Title

Effects of exercise mode on postprandial metabolism in humans with chronic paraplegia

Short Title

Exercise and postprandial metabolism in SCI

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ABSTRACT

Purpose: The purpose of this study was to assess the acute effects of exercise mode and intensity on postprandial macronutrient metabolism. **Methods:** Ten healthy males age 39 ± 10 yr with chronic paraplegia (~~>1~~13.2 ± 8.8 yr, ASIA A-C) completed 3 isocaloric bouts of upper-body exercise and a resting control. Following an overnight fast, participants completed circuit resistance exercise (CRE) first and the following conditions in a randomized order, separated by >48 h: i) control (CON), ~45 min seated rest; ii) moderate intensity continuous exercise (MICE), ~40 min arm cranking at a resistance equivalent to ~30% peak power output (PPO) and; iii) high intensity interval exercise (HIIE), ~30 min arm cranking with resistance alternating every 2 min between 10% PPO and 70% PPO. After each condition, participants completed a mixed meal tolerance test ~~(MMTT)~~ consisting of a 2,510 kJ liquid meal (35% Fat, 50% ~~CHO~~Carbohydrate, 15% Protein). Blood and expired gas samples were collected at baseline and regular intervals for 150 min post-meal. **Results:** ~~Postprandial energy expenditure was greater in HIIE than CON (*P*=.039).~~ An interaction (*P*<.001) was observed ~~for~~ with rates of lipid oxidation ~~(Lox), with~~ being elevated above CON ~~only~~ in HIIE until 60 min post-meal and in CRE ~~having greater Lox than CON~~ at all postprandial time points up to 150 min post-meal. Postprandial blood glycerol was greater in MICE (*P*=.020) and CRE (*P*=.001) compared to CON. Furthermore, non-esterified fatty acid area under the curve had a moderate-to-strong effect in CRE vs MICE and HIIE (Cohen's d: -.76 and -.50, respectively). **Conclusion:** In persons with paraplegia, high intensity exercise increased postprandial energy expenditure independent of the energy cost of exercise. Furthermore, exercise combining resistance and endurance modes (CRE) showed the greater impact on postprandial Lox.

Key Words: spinal cord injury; interval exercise; circuit resistance exercise; upper-body exercise; exercise intensity; mixed meal tolerance test

INTRODUCTION

Spinal cord injury (SCI) results in dysregulation of energy metabolism that increases risk of cardiometabolic disease (CMD) (1). The Consortium for Spinal Cord Medicine's Clinical Practice Guidelines recommend exercise as primary management strategy for combating CMD in SCI (1). Furthermore, recent AGREE II evidence-based guidelines found moderate to high GRADE confidence ratings for the effect of exercise on cardiometabolic health in persons with SCI (2). Specifically, circuit resistance training has been shown to improve the clinical lipid profile (3) and high intensity interval training is an emerging exercise strategy to target CMD (4) in persons with SCI. While the above guidelines and evidence highlight the importance of exercise for metabolic health in SCI, it is possible that lifestyle monotherapies are insufficient to modify the component risks of cardiometabolic syndrome (5). Considered in conjunction with the unique nutritional considerations in SCI (6), a further understanding of the interaction of nutrition and physical activity is warranted in this population.

Clinical and laboratory tests of macronutrient handling have shown glycemic and lipemic dysregulation in persons with SCI. Oral glucose tolerance testing (OGTT) (7-9) has shown that persons with SCI who have "normal" fasted blood glucose (<5.5 $mmol \cdot L^{-1}$) likely still experience impaired glycemic regulation. The finding of dysglycemia despite normal fasted glucose levels demonstrate how metabolic changes following SCI seem dormant until the system is presented with a challenge. Similarly, dyslipidemia can occur in SCI despite a "normal" serum triglyceride concentration (10), while laboratory postprandial lipemia tests have consistently shown an impaired ability to handle an oral lipid challenge (11-14). When also considering obesity and intermuscular fat accumulation (15) in this population, it seems that disorders of fat metabolism are paramount in the development of CMD in SCI. Evidence for disordered macronutrient handling in SCI is based primarily on single-nutrient (e.g., 75 g glucose) feeding challenges. Dysglycemia following consumption of a liquid mixed macronutrient meal has been documented in persons with tetraplegia (16) but evidence is lacking in persons with paraplegia. Further evidence is required to understand the effect of SCI

on substrate handling following consumptions of a mixed-meal, and to identify ways to influence postprandial metabolism in this population.

In non-injured humans, pre-meal exercise has a robust effect on postprandial glycemia (17) and lipemia (18). To our knowledge only one study has looked at the acute interaction of feeding and exercise in persons with SCI (19). Twenty ~~P~~ persons with and without chronic SCI (85% motor complete, 90% paraplegia) consumed a high fat meal (48 g fat, 37 % fat by kcal) and 30-min later, performed ~50 min of aerobic exercise. A blood sample was obtained ~4 hr post-meal that showed no difference in 4-hr blood glucose or triglyceride concentrations between people with and without SCI. However, a single blood draw is insufficient to quantify the dynamic glycemic and lipemic response to feeding. Furthermore, rates of postprandial substrate oxidation were not measured. It is possible that the exercise employed in this study (19) was of an insufficient energy cost to modify postprandial metabolism. However, it is also possible that the mode and/or intensity of exercise was not optimal for influencing energy expenditure during recovery from exercise. Previous studies have determined that, independent of total energy cost, exercise mode (20) and intensity (21) modulate changes in post-exercise metabolism in ~~neurologically intact~~non-injured individuals. However, the optimal exercise mode and intensity for influencing postprandial metabolism in persons with SCI has yet to be determined.

It remains unknown whether, in persons with SCI, the mode or intensity of exercise influences the metabolic handling and oxidation of macronutrients during a mixed meal tolerance test (MMTT). The objectives of this study were therefore to compare the effects of resting control (CON), moderate intensity continuous exercise (MICE), high intensity interval exercise (HIIE) and continuous resistance exercise (CRE) on (1) Fasting systemic concentrations of metabolites and hormones, (2) postprandial systemic concentrations of metabolites and hormones, and (3) postprandial energy expenditure (EE) and whole-body substrate oxidation rates. We hypothesized that higher intensity modes of intermittent upper-body exercise (i.e. HIIE and

CRE) will enhance measures of fasting and postprandial insulin sensitivity, compared to moderate intensity exercise (MICE) or rest (CON).

METHODS

This study is a partially randomized repeated measures counter-balanced design. It is registered with ClinicalTrials.gov (NCT03545867) and procedures were in accordance with the Human Subjects Research Office, University of Miami Miller School of Medicine. The protocol has been published in full (22), with trial enrollment and eligibility testing all conducted in accordance with Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (22). A flow diagram has been provided (Figure 1).

Participants

Eleven individuals with chronic SCI provided written consent to participate in this study, which was approved by institutional ethical authorities. Participants were male aged ≥ 18 years old with neurologically stable spinal cord injury (ASIA Impairment Scale A-C) at T1 and lower spinal levels for > 1 year who were able and willing to comply with study procedures. Exclusion criteria included ACSM contraindication to exercise, lower extremity fracture or dislocation within 6 months of participation, inability to provide informed consent, restrictions in upper extremity range of motion that would prevent an individual from achieving an unhindered arm cycling motion or moving throughout a range needed to perform resistance manoeuvres, pressure ulcer at ischial/gluteus, trochanteric, sacral, or heel sites within the last 3 months, taking any medication that might interfere with the study outcomes, or having been diagnosed with an illness/condition that might interact with study measures (e.g. diabetes, heart disease) or pose undue personal risk.

Baseline assessments and HIIE familiarization

Participants attended two preliminary sessions including baseline assessments and a HIIE familiarisation session before completing the four experimental conditions. Participants were

instructed to refrain from exercise/alcohol/caffeine for 24 h prior to testing and to arrive at the laboratory normally hydrated (500 ml of water within 1 h of testing). During their first preliminary visit, participants' cardiorespiratory fitness and muscular strength were assessed via an arm cycle graded exercise test and a 1-repetition maximum text, respectively, as previously described (22).

During their second preliminary visit to the laboratory, participants were fitted with a Hans-Rudolph Softmask and expired gases were collected and analyzed throughout arm cycle exercise (as described above). Participants conducted ACE on the same device/position as described above. The cycle ergometer was programmed to vary power output so that a warm-up and cool-down (2 min) and the active recovery intervals were completed at 10% PO_{peak}, and the working intervals completed at 70% PO_{peak}. The ratio of work to recovery intervals was 1:1. The EE data were used to calculate the duration of HIIE required to elicit an isocaloric challenge to CRE.

Experimental exercise and feeding trials

Participants completed the CRE condition first, allowing for the intensity and/or duration of the HIIE and MICE protocols to be adjusted to deliver an isocaloric exercise challenge. Prior to the first trial, participants were provided with a food journal and asked to record their dietary intake for the twenty-four hours prior to the CRE trial. Following completion of CRE, one of the study team (JLM or DWM) reviewed the food journal with the participant and the provided them with a copy of the journal that would then serve as their dietary plan for the subsequent main trials. Regardless of their ability to successfully follow the plan, they were instructed to record their actual dietary intake preceding the subsequent trials, and these food journals were reviewed and analysed. A commercial food analysis software (Food Processor v11.6, ESHA Research, Salem, OR, United States) was used to quantify participant's macronutrient intake. Following CRE, the remaining CON, MICE and HIIE conditions were completed in a randomised order, at least 48 h apart, with all four trials completed within a 1 month period.

Twenty-four hours prior to each main laboratory trial, participants were asked to abstain from caffeine, ~~and~~ alcohol ingestion, and strenuous exercise. However, physical activity habits on the day before, and leading up to, the trials was not recorded or controlled. On the morning of the main trials, participants were instructed to consume ~10 ml·kg^{-1} of water on waking and report to the laboratory following an overnight fast (≥ 10 h). Upon arrival, participants were fitted with the mask for indirect calorimetry (as described above) and remained seated in their wheelchair for 20 min to assess resting energy expenditure (REE). The final 10 min of this baseline measurement were used to determine pre-meal REE. Immediately after this, an initial 10 ml venous blood sample (T_{-45}) was drawn to determine the insulin and metabolite concentrations (see below). For the next ~30-50 min (depending on condition), expired gases and heart rate were collected while the participants rested (CON) or exercised (MICE, HIIE or CRE). Immediately after this period, an indwelling cannula was inserted in to an antecubital vein as previously described (22) and kept patent with sterile saline. An initial sample (T_0) was drawn before participants consumed a 600 kcal liquid test meal (to be ingested in ≤ 6 min) consisting of a macronutrient distribution equal to *ad libitum* published norms in SCI (35% Fat, 50% CHO, 15% Protein) (23). Further 10 ml venous blood samples were drawn 30 min following the meal (T_{30}), and at 30 min intervals after that (T_{60}, T_{90}, T_{120}, T_{150}) until 150 min post-meal. Expired gases were collected throughout the postprandial period.

Continuous resistance exercise (CRE)

Following baseline measurements, participants conducted 40.0 ± 4.6 min of CRE consisting of resistance maneuvers (weightlifting) and low-resistance, high-speed endurance activities (ACE). Each session was preceded by two minutes of ACE. Participants then performed 1 set of 10 repetitions for two of the following resistance maneuvers: (1) military press, (2) horizontal rows, (3) pectoralis ("pec") deck, (4) preacher curls (elbow flexion), (5) wide grip latissimus pull-down, and (6) seated dips. A detailed pictorial guide to the CRE is available in ref. (22) Figure 3 . Resistance maneuvers were performed in pairs and followed by 2 minutes

of ACE without applied resistance. Every time participants completed two resistance exercises they performed low-resistance, high-speed arm exercise for two minutes on a stationary cycle. Transitions between equipment occurred as quickly as possible, and a complete session involved three rounds of the cycle of six exercises. Resistive loads for the CRE session were 60% 1RM as determined during strength testing. The energy expenditure response to CRE (methods below) was used as a calorie target for the other exercise trials. The duration of CRE was used as the duration of seated rest in CON.

Resting control (CON)

During the resting control (CON) condition, participants remained seated in their wheelchair for the same duration as the CRE condition (38.9 ± 4.3 min). If they required the bathroom during this period, they were pushed to and from the room and the time recorded.

Moderate intensity continuous exercise (MICE)

The graded exercise test was used to generate a PO vs VO_2 regression equation. This individualized equation was used to estimate a power output during MICE that would elicit the same relative intensity (%VO_{2peak}) and duration as the CRE trial. The relationship between PO and VO_2 estimated that 26.1 ± 7.3 % PO_{peak} would elicit the 53.5 ± 7.0 % VO_{2peak} observed during CRE. Participants conducted 39.8 ± 4.6 min of ACE on the same device/position as described above.

High intensity interval exercise (HIIE)

Following baseline measurements, participants conducted ~~32.2 ± 6.2 min of~~ ACE for a duration (32.2 ± 6.2 min) estimated to achieve a calorie expenditure during HIIE equal to CRE ~~as described above~~. The cycle ergometer was programmed to vary the resistance to produce a power output for the warm-up, cool-down (2.5 min) and active recovery intervals equivalent to 10% PO_{peak}, and the working intervals completed at 70% PO_{peak}. The ratio of work to recovery intervals was 1:1. The energetic response to the HIIE familiarization trial was used to

estimate the number of bouts required so that total EE during HIIE was equivalent to the CRE condition.

~~*Continuous resistance exercise (CRE)*~~

~~Following baseline measurements, participants conducted 40.0 ± 4.6 min of CRE consisting of resistance maneuvers (weightlifting) and low-resistance, high-speed endurance activities (ACE). Each session was preceded by two minutes of ACE. Participants then performed 1 set of 10 repetitions for two of the following resistance maneuvers: (1) military press, (2) horizontal rows, (3) pectoralis ("pec") deck, (4) preacher curls (elbow flexion), (5) wide grip latissimus pull-down, and (6) seated dips. A detailed pictorial guide to the CRE is available in ref. (22) Figure 3 . Resistance maneuvers were performed in pairs and followed by 2 minutes of ACE without applied resistance. Every time participants completed two resistance exercises they performed low-resistance, high-speed arm exercise for two minutes on a stationary cycle. Transitions between equipment occurred as quickly as possible, and a complete session involved three rounds of the cycle of six exercises. Resistive loads for the CRE session were 60% 1RM as determined during strength testing.~~

Energy expenditure, substrate oxidation, and blood analytes

Energy expenditure and substrate oxidation rates were determined from expired gas analysis averaged over each exercise bout and in 20 min bins between postprandial blood draw time points. For example, indirect calorimetry data labelled "$Post_{0\text{-}30}$" is an average of 20 min of expired gas data between the blood draw T_0 and T_{30}. The appropriate stoichiometric equations were used (24) to calculate EE and substrate oxidation from indirect calorimetry data. These updated equations are calibrated for high intensity exercise where an estimated 80% of carbohydrate oxidation is assumed to come from intramuscular glycogen stores (24). Biochemical assays were performed by the Biomarker and Immunoassay laboratory at the Diabetes Research Institute, University of Miami. Insulin, glucose, and triglycerides measurements were performed by automated analyser on a Roche Cobas 6000 analyser (Roche

Diagnostics, Indianapolis, IN, United States) using manufacturer's reagents and following all instructions for instrument maintenance and assay calibration and test procedures. Intra- and inter-assay % CVs for insulin, glucose and TG were 1.2 and 3.8; 1.1 and 2.4; and 1.6 and 2.1, respectively. Non-esterified fatty acids (NEFA) was measured using reagents from Sekisui Diagnostics (Burlington, MA, United States) and glycerol using kits form Millpore Sigma (St Louis, MO) adapted for use in the Roche analyser. Intra- and inter-assay % CV were 4.1 and 6.5 for NEFA and 3.8 and 5.9 for glycerol determinations. American Diabetes Association (ADA) guidelines for using OGTT to determine dysglycemia (any postprandial [glucose] > 11.1 $mmol \cdot L^{-1}$) (25) were used to identify exaggerated postprandial glucose excursions. Expert panel guidelines for using oral fat tolerance test (OFTT) to determine dyslipidemia (any postprandial [TG] > 2.5 $mmol \cdot L^{-1}$) (26) were used to identify exaggerated postprandial lipid excursions.

Statistical analysis

Expired gas data during exercise and pre-trial nutritional data were analysed using a one-way analysis of variance (ANOVA) to detect differences between experimental conditions. Postprandial expired gas and blood analyte data were analysed using a two-way (condition × time) repeated measures ANOVA to detect differences between experimental conditions (CON, MICE, HIIE, and CRE) and across time (dependent on variable). Where significant interactions and main effects were observed, simple effects analysis was used to determine the location of variance. Asphericity was determined with Greenhouse-Geisser epsilon; all values were <0.75 and were corrected for with Greenhouse-Geisser correction. Serial measurements of glucose and insulin responses at baseline and in response to the rest/exercise challenge were converted into simple summary statistics (27), such as insulin sensitivity index (ISIMatsuda) (28) and the Homeostasis Model Assessment (HOMA) calculator, incorporating the updated HOMA-2 model (28). Individual metabolite area under the curves (AUC) (GraphPad Prism v5, GraphPad Software, La Jolla, CA,) were calculated for 150 min of the MMTT. Standardized effect sizes (Cohen's d) were calculated for AUC. Based on the magnitude of correlation

between trials, thresholds of >0.2 (small), >0.5 (moderate) and >0.8 (large) were used. For all the above statistical approaches, statistical significance was set at an alpha level of $p \leq 0.05$ and data are presented as mean ± SD.

RESULTS

Participant characteristics

Descriptive characteristics and basic injury characteristics of the ten men with chronic SCI who completed the trial are presented in Table 1. Following baseline assessments and HIIE familiarization, one participant was withdrawn from the study having been prescribed medication for type 2 diabetes by his physician. Participants were, on average, of "good" cardiorespiratory fitness (19.2 ± 5.2 $ml \cdot kg^{-1} \cdot min^{-1}$) based on a normative classification for persons with SCI (29), but fitness varied within the group. Peak heart rate (169 ± 16 $b \cdot min^{-1}$) suggests that injury did not result in disruption of sympathetic output to the myocardium.

Dietary intake

Participant's average pre-trial habitual dietary intake was 1,942 ± 15 $kcal \cdot day^{-1}$ at 37% Fat, 39% CHO, and 22% Protein (Table 2). There were no differences in participant reported caloric intake (*P*=.653) or dietary macronutrient content (Fat *P*=.184; CHO *P*=.729; Protein *P*=.537) in the 24 h preceding each experiment (Table 2).

Energy expenditure and substrate oxidation rates at baseline and during exercise

There were no significant differences between conditions in rates of EE or substrate oxidation at baseline (Figure 2). All exercise conditions were matched for total energy cost (116 ± 22, 117 ± 35, and 118 ± 22 kcal, respectively; *P*=.982). However, rates of EE were significantly greater in HIIE compared to MICE and CRE (*P*=.01) (Table 2). Participants achieved a significantly greater % VO_{2peak} (*P*<.001) in HIIE compared to MICE and CRE (Table 2). Respiratory exchange ratio (RER) was lower (*P*<.001) in MICE (0.90 ± 0.08) compared to HIIE and CRE (1.01 ± 0.07 and 1.05 ± 0.04, respectively) (Table 2).

Postprandial energy expenditure and substrate oxidation rates

There was a significant main effect of time (P=.039) and condition (P=.024) on rates of EE during recovery (Figure 2). However, the time-condition interaction term did not reach statistical significance (P=.374). Pairwise tests indicated that postprandial EE were significantly greater at $Post_{30\text{-}60}$ (P=.050) and $Post_{60\text{-}90}$ (P=.039) compared to baseline, and that EE during the HIIE condition was significantly greater than the CON (P=.038) condition.

There was a significant main effect of time (P=.020), condition (P=.000) and time-condition interaction (P=.000) for lipid oxidation (Lox; Figure 2). Pairwise tests indicate that the rate of Lox in CRE was significantly greater than CON at all time points ($Post_{0\text{-}30}$, P= .000; $Post_{30\text{-}60}$, P=.002; $Post_{60\text{-}90}$, P=.019; $Post_{90\text{-}120}$ P=.027; $Post_{120\text{-}150}$, P=.044), significantly greater than MICE at $Post_{0\text{-}30}$ (P=.030) and $Post_{30\text{-}60}$ (P=.039) and significantly greater than HIIE at $Post_{60\text{-}90}$ (P=.014). Lox in HIIE was significantly greater than CON at $Post_{0\text{-}30}$ (P=.007) and $Post_{30\text{-}60}$ (P=.015).

~~There was a significant main effect of time~~ F~~f~~or carbohydrate oxidation there was a significant main effect of time (P=.000) and a time-condition interaction (P=.006). ~~in which the r~~Rate of oxidation was significantly lower at $Post_{0\text{-}30}$ compared to all time points (all P=.000) and $Post_{30\text{-}60}$ was significantly lower than time $Post_{60\text{-}90}$ (P=.000), $Post_{90\text{-}120}$ (P=.000) and $Post_{120\text{-}150}$ (P=.003).

Metabolite concentrations in the fasted and postprandial states

Metabolite concentrations measured in the fasted state (T_{-45}) and post-exercise (T_0) are shown in Table 3. There was no significant main effect of condition or time-condition interaction for concentrations of glucose, insulin, TG and NEFA. There was a significant main effect of time in which glucose concentration at T_0 was significantly greater than T_{-45} (P=.023). There was a significant main effect of time (P=.015), condition (P=.000) and a time-condition interaction

306 (*P*=.040) for glycerol. Simple effects analysis indicated a significant increase from T_{-45} to T_0 in
307 the MICE (*P*=.008) and HIIE (*P*=.004) conditions only. There were no differences observed in
308 HOM2-IR insulin sensitivity (Table 3).
309
310 Based on ADA guidelines for OGTT (25), no participants displayed signs of postprandial
311 dysglycemia (any postprandial [glucose] > 11.1 $mmol \cdot L^{-1}$). Based on expert panel guidelines
312 for oral fat tolerance test (OFTT) (26), three of 10 participants displayed signs of postprandial
313 lipemia (PPL) (any postprandial [TG] > 2.5 $mmol \cdot L^{-1}$).
314
315 *Postprandial metabolite responses*
316 Figure 3 A-E shows the 2-hour MMTT AUC across all conditions representing changes in post-
317 load concentrations of glucose (A), insulin (B), TG (C), NEFA (D) and glycerol (E).
318 Considering the whole 150-min post-load experiment, there were no significant differences in
319 AUC in any metabolite between conditions (Table 4). Cohen's d effect sizes showed a
320 moderate-to-strong effect for comparisons between conditions for TG (CRE vs HIIE = -.44),
321 NEFA (CRE vs MICE = -.76; CRE vs HIIE = -.50), and Glycerol (MICE vs CON = -.49; CRE
322 vs CON = -.71). All other comparisons had a weak (Coden's d <±.40) effect size.
323
324 **DISCUSSION**
325 The primary finding of this study is that, independent of total exercise energy cost, exercise
326 intensity and mode modulate postprandial EE and Lox in persons with paraplegia. Secondarily,
327 our data provide provisional evidence suggesting that exercise mode modulates the
328 postprandial lipemic response. There were no differences between conditions in terms of
329 postprandial glucose or insulin responses.
330
331 *Postprandial metabolism*
332 This is one of the few scientific studies examining postprandial macronutrient metabolism in
333 response to MMTT in persons with chronic paraplegia. Previous studies of postprandial

metabolism in SCI used OGTT or OFTT that have atypical macronutrient compositions, often relying on a single macronutrient as the sole stimulus. In contrast, our MMTT was designed to reflect the energy and macronutrient content of an *ad lib* meal in persons with SCI (23).

Based on ADA guidelines for OGTT (25), no participants displayed signs of postprandial dysglycemia. Compared to a standard 75 g OGTT (25), the MMTT used in the current study contained a similar total carbohydrate load (75.5 g), but was comprised of different carbohydrate (glucose polymer from the carbohydrate powder, and a mix of sucrose/fructose/glucose from the banana) as opposed to homogeneous anhydrous glucose in an OGTT. Furthermore, the insoluble fiber and other macronutrients in our meal likely reduced the peak amplitude of the postprandial glucose and insulin response compared to OGTT (30). However, while absolute values differ, peak glucose excursions after OGTT and MMTT are well correlated (30) and insulin resistance calculated from MMTT can be effectively compared to insulin sensitivity during OGTT (31). Moreover, MMTT has a similar C-peptide response (30) and might therefore reflect pancreatic functions better compared to OGTT (31). Therefore, because our MMTT has a similar total carbohydrate content as an OGTT, the results of postprandial glucose metabolism in this study indicate that none of our participants had impaired glucose handling.

Based on expert panel guidelines for OFTT (26), three of 10 participants displayed signs of PPL. This impaired postprandial fat metabolism was seen even though the MMTT used in the current study contained ~250 less kilocalories and ~50 g less fat than the OFTT upon which the guidelines are based (26). These postprandial fat excursion data indicate that postprandial fat metabolism was relatively more impaired than postprandial glucose metabolism in our sample.

Pre-meal exercise and postprandial energy utilization

Exercise performed prior to a meal has the ability to increase postprandial EE (32). Without prior feeding, exercise intensity is the primary determinant of post-exercise EE especially when an exercise session is limited in duration (33). For example, in ~~neurologically intact~~non-injured cyclists, sprint interval training (SIT) lasting 14 min and costing 132 kcal elicited a total post-exercise energy expenditure equal to that seen after 30 min of continuous exercise at 85% VO_{2peak} costing 493 kcal (34). The effects of pre-meal exercise on postprandial EE are also intensity dependent, with previous studies suggesting a minimum intensity threshold of ~60% VO_{2peak} (35). In the current study HIIE was above the posited % VO_{2peak} threshold, contributing to why postprandial EE was elevated above CON in the HIIE condition. The % VO_{2peak} intensity of MICE and CRE (53.0 ± 6.6 and 53.5 ± 7.0% VO_{2peak}, respectively) in the current study were below this threshold. However, at $Post_{30-60}$ EE was elevated in CRE vs CON (Figure 2). This finding can be explained by the intensity of contraction during the resistance maneuvers, which resulted in local cellular stress that is not fully reflected in % VO_{2peak}. Given that the total cost of exercise was similar in all conditions and the % VO_{2peak} intensity was similar in MICE and CRE, our findings show that in persons with paraplegia exercise that is more reliant on carbohydrate oxidation ~~the amount of carbohydrate reliance during exercise best explains~~has a great impact on ~~the effects of pre-meal exercise on~~ postprandial EE.

Exercise mode and intensity had robust effects on postprandial substrate oxidation (Figure 2). During exercise rates of carbohydrate and Lox can be changed dramatically, with HIIE and CRE having a greater ~~reliance on~~ carbohydrate ~~use~~ oxidation (36). During recovery from exercise the body transitions to an increased reliance on fat that persists for hours (37) to days (38). Kuo et al (37) showed that in ~~neurologically intact~~non-injured persons exercise energy expenditure during MICE determined post-exercise fat use independent of exercise intensity. MICE can only be conducted within a limited range of intensities, and thus when compared to HIIE it is often found that MICE has a lesser effect on post-exercise metabolism (21). Furthermore, in ~~neurologically intact~~non-injured persons, a session of resistance exercise with approximately half the energy cost as MICE resulted in similar attenuation of PPL due in part

to increasing exogenous Lox (39). However, the effect of pre-meal exercise on postprandial fuel partitioning had yet to be determined in persons with SCI. In our study postprandial Lox was greater in CRE and HIIE compared to MICE and CON, and only CRE resulted in elevated Lox at the 2 hr postprandial timepoint. Similar to postprandial EE, our data show that that pre-meal exercise influences postprandial substrate oxidation in a manner dependent on the degree of carbohydrate ~~reliance~~ oxidation during exercise.

Pre-meal exercise and postprandial metabolite concentrations

Our data show little effect of pre-meal exercise on postprandial glucose concentration following MMTT (Figure 3). The circulating concentrations of glucose and insulin in all conditions, including CON, show that glucose homeostasis was well maintained. The participants in this study were relatively fit and did not have evidence of glycemic dysregulation based on fasted and postprandial (Table 3 and Figure 3) glucoses. Thus the finding that exercise had little effect on circulating glucose and insulin might be due to a floor effect due to the lower peak glycemic response to the MMTT and the lack of glycemic dysregulation in our participants. Furthermore, compared to a standard bout of exercise in ~~neurologically intact~~non-injured persons where 45 min of exercise results in ~200-600 kcal expenditure (40), the energy cost of our exercise was relatively low (~120 kcal). The results of our study may suggest that there is a minimum energy expenditure threshold required for pre-meal exercise to influence postprandial metabolite concentrations. This possibility needs to be considered in the context of exercise as a strategy for improving cardiometabolic health in persons with SCI. Obligatory upper extremity exercise and increased potential for overuse injuries in persons with SCI place a practical limit on the total exercise energy expenditure.

With respect to postprandial circulating lipids and their metabolites, statistical differences were observed only for glycerol where MICE (P=.020) and CRE (P=.001) were greater than CON. Postprandial lipemia based on peak triglyceride ≥ 2.5 mmol·L^{-1} (26) was observed after CON (3 participants), MICE (2 participants), and HIIE (3 participants). After CRE no participants

had triglyceride concentration above 2.5 mmol·L^{-1}. Therefore, CRE seemed to partially accommodate for the disordered postprandial fat metabolism inherent to SCI and observed in the current study. This response might be explained by an increased catecholamine response to CRE compared to other modes of exercise, although these were not measured. Only four of our participants had SCI above the neurological level whereby the catecholamine response to exercise is impaired (<T4) (41), and all of these participants had normal cardioacceleratory capacity (Table 1) suggesting intact SNS signaling. Beyond endocrine signaling, ~~CRE~~ our results could ~~have a greater effect on postprandial Lox due to~~be explained by local factors produced during exercise. Greater skeletal muscle glycogen reduction results in increased Lox by exercised muscles as glucose is preferentially used for glycogen resynthesis during recovery (42). ~~I~~While we did not measure glycogen utilization it is well established that HIIE and high intensity contraction in general result in greater glycogen reductions (42), but there is little data examining glycogen metabolism during circuit-style exercise. ~~I~~While there is also no current data in this area, it is also possible that contraction resulted in the release of myogenic signaling molecules (43) labeled for specific target tissues related to energy metabolism (44).

Methodological considerations

The purpose of this study was to identify the optimal exercise strategies for influencing metabolic function in persons with SCI. We aimed to control for nutritional intake for 24-hr before each trial to isolate the effects of select exercise parameters. Our strategy utilized a self-reported food journal, completed before the first trial and then reproduced before the following experimental trials. Participants reported similar energy and macronutrient intake before each trial, however, there is an inherent source of error associated with self-reported food journals. Furthermore, it is possible that nutritional differences in the >24 h preceding the trials may influence metabolism during our testing. ~~Total energy expenditure during exercise sessions was matched within ~3 kcal between trials to further isolate the effects of exercise intensity and mode. CRE was conducted first because CRE does not lend itself to predictions of energy expenditure, while there are methodologies for predicting energy expenditure during cycling~~

~~type activity(24).(45)~~ While the HIIE prescription is not conventional (10:70 %PO_{peak}), the physiological response confirms that HIIE occurred at a high intensity as 29.4 ± 7.7 % of the duration of the session was spent at or above 80 % VO_{2peak}. Finally, one further methodological consideration should be considered when interpreting our results. Our previous data indicate that CRE will elicit a mean exercise energy expenditure of ~170 kcal in persons with paraplegia (46), while the data from the current study show an expenditure of ~120 kcal. Most importantly, all previous CRE studies in SCI (3, 46, 47) calculated energy expenditure using stochiometric equations (24) that assume that the blood glucose is the only type of carbohydrate contributing to carbohydrate oxidation. In the current study we employed more appropriate calculations for glycolytic exercise (24) that assume 80% of carbohydrate use is due to utilization of muscle glycogen. Muscle glycogen utilization is energetically more efficient, thus yielding a calculation of carbohydrate oxidation that is approximately 10% lower than the calculations applied to the previous data. Given the average RER of 1.01 in the CRE condition, carbohydrate oxidation accounts for nearly all of the total energy expenditure, thus exacerbating the difference in the calculations. Furthermore, the participants in the previous study (46) were ~4 kg heavier and, assuming this difference was related to lean tissue mass, this likely contributed to a greater total energy expenditure.

Limitations

We used indirect calorimetry to match exercise EE, and CRE violates the assumptions of the stoichiometric equations used to calculate EE from indirect calorimetry (24). Accordingly, it is possible that EE was underestimated in CRE. This difference in exercise EE is an important consideration. However, studies that have used blood lactate and excess postexercise oxygen consumption suggest that if indirect calorimetry does underestimate EE during resistance exercise, the differences are relatively small (45). Based on current American Diabetes Association (25) guidelines, neither fasted blood glucose nor triglycerides (Table 3) were elevated. Furthermore, HOMA-IR was within a “normal” range. These findings of a “healthy” fasted metabolic profile in our participants means that the results of our study are not

generalizable to the large portion of the SCI population that live with stark diabetes and dyslipidemia (1). Furthermore, 40% of our participants classified as having "good" or better CRF (29), limiting the application of our results to the considerable portion of the SCI population that exists at the lowest end of the spectrum of CRF (29). Future studies should aim to understand the interaction of feeding and exercise in a population of persons with SCI who have greater metabolic impairments and thus are more representative candidates for lifestyle interventions targeting metabolic health.

Conclusions

This study is the first to demonstrate that pre-meal exercise influences postprandial metabolism in persons with SCI. Importantly, exercise intensity and mode modulate postprandial energy expenditure and substrate utilization independent of the energy cost of exercise. Furthermore, our data demonstrate that pre-meal exercise has a limited effect on macronutrient handling in paraplegics with good fitness and relatively healthy postprandial glycemic and lipemic responses. However, ~~provide provisional evidence suggesting that~~only circuit-style exercise ~~has the greatest potential to decrease systemic concentrations of blood-borne fats during the~~resulted in ~~postprandial period~~all participants have peak postprandial triglycerides being below the postprandial lipemia (2.5 mmol·L^{-1}) cutoff.

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