ± 289.1 *	1679.2 ± 310.8 *
Low-Fat	1534.3 ± 366.1	2705.4 ± 575.2 *	1764.2 ± 306.1 *	1708.5 ± 315.0 *
High-Fat	1558.6 ± 239.7	2949.9 ± 626.2 *	1735.1 ± 232.0 *	1747.8 ± 223.2 *
Low-PRO	1502.4 ± 353.5	2652.0 ± 592.5 *	1757.1 ± 321.3 *	1736.6 ± 340.7 *
High-PRO	1590.5 ± 250.1	3003.3 ± 580.3 *	1742.2 ± 211.3 *	1719.7 ± 183.5 *

Raw values for energy expenditure (kcal/day) at rest (PRE), immediately post-exercise (IP), 30 minutes post-exercise (30min), and 60 minutes post-exercise (60min).

* Denotes main effect for time; pooled values significantly greater than PRE ($p<0.05$) when covaried for LBM.