University of Bath

Title

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

Short Title

Exercise and postprandial metabolism in SCI

Authors

[1,2]McMillan DW, [3]Maher JL, [4]Jacobs KA, [5]Mendez AJ, [1]Nash MS, [3]Bilzon JLJ

Contact Information and Affiliations

[1]The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, Miami FL, USA; [2]Department of Physical Medicine and Rehabilitation, University of Miami Miller School of Medicine, Miami FL, USA; [3]Department for Health, University of Bath, Bath, Somerset, UK; [4]Department of Kinesiology and Sport Sciences, University of Miami, Miami, FL, USA; [5]Division of Endocrinology, Diabetes and Metabolism, University of Miami Miller School of Medicine, Miami, FL, USA

Author and Address for correspondence:

David W McMillan, Christine E. Lynn Rehabilitation Center for the Miami Project to Cure Paralysis, 1611 NW 12th Ave, Room 3.163, Miami, FL 33136; phone: 305-248-6320, e-mail: dmcmillan@med.miami.edu

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% CHOCarbohydrate, 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·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

54 on substrate handling following consumptions of a mixed-meal, and to identify ways to

55 influence postprandial metabolism in this population.

56

57 In non-injured humans, pre-meal exercise has a robust effect on postprandial glycemia (17)

58 and lipemia (18). To our knowledge only one study has looked at the acute interaction of

59 feeding and exercise in persons with SCI (19). Twenty P persons with and without chronic SCI

60 (85% motor complete, 90% paraplegia) consumed a high fat meal (48 g fat, 37 % fat by kcal)

61 and 30-min later, performed ~50 min of aerobic exercise. A blood sample was obtained ~4 hr

62 post-meal that showed no difference in 4-hr blood glucose or triglyceride concentrations

63 between people with and without SCI. However, a single blood draw is insufficient to quantify

64 the dynamic glycemic and lipemic response to feeding. Furthermore, rates of postprandial

65 substrate oxidation were not measured. It is possible that the exercise employed in this study

66 (19) was of an insufficient energy cost to modify postprandial metabolism. However, it is also

67 possible that the mode and/or intensity of exercise was not optimal for influencing energy

68 expenditure during recovery from exercise. Previous studies have determined that, independent

69 of total energy cost, exercise mode (20) and intensity (21) modulate changes in post-exercise

70 metabolism in neurologically intactnon-injured individuals. However, the optimal exercise

71 mode and intensity for influencing postprandial metabolism in persons with SCI has yet to be

72 determined.

73

74 It remains unknown whether, in persons with SCI, the mode or intensity of exercise influences

75 the metabolic handling and oxidation of macronutrients during a mixed meal tolerance test

76 (MMTT). The objectives of this study were therefore to compare the effects of resting control

77 (CON), moderate intensity continuous exercise (MICE), high intensity interval exercise (HIIE)

78 and continuous resistance exercise (CRE) on (1) Fasting systemic concentrations of metabolites

79 and hormones, (2) postprandial systemic concentrations of metabolites and hormones, and (3)

80 postprandial energy expenditure (EE) and whole-body substrate oxidation rates. We

81 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 $\geq$ 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

110 instructed to refrain from exercise/alcohol/caffeine for 24 h prior to testing and to arrive at the

111 laboratory normally hydrated (500 ml of water within 1 h of testing). During their first

112 preliminary visit, participants' cardiorespiratory fitness and muscular strength were assessed

113 via an arm cycle graded exercise test and a 1-repetition maximum text, respectively, as

114 previously described (22).

115

116 During their second preliminary visit to the laboratory, participants were fitted with a Hans-

117 Rudolph Softmask and expired gases were collected and analyzed throughout arm cycle

118 exercise (as described above). Participants conducted ACE on the same device/position as

119 described above. The cycle ergometer was programmed to vary power output so that a warm-

120 up and cool-down (2 min) and the active recovery intervals were completed at 10% PO_{peak}, and

121 the working intervals completed at 70% PO_{peak}. The ratio of work to recovery intervals was

122 1:1. The EE data were used to calculate the duration of HIIE required to elicit an isocaloric

123 challenge to CRE.

124

125 *Experimental exercise and feeding trials*

126 Participants completed the CRE condition first, allowing for the intensity and/or duration of

127 the HIIE and MICE protocols to be adjusted to deliver an isocaloric exercise challenge. Prior

128 to the first trial, participants were provided with a food journal and asked to record their dietary

129 intake for the twenty-four hours prior to the CRE trial. Following completion of CRE, one of

130 the study team (JLM or DWM) reviewed the food journal with the participant and the provided

131 them with a copy of the journal that would then serve as their dietary plan for the subsequent

132 main trials. Regardless of their ability to successfully follow the plan, they were instructed to

133 record their actual dietary intake preceding the subsequent trials, and these food journals were

134 reviewed and analysed. A commercial food analysis software (Food Processor v11.6, ESHA

135 Research, Salem, OR, United States) was used to quantify participant's macronutrient intake.

136 Following CRE, the remaining CON, MICE and HIIE conditions were completed in a

137 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 ($\geq$ 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 $\leq$ 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

166 of ACE without applied resistance. Every time participants completed two resistance exercises

167 they performed low-resistance, high-speed arm exercise for two minutes on a stationary cycle.

168 Transitions between equipment occurred as quickly as possible, and a complete session

169 involved three rounds of the cycle of six exercises. Resistive loads for the CRE session were

170 60% 1RM as determined during strength testing. The energy expenditure response to CRE

171 (methods below) was used as a calorie target for the other exercise trials. The duration of CRE

172 was used as the duration of seated rest in CON.

173

174 *Resting control (CON)*

175 During the resting control (CON) condition, participants remained seated in their wheelchair

176 for the same duration as the CRE condition (38.9 ± 4.3 min). If they required the bathroom

177 during this period, they were pushed to and from the room and the time recorded.

178

179 *Moderate intensity continuous exercise (MICE)*

180 The graded exercise test was used to generate a PO vs VO_2 regression equation. This

181 individualized equation was used to estimate a power output during MICE that would elicit the

182 same relative intensity (%VO_{2peak}) and duration as the CRE trial. The relationship between PO

183 and VO_2 estimated that 26.1 ± 7.3 % PO_{peak} would elicit the 53.5 ± 7.0 % VO_{2peak} observed

184 during CRE. Participants conducted 39.8 ± 4.6 min of ACE on the same device/position as

185 described above.

186

187 *High intensity interval exercise (HIIE)*

188 Following baseline measurements, participants conducted ~~32.2 ± 6.2 min of~~ ACE for a duration

189 (32.2 ± 6.2 min) estimated to achieve a calorie expenditure during HIIE equal to CRE ~~as~~

190 ~~described above~~. The cycle ergometer was programmed to vary the resistance to produce a

191 power output for the warm-up, cool-down (2.5 min) and active recovery intervals equivalent

192 to 10% PO_{peak}, and the working intervals completed at 70% PO_{peak}. The ratio of work to

193 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 "$Post_{0-30}$" 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

222 Diagnostics, Indianapolis, IN, United States) using manufacturer's reagents and following all

223 instructions for instrument maintenance and assay calibration and test procedures. Intra- and

224 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,

225 respectively. Non-esterified fatty acids (NEFA) was measured using reagents from Sekisui

226 Diagnostics (Burlington, MA, United States) and glycerol using kits form Millpore Sigma (St

227 Louis, MO) adapted for use in the Roche analyser. Intra- and inter-assay % CV were 4.1 and

228 6.5 for NEFA and 3.8 and 5.9 for glycerol determinations. American Diabetes Association

229 (ADA) guidelines for using OGTT to determine dysglycemia (any postprandial [glucose] >

230 11.1 mmol·L^{-1}) (25) were used to identify exaggerated postprandial glucose excursions. Expert

231 panel guidelines for using oral fat tolerance test (OFTT) to determine dyslipidemia (any

232 postprandial [TG] > 2.5 mmol·L^{-1}) (26) were used to identify exaggerated postprandial lipid

233 excursions.

234

235 *Statistical analysis*

236 Expired gas data during exercise and pre-trial nutritional data were analysed using a one-way

237 analysis of variance (ANOVA) to detect differences between experimental conditions.

238 Postprandial expired gas and blood analyte data were analysed using a two-way (condition ×

239 time) repeated measures ANOVA to detect differences between experimental conditions

240 (CON, MICE, HIIE, and CRE) and across time (dependent on variable). Where significant

241 interactions and main effects were observed, simple effects analysis was used to determine the

242 location of variance. Asphericity was determined with Greenhouse-Geisser epsilon; all values

243 were <0.75 and were corrected for with Greenhouse-Geisser correction. Serial measurements

244 of glucose and insulin responses at baseline and in response to the rest/exercise challenge were

245 converted into simple summary statistics (27), such as insulin sensitivity index (ISIMatsuda)

246 (28) and the Homeostasis Model Assessment (HOMA) calculator, incorporating the updated

247 HOMA-2 model (28). Individual metabolite area under the curves (AUC) (GraphPad Prism v5,

248 GraphPad Software, La Jolla, CA,) were calculated for 150 min of the MMTT. Standardized

249 effect sizes (Cohen's d) were calculated for AUC. Based on the magnitude of correlation

250   between trials, thresholds of >0.2 (small), >0.5 (moderate) and >0.8 (large) were used. For all

251   the above statistical approaches, statistical significance was set at an alpha level of $p \leq 0.05$

252   and data are presented as mean $\pm$ SD.

253

254   **RESULTS**

255   *Participant characteristics*

256   Descriptive characteristics and basic injury characteristics of the ten men with chronic SCI who

257   completed the trial are presented in Table 1. Following baseline assessments and HIIE

258   familiarization, one participant was withdrawn from the study having been prescribed

259   medication for type 2 diabetes by his physician. Participants were, on average, of "good"

260   cardiorespiratory fitness (19.2 ± 5.2 ml·kg^{-1}·min^{-1}) based on a normative classification for

261   persons with SCI (29), but fitness varied within the group. Peak heart rate (169 ± 16 b·min^{-1})

262   suggests that injury did not result in disruption of sympathetic output to the myocardium.

263

264   *Dietary intake*

265   Participant's average pre-trial habitual dietary intake was $1,942 \pm 15$ kcal·day^{-1} at 37% Fat,

266   39% CHO, and 22% Protein (Table 2). There were no differences in participant reported caloric

267   intake ($P=.653$) or dietary macronutrient content (Fat $P=.184$; CHO $P=.729$; Protein $P=.537$)

268   in the 24 h preceding each experiment (Table 2).

269

270   *Energy expenditure and substrate oxidation rates at baseline and during exercise*

271   There were no significant differences between conditions in rates of EE or substrate oxidation

272   at baseline (Figure 2). All exercise conditions were matched for total energy cost (116 ± 22,

273   117 ± 35, and 118 ± 22 kcal, respectively; $P=.982$). However, rates of EE were significantly

274   greater in HIIE compared to MICE and CRE ($P=.01$) (Table 2). Participants achieved a

275   significantly greater % VO$_{2peak}$ ($P<.001$) in HIIE compared to MICE and CRE (Table 2).

276   Respiratory exchange ratio (RER) was lower ($P<.001$) in MICE (0.90 ± 0.08) compared to

277   HIIE and CRE (1.01 ± 0.07 and 1.05 ± 0.04, respectively) (Table 2).

278

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 $Post_{30-60}$ ($P=.050$) and $Post_{60-90}$ ($P=.039$) 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 ($Post_{0-30}$, $P=.000$; $Post_{30-60}$, $P=.002$; $Post_{60-90}$, $P=.019$; $Post_{90-120}$ $P=.027$; $Post_{120-150}$, $P=.044$), significantly greater than MICE at $Post_{0-30}$ ($P=.030$) and $Post_{30-60}$ ($P=.039$) and significantly greater than HIIE at $Post_{60-90}$ ($P=.014$). Lox in HIIE was significantly greater than CON at $Post_{0-30}$ ($P=.007$) and $Post_{30-60}$ ($P=.015$).

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

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·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·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

334 metabolism in SCI used OGTT or OFTT that have atypical macronutrient compositions, often

335 relying on a single macronutrient as the sole stimulus. In contrast, our MMTT was designed to

336 reflect the energy and macronutrient content of an *ad lib* meal in persons with SCI (23).

337

338 Based on ADA guidelines for OGTT (25), no participants displayed signs of postprandial

339 dysglycemia. Compared to a standard 75 g OGTT (25), the MMTT used in the current study

340 contained a similar total carbohydrate load (75.5 g), but was comprised of different

341 carbohydrate (glucose polymer from the carbohydrate powder, and a mix of

342 sucrose/fructose/glucose from the banana) as opposed to homogeneous anhydrous glucose in

343 an OGTT. Furthermore, the insoluble fiber and other macronutrients in our meal likely reduced

344 the peak amplitude of the postprandial glucose and insulin response compared to OGTT (30).

345 However, while absolute values differ, peak glucose excursions after OGTT and MMTT are

346 well correlated (30) and insulin resistance calculated from MMTT can be effectively compared

347 to insulin sensitivity during OGTT (31). Moreover, MMTT has a similar C-peptide response

348 (30) and might therefore reflect pancreatic functions better compared to OGTT (31). Therefore,

349 because our MMTT has a similar total carbohydrate content as an OGTT, the results of

350 postprandial glucose metabolism in this study indicate that none of our participants had

351 impaired glucose handling.

352

353 Based on expert panel guidelines for OFTT (26), three of 10 participants displayed signs of

354 PPL. This impaired postprandial fat metabolism was seen even though the MMTT used in the

355 current study contained ~250 less kilocalories and ~50 g less fat than the OFTT upon which

356 the guidelines are based (26). These postprandial fat excursion data indicate that postprandial

357 fat metabolism was relatively more impaired than postprandial glucose metabolism in our

358 sample.

359

360 *Pre-meal exercise and postprandial energy utilization*

361 Exercise performed prior to a meal has the ability to increase postprandial EE (32). Without

362 prior feeding, exercise intensity is the primary determinant of post-exercise EE especially when

363 an exercise session is limited in duration (33). For example, in ~~neurologically intact~~non-injured

364 cyclists, sprint interval training (SIT) lasting 14 min and costing 132 kcal elicited a total post-

365 exercise energy expenditure equal to that seen after 30 min of continuous exercise at 85%

366 VO_{2peak} costing 493 kcal (34). The effects of pre-meal exercise on postprandial EE are also

367 intensity dependent, with previous studies suggesting a minimum intensity threshold of ~60%

368 VO_{2peak} (35). In the current study HIIE was above the posited % VO_{2peak} threshold, contributing

369 to why postprandial EE was elevated above CON in the HIIE condition. The % VO_{2peak}

370 intensity of MICE and CRE (53.0 ± 6.6 and $53.5 \pm 7.0\%$ VO_{2peak}, respectively) in the current

371 study were below this threshold. However, at $Post_{30-60}$ EE was elevated in CRE vs CON (Figure

372 2). This finding can be explained by the intensity of contraction during the resistance

373 maneuvers, which resulted in local cellular stress that is not fully reflected in % VO_{2peak}. Given

374 that the total cost of exercise was similar in all conditions and the % VO_{2peak} intensity was

375 similar in MICE and CRE, our findings show that in persons with paraplegia exercise that is

376 more reliant on carbohydrate oxidation ~~the amount of carbohydrate reliance during exercise~~

377 ~~best explains~~has a great impact on ~~the effects of pre-meal exercise on~~ postprandial EE.

378

379 Exercise mode and intensity had robust effects on postprandial substrate oxidation (Figure 2).

380 During exercise rates of carbohydrate and Lox can be changed dramatically, with HIIE and

381 CRE having a greater ~~reliance on~~ carbohydrate ~~use~~ oxidation (36). During recovery from

382 exercise the body transitions to an increased reliance on fat that persists for hours (37) to days

383 (38). Kuo et al (37) showed that in ~~neurologically intact~~non-injured persons exercise energy

384 expenditure during MICE determined post-exercise fat use independent of exercise intensity.

385 MICE can only be conducted within a limited range of intensities, and thus when compared to

386 HIIE it is often found that MICE has a lesser effect on post-exercise metabolism (21).

387 Furthermore, in ~~neurologically intact~~non-injured persons, a session of resistance exercise with

388 approximately half the energy cost as MICE resulted in similar attenuation of PPL due in part

389 to increasing exogenous Lox (39). However, the effect of pre-meal exercise on postprandial

390 fuel partitioning had yet to be determined in persons with SCI. In our study postprandial Lox

391 was greater in CRE and HIIE compared to MICE and CON, and only CRE resulted in elevated

392 Lox at the 2 hr postprandial timepoint. Similar to postprandial EE, our data show that that pre-

393 meal exercise influences postprandial substrate oxidation in a manner dependent on the degree

394 of carbohydrate ~~reliance~~ oxidation during exercise.

395

396 *Pre-meal exercise and postprandial metabolite concentrations*

397 Our data show little effect of pre-meal exercise on postprandial glucose concentration

398 following MMTT (Figure 3). The circulating concentrations of glucose and insulin in all

399 conditions, including CON, show that glucose homeostasis was well maintained. The

400 participants in this study were relatively fit and did not have evidence of glycemic

401 dysregulation based on fasted and postprandial (Table 3 and Figure 3) glucoses. Thus the

402 finding that exercise had little effect on circulating glucose and insulin might be due to a floor

403 effect due to the lower peak glycemic response to the MMTT and the lack of glycemic

404 dysregulation in our participants. Furthermore, compared to a standard bout of exercise in

405 ~~neurologically intact~~non-injured persons where 45 min of exercise results in ~200-600 kcal

406 expenditure (40), the energy cost of our exercise was relatively low (~120 kcal). The results of

407 our study may suggest that there is a minimum energy expenditure threshold required for pre-

408 meal exercise to influence postprandial metabolite concentrations. This possibility needs to be

409 considered in the context of exercise as a strategy for improving cardiometabolic health in

410 persons with SCI. Obligatory upper extremity exercise and increased potential for overuse

411 injuries in persons with SCI place a practical limit on the total exercise energy expenditure.

412

413 With respect to postprandial circulating lipids and their metabolites, statistical differences were

414 observed only for glycerol where MICE (P=.020) and CRE (P=.001) were greater than CON.

415 Postprandial lipemia based on peak triglyceride ≥ 2.5 mmol·L^{-1} (26) was observed after CON

416 (3 participants), MICE (2 participants), and HIIE (3 participants). After CRE no participants

417 had triglyceride concentration above 2.5 mmol·L^{-1}. Therefore, CRE seemed to partially

418 accommodate for the disordered postprandial fat metabolism inherent to SCI and observed in

419 the current study. This response might be explained by an increased catecholamine response to

420 CRE compared to other modes of exercise, although these were not measured. Only four of our

421 participants had SCI above the neurological level whereby the catecholamine response to

422 exercise is impaired (<T4) (41), and all of these participants had normal cardioacceleratory

423 capacity (Table 1) suggesting intact SNS signaling. Beyond endocrine signaling, ~~CRE~~ our

424 results could ~~have a greater effect on postprandial Lox due to~~be explained by local factors

425 produced during exercise. Greater skeletal muscle glycogen reduction results in increased Lox

426 by exercised muscles as glucose is preferentially used for glycogen resynthesis during recovery

427 (42). ~~I~~While we did not measure glycogen utilization it is well established that HIIE and high

428 intensity contraction in general result in greater glycogen reductions (42), but there is little data

429 examining glycogen metabolism during circuit-style exercise. ~~I~~While there is also no current

430 data in this area, it is also possible that contraction resulted in the release of myogenic signaling

431 molecules (43) labeled for specific target tissues related to energy metabolism (44).

432

433 *Methodological considerations*

434 The purpose of this study was to identify the optimal exercise strategies for influencing

435 metabolic function in persons with SCI. We aimed to control for nutritional intake for 24-hr

436 before each trial to isolate the effects of select exercise parameters. Our strategy utilized a self-

437 reported food journal, completed before the first trial and then reproduced before the following

438 experimental trials. Participants reported similar energy and macronutrient intake before each

439 trial, however, there is an inherent source of error associated with self-reported food journals.

440 Furthermore, it is possible that nutritional differences in the >24 h preceding the trials may

441 influence metabolism during our testing. ~~Total energy expenditure during exercise sessions~~

442 ~~was matched within ~3 kcal between trials to further isolate the effects of exercise intensity~~

443 ~~and mode. CRE was conducted first because CRE does not lend itself to predictions of energy~~

444 ~~expenditure, while there are methodologies for predicting energy expenditure during cycling~~

445 ~~type activity(24).(45)~~ While the HIIE prescription is not conventional (10:70 %PO_{peak}), the

446 physiological response confirms that HIIE occurred at a high intensity as 29.4 ± 7.7 % of the

447 duration of the session was spent at or above 80 % VO_{2peak}. Finally, one further methodological

448 consideration should be considered when interpreting our results. Our previous data indicate

449 that CRE will elicit a mean exercise energy expenditure of ~170 kcal in persons with paraplegia

450 (46), while the data from the current study show an expenditure of ~120 kcal. Most importantly,

451 all previous CRE studies in SCI (3, 46, 47) calculated energy expenditure using stochiometric

452 equations (24) that assume that the blood glucose is the only type of carbohydrate contributing

453 to carbohydrate oxidation. In the current study we employed more appropriate calculations for

454 glycolytic exercise (24) that assume 80% of carbohydrate use is due to utilization of muscle

455 glycogen. Muscle glycogen utilization is energetically more efficient, thus yielding a

456 calculation of carbohydrate oxidation that is approximately 10% lower than the calculations

457 applied to the previous data. Given the average RER of 1.01 in the CRE condition, carbohydrate

458 oxidation accounts for nearly all of the total energy expenditure, thus exacerbating the

459 difference in the calculations. Furthermore, the participants in the previous study (46) were ~4

460 kg heavier and, assuming this difference was related to lean tissue mass, this likely contributed

461 to a greater total energy expenditure.

462

463 *Limitations*

464 We used indirect calorimetry to match exercise EE, and CRE violates the assumptions of the

465 stoichiometric equations used to calculate EE from indirect calorimetry (24). Accordingly, it

466 is possible that EE was underestimated in CRE. This difference in exercise EE is an important

467 consideration. However, studies that have used blood lactate and excess postexercise oxygen

468 consumption suggest that if indirect calorimetry does underestimate EE during resistance

469 exercise, the differences are relatively small (45). Based on current American Diabetes

470 Association (25) guidelines, neither fasted blood glucose nor triglycerides (Table 3) were

471 elevated. Furthermore, HOMA-IR was within a "normal" range. These findings of a "healthy"

472 fasted metabolic profile in our participants means that the results of our study are not

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

480

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

491

492 *Funding Source and Disclaimers*
493 This study was funded by the Miami Project to Cure Paralysis and the University of Miami
494 Department of Kinesiology and Sport Sciences. The authors declare no conflicts of interest.
495 The results of this study do not constitute endorsement by the ACSM. The results of this study
496 are presented clearly and honestly without fabrication, falsification, or inappropriate data
497 manipulation.

498

499 **REFERENCES**

500   1.   Nash MS, Groah SL, Gater DR et al. Identification and Management of
501   Cardiometabolic Risk after Spinal Cord Injury: Clinical Practice Guideline for Health
502   Care Providers. *J Spinal Cord Med*. 2019:1-35.
503   2.   Martin Ginis KA, van der Scheer JW, Latimer-Cheung AE et al. Evidence-based
504   scientific exercise guidelines for adults with spinal cord injury: an update and a new
505   guideline. *Spinal Cord*. 2018;56(4):308-21.
506   3.   Nash MS, Jacobs PL, Mendez AJ, Goldberg RB. Circuit resistance training improves
507   the atherogenic lipid profiles of persons with chronic paraplegia. *J Spinal Cord Med*.
508   2001;24(1):2-9.
509   4.   Nightingale TE, Metcalfe RS, Vollaard NB, Bilzon JL. Exercise Guidelines to Promote
510   Cardiometabolic Health in Spinal Cord Injured Humans: Time to Raise the Intensity?
511   *Arch Phys Med Rehabil*. 2017;98(8):1693-704.
512   5.   de Groot S, Adriaansen JJ, Tepper M, Snoek GJ, van der Woude LH, Post MW.
513   Metabolic syndrome in people with a long-standing spinal cord injury: associations
514   with physical activity and capacity. *Appl Physiol Nutr Metab*. 2016;41(11):1190-6.
515   6.   Bigford G, Nash MS. Nutritional Health Considerations for Persons with Spinal Cord
516   Injury. *Top Spinal Cord Inj*. 2017;23(3):188-206.
517   7.   Segal JL, Thompson JF, Tayek JA. Effects of long-term 4-aminopyridine therapy on
518   glucose tolerance and glucokinetics in patients with spinal cord injury.
519   *Pharmacotherapy*. 2007;27(6):789-92.
520   8.   Yarar-Fisher C, Bickel CS, Windham ST, McLain AB, Bamman MM. Skeletal muscle
521   signaling associated with impaired glucose tolerance in spinal cord-injured men and
522   the effects of contractile activity. *J Appl Physiol*. 2013;115(5):756-64.
523   9.   Elder CP, Apple DF, Bickel CS, Meyer RA, Dudley GA. Intramuscular fat and glucose
524   tolerance after spinal cord injury - a cross-sectional study. *Spinal Cord*.
525   2004;42(12):711-6.
526   10. La Fountaine MF, Cirnigliaro CM, Hobson JC et al. Establishing a threshold to predict
527   risk of cardiovascular disease from the serum triglyceride and high-density
528   lipoprotein concentrations in persons with spinal cord injury. *Spinal Cord*.
529   2018;56(11):1051-8.
530   11. Nash MS, DeGroot J, Martinez-Arizala A, Mendez AJ. Evidence for an exaggerated
531   postprandial lipemia in chronic paraplegia. *J Spinal Cord Med*. 2005;28(4):320-5.
532   12. Ellenbroek D, Kressler J, Cowan RE, Burns PA, Mendez AJ, Nash MS. Effects of
533   prandial challenge on triglyceridemia, glycemia, and pro-inflammatory activity in
534   persons with chronic paraplegia. *J Spinal Cord Med*. 2014.
535   13. Emmons RR, Garber CE, Cirnigliaro CM et al. The Influence of Visceral Fat on the
536   Postprandial Lipemic Response in Men with Paraplegia. *J Am Coll Nutr*.
537   2010;29(5):476-81.
538   14. Emmons RR, Cirnigliaro CM, Kirshblum SC, Bauman WA. The relationship between
539   the postprandial lipemic response and lipid composition in persons with spinal cord
540   injury. *J Spinal Cord Med*. 2014;37(6):765-73.
541   15. Gorgey AS, Dudley GA. Skeletal muscle atrophy and increased intramuscular fat after
542   incomplete spinal cord injury. *Spinal Cord*. 2007;45(4):304-9.
543   16. Aksnes AK, Brundin T, Hjeltnes N, Maehlum S, Wahren J. Meal-Induced Rise in
544   Resting Energy-Expenditure in Patients with Complete Cervical Spinal-Cord Lesions.
545   *Paraplegia*. 1993;31(7):462-72.

546 17. van Dijk JW, Manders RJ, Tummers K et al. Both resistance- and endurance-type
547   exercise reduce the prevalence of hyperglycaemia in individuals with impaired
548   glucose tolerance and in insulin-treated and non-insulin-treated type 2 diabetic
549   patients. *Diabetologia*. 2012;55(5):1273-82.
550 18. Freese EC, Gist NH, Cureton KJ. Effect of prior exercise on postprandial lipemia: an
551   updated quantitative review. *J Appl Physiol*. 2014;116(1):67-75.
552 19. Yoon E, Kim H, Choo J, Park K, Jae S. Effect of an Exergaming on Postprandial
553   Endothelial Dysfunction Following a High Fat Meal in Individuals with Spinal Cord
554   Injury. *Korean J Sports Med*. 2017;35:7.
555 20. Greer BK, Sirithienthad P, Moffatt RJ, Marcello RT, Panton LB. EPOC Comparison
556   Between Isocaloric Bouts of Steady-State Aerobic, Intermittent Aerobic, and
557   Resistance Training. *Res Q Exercise Sport*. 2015;86(2):190-5.
558 21. Little JP, Jung ME, Wright AE, Wright W, Manders RJ. Effects of high-intensity interval
559   exercise versus continuous moderate-intensity exercise on postprandial glycemic
560   control assessed by continuous glucose monitoring in obese adults. *Appl Physiol Nutr
561   Metab*. 2014;39(7):835-41.
562 22. McMillan DW, Maher JL, Jacobs KA, Mendez AJ, Nash MS, Bilzon JLJ. Influence of
563   upper-body continuous, resistance or high-intensity interval training (CRIT) on
564   postprandial responses in persons with spinal cord injury: study protocol for a
565   randomised controlled trial. *Trials*. 2019;20(1).
566 23. Groah SL, Nash MS, Ljungberg IH et al. Nutrient intake and body habitus after spinal
567   cord injury: an analysis by sex and level of injury. *J Spinal Cord Med*. 2009;32(1):25-
568   33.
569 24. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by
570   means of gas exchange measurements. *Int J Sports Med*. 2005;26 Suppl 1:S28-37.
571 25. Cefalu WT, Berg EG, Saraco M et al. Classification and Diagnosis of Diabetes:
572   Standards of Medical Care in Diabetes-2019. *Diabetes Care*. 2019;42:S13-S28.
573 26. Kolovou GD, Mikhailidis DP, Kovar J et al. Assessment and Clinical Relevance of Non-
574   Fasting and Postprandial Triglycerides: An Expert Panel Statement. *Curr Vasc
575   Pharmacol*. 2011;9(3):258-70.
576 27. Wolever TM, Jenkins DJ. The use of the glycemic index in predicting the blood
577   glucose response to mixed meals. *Am J Clin Nutr*. 1986;43(1):167-72.
578 28. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose
579   tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*.
580   1999;22(9):1462-70.
581 29. Simmons OL, Kressler J, Nash MS. Reference Fitness Values in the Untrained Spinal
582   Cord Injury Population. *Arch Phys Med Rehabil*. 2014;95(12):2272-8.
583 30. Meier JJ, Baller B, Menge BA, Gallwitz B, Schmidt WE, Nauck MA. Excess glycaemic
584   excursions after an oral glucose tolerance test compared with a mixed meal
585   challenge and self-measured home glucose profiles: is the OGTT a valid predictor of
586   postprandial hyperglycaemia and vice versa? *Diabetes Obes Metab*. 2009;11(3):213-
587   22.
588 31. Selimoglu H, Duran C, Kiyici S et al. Comparison of composite whole body insulin
589   sensitivity index derived from mixed meal test and oral glucose tolerance test in
590   insulin resistant obese subjects. *Endocrine*. 2009;36(2):299-304.
591 32. Melanson EL, Sharp TA, Seagle HM et al. Effect of exercise intensity on 24-h energy
592   expenditure and nutrient oxidation. *J Appl Physiol*. 2002;92(3):1045-52.

593 33. Borsheim E, Bahr R. Effect of exercise intensity, duration and mode on post-exercise
594   oxygen consumption. *Sports Med*. 2003;33(14):1037-60.
595 34. Islam H, Townsend LK, Hazell TJ. Excess Postexercise Oxygen Consumption and Fat
596   Utilization Following Submaximal Continuous and Supramaximal Interval Running.
597   *Res Q Exercise Sport*. 2018;89(4):450-6.
598 35. Treadway JL, Young JC. Failure of prior low-intensity exercise to potentiate the
599   thermic effect of glucose. *Eur J Appl Physiol Occup Physiol*. 1990;60(5):377-81.
600 36. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise:
601   the "crossover" concept. *J Appl Physiol*. 1994;76(6):2253-61.
602 37. Kuo CC, Fattor JA, Henderson GC, Brooks GA. Lipid oxidation in fit young adults
603   during postexercise recovery. *J Appl Physiol*. 2005;99(1):349-56.
604 38. Henderson GC, Alderman BL. Determinants of resting lipid oxidation in response to a
605   prior bout of endurance exercise. *J Appl Physiol*. 2014;116(1):95-103.
606 39. Davitt PM, Arent SM, Tuazon MA, Golem DL, Henderson GC. Postprandial triglyceride
607   and free fatty acid metabolism in obese women after either endurance or resistance
608   exercise. *J Appl Physiol*. 2013;114(12):1743-54.
609 40. Ainsworth BE, Haskell WL, Herrmann SD et al. 2011 Compendium of Physical
610   Activities: a second update of codes and MET values. *Med Sci Sports Exerc*.
611   2011;43(8):1575-81.
612 41. Schmid A, Huonker M, Stahl F et al. Free plasma catecholamines in spinal cord
613   injured persons with different injury levels at rest and during exercise. *J Auton Nerv
614   Syst*. 1998;68(1-2):96-100.
615 42. Jensen TE, Richter EA. Regulation of glucose and glycogen metabolism during and
616   after exercise. *J Physiol*. 2012;590(5):1069-76.
617 43. Pedersen BK. Muscles and their myokines. *J Exp Biol*. 2011;214(2):337-46.
618 44. Whitham M, Parker BL, Friedrichsen M et al. Extracellular Vesicles Provide a Means
619   for Tissue Crosstalk during Exercise. *Cell Metab*. 2018;27(1):237-51 e4.
620 45. Scott CB. Contribution of blood lactate to the energy expenditure of weight training.
621   *J Strength Cond Res*. 2006;20(2):404-11.
622 46. Jacobs PL, Mahoney ET, Nash MS, Green BA. Circuit resistance training in persons
623   with complete paraplegia. *J Rehabil Res Dev*. 2002;39(1):21-8.
624 47. Nash MS, Jacobs PL, Woods JM, Clark JE, Pray TA, Pumarejo AE. A comparison of 2
625   circuit exercise training techniques for eliciting matched metabolic responses in
626   persons with paraplegia. *Arch Phys Med Rehabil*. 2002;83(2):201-9.
627