Are post-exercise plasma glucose elevations involved in exercise-induced appetite suppression?

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ABSTRACT

Changes in glucose and insulin are potentially involved in the appetite-regulatory effects of exercise considering their role post-prandially. *Purpose*: To examine if glucose and insulin play a role in post-exercise appetite regulation. *Methods*: 12 participants (M=8; 26±5 y) completed 3 experimental sessions in a systematically rotated randomized crossover design: 1) no-exercise control (CTRL); 2) moderate-intensity continuous training (MICT; 30-min, 70% maximal oxygen consumption (VO_{2max})); and 3) sprint interval training (SIT; 4 x 30-s "all-out" sprints, interspersed with 4-min rest). Plasma glucose, insulin, acylated ghrelin, active peptide tyrosine tyrosine (PYY), active glucagon-like peptide-1 (GLP-1), and overall appetite perceptions were measured pre-exercise, 0-, 30-, 60-, and 120-min post-exercise. Energy intake was recorded the day before, of, and after experimental sessions. *Results*: Glucose was elevated 0-min post-exercise (p<0.097, d>0.52) compared to CTRL with no differences between exercise bouts. Acylated ghrelin was suppressed by MICT (60-, 120-min) and SIT (0-, 30-, 60-, 120-min; p<0.080, d> 0.56) compared to CTRL, while also suppressed in SIT compared to MICT at 30-, 60-, 120-min (p<0.026, d>0.74). GLP-1 was elevated following MICT (0-, 30-, 60-min) and SIT (60-min; p<0.094, d>0.53) compared to CTRL and following MICT compared to SIT (0-min; p=0.005, d=1.03). Overall appetite was suppressed by SIT post-exercise (p<0.058, d>0.61) compared to CTRL and MICT, and by MICT 0-min post-exercise compared to CTRL (p=0.036, d=0.71). There were no exercise effects on insulin, PYY, or free-living energy intake (p>0.217, η_p^2<0.130). *Conclusion*: Glucose and insulin do not appear to play a role in exercise-induced appetite suppression.

Keywords: appetite-regulating peptides, high-intensity interval training, food intake, blood lactate, exercise intensity, hunger, satiety

INTRODUCTION

Energy balance is broadly understood as the equilibrium of energy intake and energy expenditure (Hall et al., 2012), however this relationship can be uncoupled from homeostatic norms (Shook et al., 2015). Energy intake is controlled by a complex system involving behavioural, environmental, and physiological factors (King et al., 2012), with the physiological component regulated by interactions between peripheral gastrointestinal signals and brain regions such as the hypothalamus and brainstem that integrate these signals to maintain energy homeostasis (Murphy and Bloom, 2006). These peripheral gastrointestinal signals regulating energy intake are in part comprised of peripheral appetite hormones such as acylated ghrelin, the only established episodic orexigenic (appetite stimulating) hormone (Cummings and Overduin, 2007) as well as active peptide tyrosine tyrosine (PYY) and active glucagon-like peptide-1 (GLP-1), which are both acute anorexigenic (appetite-inhibiting) hormones (Batterham et al., 2002, Holst, 2007). These appear to be the key appetite-regulating hormones that have been measured in response to acute exercise (Schubert et al., 2014, McCarthy et al., 2024b, Hazell et al., 2016).

Acute exercise bouts (30-90 min) have demonstrated acylated ghrelin suppression while elevating GLP-1 and PYY concentrations (active and total) that correspond with transient reductions in subjective appetite perceptions (Schubert et al., 2013, Schubert et al., 2014, Bornath et al., 2023). These effects appear more consistent with increased exercise intensities (≥70% VO_{2max}) suggesting a continuum for appetite regulation depending on the exercise stimulus (Broom et al., 2017, Broom et al., 2007, Hazell et al., 2016, Hazell et al., 2017, Islam et al., 2017, McCarthy et al., 2023, Ueda et al., 2009, Sim et al., 2014, Panissa et al., 2016, Deighton et al., 2013a, Deighton et al., 2013b, Holliday and Blannin, 2017, Moniz et al., 2023) irrespective of exercising in fasted or fed states. Acute moderate-intensity continuous training (MICT) bouts of

differing work rates (~50% $\dot{V}O_{2max}$ vs 75% $\dot{V}O_{2max}$) have displayed intensity-dependent differences in acylated ghrelin (Broom et al., 2017) and total PYY (Ueda et al., 2009) and comparisons of MICT and high-intensity (HIIT; intermittent exercise bouts performed above moderate intensity)/sprint interval training (SIT; repeated bouts performed with near-maximal to "all out" effort) also demonstrated more potent acylated ghrelin suppression with GLP-1 and PYY (active and total) elevations (Hazell et al., 2017, Islam et al., 2017, McCarthy et al., 2023, Deighton et al., 2013a, Sim et al., 2014, Panissa et al., 2016, Hazell et al., 2016). Despite important research characterizing the appetite hormone responses to different exercise protocols, the specific physiological mechanisms underpinning these responses are not well understood (Hazell et al., 2016, McCarthy et al., 2024b).

Several mechanisms are purported to influence appetite-regulating hormones post-exercise (Hazell et al., 2016, McCarthy et al., 2024b) and though blood lactate accumulation has garnered the most support for its involvement in exercise-induced appetite suppression (McCarthy et al., 2024a, Islam et al., 2017, McCarthy et al., 2023, McCarthy et al., 2020, Vanderheyden et al., 2020), glucose and insulin are also known to have appetite-regulating properties post-prandially (Campfield and Smith, 2003, Campfield et al., 1992, Melanson et al., 1999, Smith and Campfield, 1993, Wyatt et al., 2021, Shiiya et al., 2002, Broglio et al., 2004, Nakagawa et al., 2002, Lu et al., 2021, Djurhuus et al., 2002, Holst, 2007, Mayer, 1955, van der Lely et al., 2004). With regards to exercise, glucose and insulin exhibit intensity-dependent increases for brief (≤ 30 min) periods post-exercise (Peake et al., 2014, Marliss et al., 2000, Marliss et al., 1991, Marliss and Vranic, 2002, Sigal et al., 1996). Post-prandially glucose elevations increase active GLP-1 (Holst, 2007, Lu et al., 2021, Djurhuus et al., 2002) and suppress acylated ghrelin (van der Lely et al., 2004, Broglio et al., 2004, Shiiya et al., 2002, Nakagawa et al., 2002, Djurhuus et al., 2002),

coinciding with decreased appetite perceptions and energy intake (Wyatt et al., 2021, Campfield and Smith, 2003, Smith and Campfield, 1993, Mayer, 1955). However, these post-prandial elevations are brief due to continued intracellular transport of glucose, returning circulating concentrations to normal, or over prolonged periods slightly hypoglycemic conditions evoking an inverse response increasing perceptions of hunger and meal initiation (Mayer, 1955, Wyatt et al., 2021, van der Lely et al., 2004, Campfield and Smith, 2003, Smith and Campfield, 1993). Despite substantial evidence supporting glucose and insulin's involvement in appetite regulation post-feeding, their potential role post-exercise remains largely unknown (Peake et al., 2014, Frampton et al., 2023, Sim et al., 2014, Broom et al., 2017, Broom et al., 2007). Therefore, we aimed to explore the potential role of glucose and insulin on exercise-induced appetite suppression using an exercise intensity dose-response paradigm (rest, moderate, high) in recreationally active males and females.

METHODS

Participants

A sample size calculation was completed *a priori* (GPower 3.1) based on previous research examining differences in acylated ghrelin, PYY, GLP-1, and glucose in males (Bornath et al., 2023, Islam et al., 2017, Hazell et al., 2017, McCarthy et al., 2023, Vanderheyden et al., 2020) and females in the follicular phase (FP) (Moniz et al., 2023, Hazell et al., 2017). Based on these effect sizes, α=0.05, and power=0.80, a sample size of 10 participants were necessary to detect differences. Fifteen young adults (n = 5 females, n = 10 males) volunteered to participate in this study, however three participants withdrew due to a lack of availability. Thus, twelve participants (n = 4 females, n = 8 males) completed the study (Table 1). All participants were deemed "healthy"

as per the Canadian Society for Exercise Physiology (CSEP) Get Active Questionnaire, recreationally active completing ~150 min/week of physical activity as per the CSEP Physical Activity and Sedentary Behaviour Questionnaire and were non-smoking. All female participants provided a detailed history of their prior 3 menstrual cycles and were eumenorrheic as defined by consistent naturally occurring menstrual cycles between 21-35 d in length for a minimum of 1 y and be menstruating for a minimum of 3 y (Elliott-Sale et al., 2021) . Participants were excluded if they had a diagnosis of any eating disorder or metabolic disease, or if they were currently consuming pharmaceuticals or supplements known to alter metabolism. Additionally, females were excluded if they were currently using contraceptives that delivered continuous dosages of exogenous ovarian hormones (i.e., skin patch or contraceptive injection) or had done so within the last 3 months, or if they had been pregnant within the last 3 y or had plans to become pregnant prior to completion of study participation. Day 1 of their menstrual cycle was defined as the onset of menses and experimental sessions for female participants were scheduled 2-5 days following this, with the average experimental session occurred on day 3±1 of their menstrual cycle in their early-FP. All participants provided written informed consent after all experimental details were explained. This study was approved by the Research Ethics Board at Wilfrid Laurier University.

Study Design

Participants completed the 3 experimental sessions (no-exercise control (CTRL), MICT, SIT; ~4-h each) in a randomized, systematically rotated crossover order. To do this, the 6 possible orders the 3 sessions could be completed in were numbered 1-6. When a new participant began the study a random number generator was used to determine which order they would follow. Once an order was used, it was not used again until all the other orders had been used once (the first 6

participants). Blood samples and subjective appetite perceptions were obtained at several time points during each session. Energy intake (food and beverage consumption) was recorded over a 3-day period surrounding the experimental session (day before, day of, and day after), and participants were instructed to replicate their energy intake 24-h prior to each experimental session. Participants were instructed to refrain from strenuous physical activity and alcohol 24-h prior, and caffeine 12-h prior to the experimental session.

Pre-experimental Procedures

Prior to the experimental session, all participants completed one familiarization session where they completed informed consent, were screened for exclusion criteria, had anthropometric measurements of height (cm) and weight (kg) taken using a mechanical scale (Health-o-meter Professional, Sunbeam Products Inc., IL, USA), as well as introduced to the study protocol and equipment. After completion of the required consent and intake questionnaires, participants performed a graded exercise test to exhaustion on a motorized treadmill (4Front, Woodway, WI, USA) for the determination of maximal oxygen consumption ($\dot{V}O_{2max}$). Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were continuously measured using a breath-by-breath gas collection and analysis system (Quark-CPET, Cosmed, RM, Italy). Prior to data collection the gas collection analyzer was calibrated with gases of known concentrations and a 3-L syringe for flow. Each participant was fitted with a silicon facemask (7400 series Vmask, Hans Rudolph Inc., KS, USA) to ensure comfort and prevent leaking during gas measurements. Heart rate (HR) was recorded using an integrated HR monitor (H6, Coospo, GD, China). Participants began the test with a standardized warm-up, after which they ran at a self-selected pace between 5-7 $mi \cdot h^{-1}$ (8-11 $km \cdot h^{-1}$) for the remainder of the test. Incremental increases in grade (2%) were

applied every 2-min until volitional fatigue and ratings of perceived exertion (RPE) were recorded at the end of each 2-min stage. After a 5-min cool-down a verification phase was completed at 105% of their maximal work rate achieved in the previous test until volitional fatigue to ensure the attainment of $\dot{V}O_{2max}$ (McCarthy et al., 2021). $\dot{V}O_{2max}$ was the greatest 30-s average value during the graded exercise test at which $\dot{V}O_2$ values plateaued where a plateau was defined as an increase ≤ half of the expected increase in $\dot{V}O_2$ between stages as determined using the ACSM running equation (Glass et al., 2007). When a plateau was not present, two of the following secondary criteria were necessary: 1) respiratory exchange ratio (RER) >1.15; 2) maximal HR is within ±10 bpm of age-predicted maximum (defined as 220 – age); or 3) a RPE ≥19. A plateau was achieved for 8 of the 12 participants, and for those participants who did not achieve a plateau (2M, 2F) they all met at least two of the secondary criteria requirements. The subsequent verification phase $\dot{V}O_{2max}$ value confirmed the value from the graded exercise test if they did not differ by >1.59 $mL \cdot kg^{-1} \cdot min^{-1}$ (typical error value calculated in our laboratory) (McCarthy et al., 2021). Following the determination of $\dot{V}O_{2max}$, ~70% of this value was calculated as the target intensity during the MICT protocol. Participants were provided a brief rest period (minimum of ~10-min), then acclimated with the treadmill and efforts associated with 70% $\dot{V}O_{2max}$ and "all-out" self-propelled sprints for the subsequent exercise protocols to reduce any potential learning effects.

Experimental Sessions

Participants arrived at the laboratory at 0800 h following a 12-h overnight fast and remained in the laboratory for ~3-h (Figure 1). Upon arrival participants were provided a standardized breakfast smoothie consisting of 7 $kcal \cdot kg^{-1}$ body mass (56% carbohydrate, 23% fat, and 21% protein; Gruppo, ON, Canada) and were allotted 15-min to consume and 45-min to digest.

198 Water was consumed ad libitum throughout the session. From ~0910-0950 h participants either
199 completed the no-exercise CTRL session or one of the two running-based exercise sessions at
200 differing intensities: 1) MICT (30-min at 70% $\dot{V}O_{2max}$); and 2) SIT (14-min comprised of 4 x 30-
201 s "all-out" efforts interspersed with 4-min rest). The exercise sessions both commenced with a 5-
202 min standardized warm-up and finished with a 5-min cool-down. The SIT session was delayed by
203 ~16-min to ensure all sessions ended at the same time and during CTRL session participants sat
204 quietly (i.e., reading, using electronic devices) in the laboratory for 40-min. Gas exchange ($\dot{V}O_2$
205 and $\dot{V}CO_2$) and HR were measured continuously throughout the CTRL, MICT, and SIT protocols
206 and during the post-exercise period with the same gas collection system described earlier. Venous
207 blood samples were obtained at five time points: 0900 h (pre-exercise), 0950 h (0-min post-
208 exercise), 1020 h (30-min post-exercise), 1050 h (60-min post-exercise), and 1150 h (120-min
209 post-exercise). Subjective perceptions of appetite were assessed prior to each blood sampling time
210 point using a visual analogue scale (VAS) that has previously been validated (Flint et al., 2000)
211 and used by our laboratory in male (Islam et al., 2017, Vanderheyden et al., 2020, Bornath et al.,
212 2023, McCarthy et al., 2024a) and female (Broad et al., 2020, Hallworth et al., 2017, Hazell et al.,
213 2017, Moniz et al., 2023) participants of a similar age.

214 **[FIGURE 1 ABOUT HERE]**

215

216 **Exercise protocols**

217 All exercise protocols were performed on the same treadmill with both motorized and
218 dynamic (self-propelled) settings and consisted of a standardized 5-min warm-up (3.5 $mi \cdot h^{-1}$) and
219 ended with a self-selected paced 5-min cool-down. The MICT session consisted of 30-min of
220 continuous running at a target workload of 70% $\dot{V}O_{2max}$, while the SIT session consisted of 4 x 30-

s "all-out" efforts interspersed with 4-min rest for a total duration of 14-min (last 4-min rest period is not taken). A pre-determined work rate was calculated using a mode-specific standardized equation (the ACSM running equation) using the speed and $\dot{V}O_2$ data from the graded exercise test to determine the speed necessary to elicit the target intensity of 70% $\dot{V}O_{2max}$ (Glass et al., 2007). To ensure the achieved intensity matched the target intensity, $\dot{V}O_2$ was monitored continuously adjusting the speed as necessary to elicit the desired work rate.

Blood processing and analysis

Blood samples were collected via antecubital venipuncture while participants were in a supine position for the measurement of acylated ghrelin, active PYY (PYY_{3-36}), active GLP-1 ($GLP\text{-}1_{7-36}$ and $GLP\text{-}1_{7-37}$), plasma glucose, insulin, and blood lactate. Whole blood samples were collected into two separate pre-chilled 3 mL Vacutainer tubes pre-coated with K_2 EDTA (5.4 mg) and one additional Vacutainer pre-coated with lithium heparin (1; 75 USP units) at each time point. A droplet of whole blood was taken from one vacutainer prior to the addition of inhibitors and was placed on a lactate strip to measure blood lactate concentrations using a handheld lactate analyzer which was calibrated prior to use (Lactate Plus, Nova Biomedical, MA, USA). To prevent the degradation of acylated ghrelin via proteolytic enzyme activity 40 µL of AEBSF (25 $mg \cdot mL^{-1}$) per mL of whole blood was added to the first tube. The second tube received 10 µL of dipeptidyl peptidase-4 (DPP-IV) and 500 KIU of aprotinin per mL of whole blood to prevent the inactivation of GLP-1 and facilitate the ex-vivo conversion of PYY_{1-36} to PYY_{3-36}. Plasma glucose and insulin samples were analyzed from the pre-coated lithium heparin Vacutainers. All tubes were inverted 10 times and centrifuged at 2300 *g* for 15 min at 4° C, after which plasma supernatant was aliquoted into Eppendorf tubes. Additionally, the plasma from the vacutainer containing AEBSF for the

analysis of acylated ghrelin was acidified with 100 μL of HCl per mL of plasma. All samples were stored at -80ºC for subsequent analysis, whereby commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to determine the plasma concentrations of acylated ghrelin (EZGRA-88K, EMD Millipore, MA, USA), active PYY (EK-059-02, Phoenix Pharmaceuticals, CA, USA), active GLP-1 (EZGLPHS-35K, Millipore, MA, USA), and insulin (80-INSHU-E01.1, ALPCO, NH, USA) in accordance with the manufacturer's instructions. Plasma glucose was analyzed (Gillen et al., 2021) photometrically using the glucose oxidase reaction in conjunction with an auxiliary (peroxidase) reaction (Infinity, ThermoScientific, Canada). Briefly, 10 μL of sample and 200 μL of assay reagent were added to a 96-well plate. Following a 30-s shake, plates were incubated at 37°C for 3-min and read at 340nm. Glucose standards (0, 2.5, 5.0, 7.5, and 10 $mmol \cdot L^{-1}$) were prepared using laboratory grade glucose anhydrous (D-(+)-glucose, Sigma-Aldrich, ON, Canada), and a glucose control (5.56 $mmol \cdot L^{-1}$; glucose standard, Sigma-Aldrich, ON, Canada) were added to verify accuracy of the standard curve. All samples were analyzed in duplicate, except for a random 25% which were run in triplicate, and batch analyzed for each participant to eliminate inter-assay variation. The intra-assay coefficients of variation for acylated ghrelin, active PYY, active GLP-1, plasma glucose, and plasma insulin were 7.4%, 6.7%, 5.3%, 8.5%, and 4.1%, respectively.

Appetite Perceptions

Appetite perceptions were assessed using a series appetite questions (Flint et al., 2000) where participants were assessed for their feelings of fullness (i.e. "How full do you feel?"), satisfaction (i.e. "How satisfied do you feel?"), hunger (i.e. "How hungry do you feel?"), and food consumption (i.e. "How much do you think you can eat?") using paper-based visual analogue

scales (VAS) with separate 100 mm lines with contrasting statements on the end of each line. The mean values of the four appetite perceptions were used to calculate an overall appetite score at each time point, with the values for satisfaction and fullness being inverted (see calculation below). Overall Appetite = (Hunger + (100 − Satisfaction) + Prospective Food Consumption + (100 − Fullness))/4

Energy Intake

Free-living energy intake was recorded over a 3-day period (day before, day of, and day after) surrounding the experimental session using the self-reported image-based dietary smart phone application Keenoa®. Participants also completed self-reported dietary food logs as a back-up in case of technical difficulties. Detailed instructions were provided and participants practiced using the app during the familiarization session, in addition to receiving a sample self-reported dietary food log to ensure accurate measurement and recording. A 24-h recall follow-up interview was also conducted on the morning of the session and at the end of the 3-day period to increase accuracy of the food log recordings. A registered dietician reviewed all food entries through the smart phone application software and edited the food diaries as needed to increase diet data accuracy (Ji et al., 2020). Absolute energy and macronutrient intake were calculated using the Keenoa® online software (Keenoa®, QC, Canada), that uses the Canadian Nutrient File (Health Canada, Government of Canada).

Statistical analysis

All data are presented as mean ± standard deviation (SD) and were analyzed using SPSS software version 26 (IBM SPSS Inc, IL, USA). A series of two-factor (session X time) repeated

290 measures analysis of variance (RM ANOVA) were conducted to examine responses of glucose,
291 insulin, acylated ghrelin, active PYY, active GLP-1, blood lactate, and overall appetite
292 perceptions. Two-factor (session X day) RM ANOVA was conducted to compare changes in free-
293 living energy intake between sessions on the day before, day of, and day after the experimental
294 session. Area under the curve (AUC) was calculated using the trapezoidal method for all blood
295 related parameters and overall appetite perceptions, and then compared between conditions using
296 a one-factor (session) RM ANOVA. Bonferroni corrections were used for post-hoc analysis where
297 necessary. Repeated measure correlations were conducted using R (version 4.4.1; Posit Software,
298 Boston, United States of America) in R-Studio (version 4.4.1) to assess the relationship between
299 glucose, insulin and appetite-related parameters. Specifically, the *rmcorr* function from the R
300 package *rmcorr* (Bakdash and Marusich, 2017) was used to examine if the change (Δ) in glucose
301 and insulin was correlated with Δ ghrelin, Δ GLP-1, Δ overall appetite perceptions, ghrelin AUC,
302 GLP-1 AUC, and overall appetite AUC. To do this, Δ glucose, Δ insulin, Δ ghrelin, Δ GLP-1, and
303 Δ overall appetite perceptions were calculated by subtracting pre-exercise values from
304 immediately post-exercise values and AUC values were calculated as described above. The
305 figures for the repeated measures correlation were prepared in Rstudio (Posit Software). Partial
306 eta-squared (η_p^2) values were calculated to estimate the effect sizes (small: 0.01, medium: 0.06,
307 and large: 0.14) for main effects and interactions where necessary (Cohen, 1992). Cohen's *d* was
308 calculated to estimate effect size (small 0.2, medium 0.5, large 0.8, very large 1.3) for post-hoc
309 comparisons (Cohen, 1992). Confidence intervals (CI) of 95% were calculated for all post-hoc
310 comparisons with the upper and lower limits provided in parentheses. A priori, differences were
311 considered important if $p<0.100$ with a corresponding effect size that was medium or greater. This
312 interpretation follows suggestions from statisticians regarding the use and interpretation of p-

values including: 1) eliminating the terms "statistically significant" or "not statistically significant" when describing data; 2) not basing scientific conclusions on p-values alone; and 3) using additional metrics or tests to support interpretations of p-values such as effect sizes or confidence intervals (Wasserstein et al., 2019). While this differs from traditional statistical interpretations, this is done in an effort to progress beyond interpreting data using p-values alone as a dichotomous variable and done objectively to allow the reader to interpret the data themselves based on the variety of information provided (i.e. p-value, effect size, mean difference).

RESULTS

Participants Characteristics

Participant attributes and exercise session data are presented in Table 1.

Plasma Glucose

Two-factor (session X time) RM ANOVA revealed an interaction (p=0.043, η_p^2=0.161) for changes in plasma glucose over time (Figure 2A). There were no differences pre-exercise between CTRL and MICT (p=0.113, d=0.50, CI [-0.15, 1.26]), CTRL and SIT (p=0.336, d=0.24, CI [-0.31, 0.83], or MICT and SIT (p=0.327, d=0.79, CI [-0.92, 0.33]. Compared to CTRL, plasma glucose at 0-min post-exercise was elevated following MICT (p=0.051, d=0.63, CI [0.00, 1.65]) and SIT (p=0.097, d=0.52, CI [-0.16, 1.66]), with no differences between MICT and SIT (p=0.788, d=0.09, CI [-0.52, 0.67]). At 30-min post-exercise, plasma glucose was greater following SIT compared to CTRL (p=0.018, d=0.82, CI [0.10, 0.88], while there was no difference between CTRL and MICT (p=0.232, d=0.53, CI [-1.05, 0.28]) or MICT and SIT (p=0.722, d=0.42, CI [-0.75, 0.54]).

At 60-min post-exercise, no differences in plasma glucose existed between CTRL and MICT (p=0.480, d=0.33, CI [-0.55, 0.28]), CTRL and SIT (p=0.812, d=0.35, CI [-1.07, 0.86]), or MICT and SIT (p=0.788, d=0.09, CI [-0.52, 0.67]). At 120-min post-exercise, plasma glucose was greater in CTRL compared to SIT (p=0.044, d=0.65, CI [-0.01, 0.77]) and in MICT compared to SIT (p=0.065, d=0.59, CI [-0.02, 0.66]), with no difference between CTRL and MICT (p=0.693, d=0.43, CI [-0.33, 0.48]). There was no effect of session on plasma glucose AUC (p=0.317, η_p^2=0.095; Figure 2B).

[FIGURE 2 ABOUT HERE]

Insulin

Two-factor (session X time) RM ANOVA revealed no interaction (p=0.520, η_p^2=0.076) for changes in insulin (Figure 2C). There was no main effect of session (p=0.652, η_p^2=0.038), however there was a main effect of time (p<0.001, η_p^2=0.745). Pre-exercise insulin was greater than 0-min (p=0.015, d=0.80, CI [4.93, 53.55]), 30-min (p<0.001, d=1.23, CI [18.15, 68.26]), 60-min (p=0.001, d=1.19, CI [-17.83, 71.03]), and 120-min post-exercise (p<0.001, d=1.42, CI [24.24, 78.24]). At 0-min post-exercise, insulin was elevated compared to 30-min (p=0.005, d=0.95, CI [3.82, 24.11]), 60-min (p=0.005, d=0.84, CI [4.24, 26.14]), and 120-min post-exercise (p<0.001, d=1.31, CI [9.62, 34.39]). There was no difference between 30-min and 60-min post-exercise (p>0.999, d=0.09, CI [-6.62, 4.17]), however insulin was greater at 30-min compared to 120-min post-exercise (p=0.024, d=0.85, CI [0.87, 15.22]). At 60-min post-exercise, no differences in insulin existed compared to 120-min post-exercise (p=0.113, d=0.55, CI [-1.03, 14.66]). There was no effect of session for insulin AUC (p=0.578, η_p^2=0.049; Figure 2D).

359 ***Acylated ghrelin***

360 Two-factor (session X time) RM ANOVA revealed an interaction (p=0.043, η_p^2=0.161) for
361 changes in acylated ghrelin (Figure 3A). There were no differences pre-exercise between CTRL
362 and MICT (p=0.722, d=0.11, CI [-43.68, 31.26]), CTRL and SIT (p=0.710, d=0.11, CI [-32.20,
363 45.73], or MICT and SIT (p=0.453, d=0.22, CI [-23.72, 49.66]. Acylated ghrelin was suppressed
364 at 0-min post-exercise following SIT compared to CTRL (p=0.002, d=1.13, CI [-190.00, -53.43]),
365 however there were no difference between CTRL and MICT (p=0.131, d=0.47, CI [-30.66,
366 207.26]) or MICT and SIT (p=0.346, d=0.28, CI [-41.23, 108.06]). At 30-min post-exercise,
367 acylated ghrelin was suppressed in SIT compared to CTRL (p=0.001, d=1.24, CI [-341.44, -
368 109.74]) and MICT (p=0.003, d=1.12, CI [-280.28, -77.58]), with no difference between CTRL
369 and MICT (p=0.442, d=0.23, CI [-81.94, 175.23]). At 60-min post-exercise, MICT (p=0.080,
370 d=0.56, CI [-7.46, 111.96]) and SIT (p<0.001, d=1.38, CI [163.08, 441.83]) were suppressed
371 compared to CTRL and SIT was also suppressed compared to MICT (p<0.001, d=1.57, CI [-
372 351.37, -149.04]). At 120-min post-exercise, MICT (p=0.051, d=0.63, CI [-0.25, 145.51]) and
373 SIT (p=0.008, d=0.93, CI [58.26, 312.05]) remained suppressed compared to CTRL with SIT also
374 being suppressed compared to MICT (p=0.026, d=0.74, CI [-208.51, -116.55]). Acylated ghrelin
375 AUC demonstrated an effect of session (p<0.001, η_p^2=0.620; Figure 3B) where SIT was
376 suppressed compared to CTRL (p=0.001, d=1.44, CI [-47416.21, -13239.33]) and MICT (p=0.004,
377 d=1.22, CI [-35733.44, -7130.55]), with no differences between CTRL and MICT (p=0.196,
378 d=0.59, CI [-3365.61, 21157.16]).

379 **[FIGURE 3 ABOUT HERE]**

380 ***Active PYY***

Two-factor (session X time) RM ANOVA revealed no interaction (p=0.547, η_p^2=0.066) for changes in active PYY (Figure 3C). There was no main effect of session (p=0.763, η_p^2=0.024), though there was a main effect of time (p=0.029, η_p^2=0.255) where there were no differences pre-exercise compared to 0-min (p>0.999, d=0.20, CI [-0.21, 0.12]), 30-min (p>0.999, d=0.20, CI [-0.12, 0.22]), 60-min (p=0.570, d=0.42, CI [-0.05, 0.22]), or 120-min post-exercise (p=0.211, d=0.36, CI [-0.03, 0.21]). There were no differences between 0-min and 30-min (p>0.999, d=0.40, CI [-0.10, 0.28]), 60-min (p=0.315, d=0.64, CI [-0.05, 0.31]), or 120-min post-exercise (p=0.245, d=0.48, CI [-0.05, 0.32]). There were also no differences between 30-min and 60-min post-exercise (p>0.999, d=0.22, CI [-0.07, 0.14]), 30-min and 120-min (p=0.559, d=0.38, CI [-0.03, 0.12]), and 60-min and 120-min (p>0.999, d=0.04, CI [-0.09, 0.11]). For active PYY AUC there was no effect of session (p=0.705, η_p^2=0.031; Figure 3D).

Active GLP-1

Two-factor (session X time) RM ANOVA revealed an interaction (p=0.004, η_p^2=0.221) for changes in active GLP-1 (Figure 3E). There were no differences pre-exercise between CTRL and MICT (p=0.295, d=0.32, CI [-1.00, 2.99]), CTRL and SIT (p=0.958, d=0.02, CI [-2.21, 2.11], or MICT and SIT (P=0.274, d=0.33, CI [-3.06, 0.96]. At 0-min post-exercise, active GLP-1 was greater following MICT compared to CTRL (p=0.094, d=0.53, CI [-0.72, 7.81]) and compared to SIT (p=0.005, d=1.03, CI [1.38, 5.86]), with no difference between CTRL and SIT (p=0.957, d=0.02, CI [-2.68, 2.82]). At 30-min post-exercise, MICT was elevated compared to CTRL (p=0.001, d=0.76, CI [0.19, 2.14]), with no differences between CTRL and SIT (p=0.723, d=0.11, CI [-2.00, 1.43]) or MICT and SIT (p=0.340, d=0.29, CI [-1.07, 2.83]). At 60-min post-exercise, MICT (p=0.014, d=0.84, CI [0.34, 2.45]) and SIT (p=0.006, d=0.99, CI [0.61, 2.82]) were elevated

compared to CTRL with no difference between MICT and SIT (p=0.261, d=0.34, CI [-0.92, 0.28]). At 120-min post-exercise, there were no differences between CTRL and MICT (p=0.764, d=0.09, CI [-1.14, 0,86]), CTRL and SIT (p=0.670, d=0.13, CI [-1.29, 0.86]), or MICT and SIT (p=0.826, d=0.07, CI [-0.80, 0.65]). Active GLP-1 AUC demonstrated an effect of session (p=0.035, η_p^2=0.262; Fig. 3F) however no differences in AUC were present between CTRL and MICT (p=0.175, d=0.61, CI [-481.97, 69.26]), CTRL and SIT (p=0.599, d=0.39, CI [-23.16, 96.99]), or MICT and SIT (p=0.116, d=0.68, CI [-23.16, 254.09]).

Repeated Measures Correlations for Appetite-regulating Hormones

Change in glucose had a negative correlation with Δ ghrelin (r_m=-0.49, p=0.013, 95% CI [-0.740,-0.114]; Figure 5A), but was not correlated with ghrelin AUC (r_m=-0.19, p=0.341, 95% CI [-0.551,0.213]; Figure 5B), Δ GLP-1 (r_m=0.09, p=0.668, 95% CI [-0.316,0.469]; Figure 5C), GLP-1 AUC (r_m=0.31, p=0.138, 95% CI [-0.102,0.625]; Figure 5D), Δ overall appetite (r_m=-0.20, p=0.335, 95% CI [-0.553,0.211]; Figure 5E), or overall appetite AUC (r_m=0.12, p=0.550, 95% CI [-0.284,-0.496]; Figure 5F).

Change in insulin was not correlated with Δ ghrelin (r_m=-0.16, p=0.457, 95% CI [-0.519,-0.255]; Figure 6a), ghrelin AUC (r_m=0.03, p=0.905, 95% CI [-0.374,0.416]; Figure 6b), Δ GLP-1 (r_m=0.04, p=0.840, 95% CI [-0.359,0.430]; Figure 6c), GLP-1 AUC (r_m=0.10, p=0.647, 95% CI [-0.311,0.473]; Figure 6d), Δ overall appetite (r_m=-0.10, p=0.642, 95% CI [-0.475,0.309]; Figure 6e), or overall appetite AUC (r_m=0.38, p=0.063, 95% CI [-0.022,-0.672]; Figure 6f).

Overall appetite perceptions

Two-factor (session X time) RM ANOVA revealed an interaction (p<0.001, η_p^2=0.265) for changes in overall appetite perceptions (Figure 4A). There were no differences pre-exercise between CTRL and MICT (p=0.998, d=0.00, CI [-14, 14]), CTRL and SIT (p=0.552, d=0.18, CI [-8, 14], or MICT and SIT (p=0.521, d=0.19, CI [-7, 13]. At 0-min post-exercise, appetite perceptions were reduced following MICT (p=0.036, d=0.71, CI [-18, -1]) and SIT (p=0.001, d=1.22, CI [-30, -9]) compared to CTRL, while SIT was also reduced compared to MICT (p=0.008, d=0.94, CI [-17, -4]). At 30-min post-exercise, SIT was reduced compared to CTRL (p<0.001, d=1.42, CI [-40, -15]) and MICT (p<0.001, d=1.38, CI [-32, -12]), with no difference between CTRL and MICT (p=0.232, d=0.36, CI [-4, 15]). At 60-min post-exercise, appetite was again reduced in SIT compared to CTRL (p=0.002, d=1.14, CI [-28, -8]) and compared to MICT (p<0.001, d=1.31, CI [-22, -8]), with no difference between CTRL and MICT (p=0.475, d=0.21, CI [-6, 12]). At 120-min post-exercise, SIT was reduced compared to CTRL (p=0.058, d=0.61, CI [-20, 0]) and MICT (p=0.09, d=0.92, CI [-18, -3]), with no difference between CTRL and MICT (p=0.711, d=0.11, CI [-8, 6]). Overall appetite AUC exhibited an effect of session (p<0.001, η_p^2=0.494; Fig. 4B) where SIT was reduced compared to CTRL (p=0.013, d=1.03, CI [-2339, -275]) and MICT (p=0.001, d=1.42, CI [-1535, -415]), with no difference between CTRL and MICT (p=0.806, d=0.34, CI [-471, 1135]).

[FIGURE 4 ABOUT HERE]

Free-Living Energy intake

Two-factor (session X day) RM ANOVA revealed no interaction (p=0.180, η_p^2=0.130) for changes in absolute energy intake (Fig. 4C). There was no main effect of session (p=0.297,

449 η_p^2=0.105), however there was a main effect of day (p=0.006, η_p^2=0.377) where energy intake was
450 greater on the day of the session compared to the day before (p=0.049, d=0.60, CI [2, 870]) and
451 the day after (p=0.009, d=0.44, CI [84, 589]). There were no differences between the day before
452 the session and the day after (p>0.999, d=0.15, CI [-449, 250]).
453
454 **[FIGURE 5 AND FIGURE 6 ABOUT HERE]**
455
456 **DISCUSSION**

457 While there are several mechanisms proposed to regulate appetite post-exercise (Hazell et
458 al., 2016, McCarthy et al., 2024b), to our knowledge this is the first study to comprehensively
459 assess the potential role of glucose and insulin. Based on established roles of glucose and insulin
460 in appetite regulation post-prandially (Shiiya et al., 2002, Broglio et al., 2004, Djurhuus et al.,
461 2002, Lu et al., 2021), we examined the influence of glucose and insulin on post-exercise appetite-
462 regulation and subsequent energy intake through an exercise intensity paradigm. The main
463 findings of this study are: 1) both exercise bouts resulted in transient plasma glucose elevations,
464 with minimal differences between exercise sessions and no effects of exercise on insulin; 2) SIT
465 elicited sustained post-exercise suppression of acylated ghrelin and overall appetite perceptions
466 across all time points, while MICT only increased active GLP-1 but only a transient suppression
467 of overall appetite perceptions immediately post-exercise; 3) the immediately post-exercise change
468 in glucose was negatively associated with changes in ghrelin but not GLP-1 and the change in
469 insulin was not associated with any appetite-regulating parameters; and 4) no effects of exercise
470 on free-living energy intake. Overall, these results suggest exercise-induced plasma glucose
471 increases did not influence appetite-regulating parameters, while insulin responses also did not

472 align with changes in appetite-regulating hormones or depict divergence between conditions
473 further indicating that glucose and insulin are unlikely to be involved in exercise-induced appetite
474 regulation.

475 Post-exercise plasma glucose elevations immediately following MICT and SIT aligned
476 with that of blood glucose in previous literature (Marliss et al., 2000, Peake et al., 2014, Sim et al.,
477 2014, Marliss et al., 1991, Marliss and Vranic, 2002, Sigal et al., 1996, Broom et al., 2007),
478 however the magnitude of our exercise-induced increases were potentially blunted by feeding prior
479 to exercise when compared to previously fasted exercise responses (Marliss et al., 2000, Broom et
480 al., 2007, Marliss et al., 1991, Marliss and Vranic, 2002, Sigal et al., 1996). Additionally,
481 previously demonstrated intensity-dependent differences in blood glucose were not reproduced in
482 our data, which could be attributed to the brief SIT protocol (4 x 30-s totaling 4 min of work) as
483 other studies either completed vigorous-intensity (>84% $\dot{V}O_{2max}$) continuous efforts for 13-15 min
484 (Marliss et al., 2000, Marliss et al., 1991, Marliss and Vranic, 2002, Sigal et al., 1996) or completed
485 HIIT protocols with additional bouts and longer work intervals (Sim et al., 2014, Peake et al.,
486 2014). Insulin concentrations were not affected by exercise and continually decreased from the
487 pre-exercise timepoint across all three sessions exhibiting effects solely related to pre-exercise
488 feeding, replicating exercise responses in a fed state (Peake et al., 2014). While his is contrary to
489 other studies displaying both exercise-induced increases with the magnitude of these responses
490 being intensity-dependent (Marliss et al., 2000, Marliss et al., 1991, Marliss and Vranic, 2002,
491 Sigal et al., 1996), these differences are likely attributed to exercise protocol completion in a fasted
492 state. Although our post-exercise glucose and insulin responses are not uncharacteristic of a
493 recreationally active, normoglycemic cohort (Gillen et al., 2021), exercise duration and feeding
494 status differences within our experimental design potentially muted the anticipated responses.

Exercise-induced suppression of acylated ghrelin was more prominent following SIT compared to MICT across all time points further supporting the intensity-dependent suppression of this orexigenic hormone (Deighton et al., 2013a, Broom et al., 2017, Holliday and Blannin, 2017, Sim et al., 2014, Hazell et al., 2016, Islam et al., 2017, McCarthy et al., 2023). Following MICT, acylated ghrelin was suppressed compared to CTRL at 60- and 120-min post-exercise replicating findings from longer duration MICT protocols of similar intensity (Broom et al., 2007), however these responses were of a lesser magnitude than SIT. Though the repeated measures correlation suggests a relationship between glucose and acylated ghrelin, both MICT and SIT generated similar increases in blood glucose post-exercise but the SIT session suppressed acylated ghrelin to a greater degree than both CTRL and MICT suggesting limited but potential involvement of glucose as a mechanism for exercise-induced acylated ghrelin suppression (Shiiya et al., 2002, Broglio et al., 2004, Nakagawa et al., 2002, Djurhuus et al., 2002). The divergence in acylated ghrelin responses between MICT and SIT throughout the post-exercise observation period (30-, 60-, 120-min post-exercise and AUC), however are likely attributed to greater blood lactate accumulation following supramaximal SIT efforts (Islam et al., 2017, McCarthy et al., 2023, see supplementary data).

Exercise-induced alterations in active PYY were not present (across time points and AUC) demonstrating a lack of an exercise effect on active PYY that aligns with previous findings employing protocols of similar intensity and duration measuring active PYY (Panissa et al., 2016, Moniz et al., 2023). While some studies have suggested exercise-induced active PYY responses (Deighton et al., 2013b, Ueda et al., 2009), their concentrations are substantially lower than the current study making it difficult to compare, but the exercise-induced appetite suppression seen in those studies is likely attributed to other appetite hormone responses. Others have displayed

exercise-induced fluctuations in total PYY (Broom et al., 2009, Deighton et al., 2013a, Larson-Meyer et al., 2012), however it is important to emphasize that active PYY is found in greater circulating concentrations and known to exert more potent anorexigenic effects than total PYY (Cummings and Overduin, 2007, Batterham et al., 2006). Additionally, the absent response of active PYY in our study to exercise-induced increase in plasma glucose continues to support the lack of relationship between plasma glucose and circulating PYY concentrations as noted previously (Batterham et al., 2002).

Despite lack of changes in PYY, active GLP-1 has exhibited increases post-exercise (0-, 30-, and 60-min) following MICT. These exercise-induced elevations post-MICT support previous work (in both total and active GLP-1) in young adults (Islam et al., 2017, Ueda et al., 2009, Hallworth et al., 2017), while the limited response of active GLP-1 following SIT aligns with responses from our group (Islam et al., 2017) and opposes the findings of others (Holliday and Blannin, 2017, Hallworth et al., 2017). However, the additional time (2 h) provided for meal digestion prior to exercise (Holliday and Blannin, 2017) and measurement of total GLP-1 (Holliday and Blannin, 2017, Hallworth et al., 2017) may have contributed to these discrepancies. GLP-1 release has previously been associated with elevations in blood glucose post-prandially and these GLP-1 increases typically augment insulin release (Lu et al., 2021, Djurhuus et al., 2002, Holst, 2007), however our data did not support such relationships as the change GLP-1 and GLP-1 AUC did not reflect the change in plasma glucose or the change in insulin. Despite the current data suggesting no relationship between GLP-1, blood glucose, and insulin when exercising in a fed state, it is important to consider the potential implications of fasted exercise conditions warranting further investigations.

Previous literature indicates blood glucose fluctuations manipulate energy intake responses and overall appetite perceptions pre- and post-prandially (Campfield and Smith, 2003, Smith and Campfield, 1993, Wyatt et al., 2021, Mayer, 1955), however our change in appetite perceptions immediately post-exercise did not correlate with the change plasma glucose (Figures 5E and 5F), and neither altered day of session absolute or relative (see supplementary material) energy intake. Appetite perception responses throughout the post-exercise observation period did coincide with acylated ghrelin reductions as SIT produced prolonged decreases in post-exercise subjective appetite perceptions (all time points), while MICT reductions were brief (0-min post-exercise), agreeing with studies in similar cohorts (Islam et al., 2017, Holliday and Blannin, 2017, Deighton et al., 2013a, Hallworth et al., 2017, Deighton et al., 2013b, Ueda et al., 2009, Panissa et al., 2016). Though our data demonstrates differences in energy intake where the experimental session day was increased compared to the day before and day after, these differences appear influenced by the ~600 kcal (~9 kcal·kg^{-1}) increase in energy intake on the day of MICT. This differs from our previous work observing free-living energy intake reductions (~300 kcal) on the day of SIT compared to CTRL in a similar cohort (Islam et al., 2017), and equivocal energy intake on the day of SIT compared to CTRL in middle-aged adults (McCarthy et al., 2023), highlighting the variability and inter-person differences which add complexity when comparing energy intake between studies. Difficulties measuring energy intake notwithstanding, overall appetite suppression following SIT coinciding with acylated ghrelin reductions and reciprocal blood lactate accumulation further support the intensity-dependent effects of exercise as well as lactate as a mechanism for acute appetite regulation (Islam et al., 2017, McCarthy et al., 2024a, Vanderheyden et al., 2020). However, limited acute exercise effects on energy intake requires further

562 investigation to determine the influence of appetite-regulating hormones and the proposed
563 mechanisms on these outcomes.

564 This study is the first to investigate the role of glucose and insulin in post-exercise appetite
565 regulation providing novel data examining the multifaceted implications of these theorized
566 mechanisms on appetite-regulating hormones, appetite perceptions, and energy intake, while also
567 providing supporting data for intensity-dependent effects on these outcomes. This study also
568 included male and female participants, with females participating in the follicular phase when
569 ovarian hormone concentrations are similar between the sexes mitigating known effects of
570 elevated ovarian hormones on appetite-regulating hormones (Moniz et al., 2023, Asarian and
571 Geary, 2013, Devries et al., 2006). Additionally, while absolute energy intake differences between
572 the sexes may exist due to body size differences (Hagobian et al., 2013, Panissa et al., 2016),
573 relative energy intake across the observation periods was consistent. Despite these strengths, it is
574 important to discuss some limitations of this current study. The fed state of all participants prior
575 to exercise was selected due to known effect on appetite when performing SIT in a fasted state
576 (Broad et al., 2020), however this may have blunted the exercise-induced increases in glucose and
577 the magnitude of divergent responses between exercise intensities previously exhibited following
578 fasted exercise (Marliss et al., 2000, Marliss et al., 1991, Marliss and Vranic, 2002, Sigal et al.,
579 1996, Sim et al., 2014). Pre-exercise energy intake appears to have prevented post-exercise insulin
580 increases despite glucose elevations as previously demonstrated (Peake et al., 2014), inhibiting our
581 ability to determine the effect of insulin in post-exercise appetite regulation. Additionally,
582 previous intensity-dependent differences in glucose were not reproduced in our data, potentially
583 due to the brief nature of the SIT session as other studies either completed vigorous-intensity
584 (>84% $\dot{V}O_{2max}$) continuous efforts for 13-15 min (Marliss et al., 2000, Marliss et al., 1991, Marliss

and Vranic, 2002, Sigal et al., 1996) or completed HIIT protocols with additional bouts and/or longer working intervals (Peake et al., 2014, Sim et al., 2014), which may have reduced the potential influences of glucose on appetite-regulating hormones in our data. While there are limitations and difficulties with measuring human free-living energy intake as previously stated (Dhurandhar et al., 2015), we utilized an image-based smart phone application software which allows dieticians to review the recorded energy intake entries (Ji et al., 2020) and we conducted 24-h recall follow-up interviews (on the morning of each session and within 48 hours after completion of the session) to improve food log accuracy (Hise et al., 2002) in an attempt to provide additional rigor to our energy intake measurements. The study design also prevented participants from consuming food and beverages post-exercise while in the lab. Though this design was chosen to examine the metabolite and appetite responses over the 120-min post-exercise without energy intake, it potentially prevented participants eating and/or drinking differently between the various conditions during the post-exercise observation period and limited our ability to observe energy intake differences between conditions. Additionally, our sample size calculation was to detect differences in appetite-regulating hormones, glucose, and insulin as these were our primary outcomes, and were not powered to detect changes in free-living energy intake (secondary outcome). Based on a mean change of 250 kcal between exercise and CTRL sessions (meaningful difference in daily energy intake) and the standard deviation observed in our data, a sample of 18 would be necessary to determine impactful changes in free-living energy intake. Considering the cost and rigorous data collection associated with blood collection, processing, and analysis this may not be feasible, however future work is warranted to assess free-living energy intake surrounding different exercise perturbations in larger sample sizes.

Though the focus of this study was the potential role of glucose and insulin, we did also measure blood lactate. This data supports previous literature demonstrating the important role of blood lactate accumulation as a mechanism for exercise-induced appetite regulation through suppression of acylated ghrelin and subjective appetite perceptions (Islam et al., 2017, Hazell et al., 2016, McCarthy et al., 2020, McCarthy et al., 2024a, McCarthy et al., 2023, Vanderheyden et al., 2020). While other mechanisms may yet be involved in exercise-induced appetite suppression, lactate accumulation appears involved in the suppression of acylated ghrelin and subjective appetite perceptions (McCarthy et al., 2020).

Conclusions

Overall, both exercise sessions increased plasma glucose and suppressed overall appetite perceptions but had no effect on energy intake. While the SIT session suppressed acylated ghrelin, the MICT session elevated GLP-1 suggesting changes in blood glucose are not involved in exercise-induced appetite suppression. No other glucose or insulin responses aligned with appetite-regulating parameters. This study also supports previously established intensity-dependent effects of exercise in suppressing acylated ghrelin and overall appetite perceptions, implicating other appetite-regulating mechanisms such as blood lactate accumulation which demonstrate more prolonged and pronounced effects. Future work should still consider a fasted exercise design to mitigate the potentially confounding effects of energy intake on these outcomes.

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Competing Interests

The authors declare there are no competing interests.

CRediT Authorship Contribution

Derek PD Bornath: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal Analysis, Data curation, Conceptualization. **Seth F McCarthy**: Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis. **Jessica AL Tucker**: Writing – review & editing, Investigation, Formal analysis. **Tamara R Cohen**: Writing – review & editing, Investigation, Formal analysis, Data curation. **Philip J Medeiros**: Writing – review & editing, Investigation, Formal analysis. **Tom J Hazell**: Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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653

654 **Data Availability**

655 Data will be made available upon reasonable request.

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861 **Figure 1.** Experimental timeline. CTRL, no-exercise control; MICT, moderate-intensity
862 continuous training; SIT, sprint interval training.
863
864 **Figure 2. A)** Absolute plasma glucose concentrations across all time points during each
865 experimental session. **B)** Area under the curve (AUC) for plasma glucose across all time points
866 during each experimental session. **C)** Absolute insulin concentrations across all time points during
867 each experimental session. **D)** AUC for insulin concentrations across all time points during each
868 experimental session. ¥ denotes differences between CTRL vs MICT at specific time points; $ denotes
869 differences between CTRL vs SIT at specific time points; # denotes differences between MICT vs SIT
870 at specific time points. Specific time point differences within sessions denoted by *a* – vs 0-min post-
871 exercise; *b* – vs 30-min post-exercise; *c*– vs 60-min post-exercise; *d* – vs 120-min post-exercise.
872 CTRL, no-exercise control; MICT, moderate-intensity continuous training; SIT, sprint interval
873 training.
874
875
876 **Figure 3. A)** Absolute acylated ghrelin concentrations across all time points during each
877 experimental session. **B)** Area under the curve (AUC) for acylated ghrelin across all time points
878 during each experimental session. **C)** Absolute active PYY concentrations across all time points
879 during each experimental session. **D)** AUC for active PYY concentrations across all time points
880 during each experimental session. **E)** Absolute active GLP-1 concentrations across all time points
881 during each experimental session. **F)** AUC for active GLP-1 concentrations across all time points
882 during each experimental session. ¥ denotes differences between CTRL vs MICT at specific time
883 points; $ denotes differences between CTRL vs SIT at specific time points; # denotes differences

884 between MICT vs SIT at specific time points. CTRL, no-exercise control; MICT, moderate-intensity
885 continuous training; SIT, sprint interval training.
886
887 **Figure 4. A)** Absolute overall appetite perceptions across all time points during each experimental
888 session. **B)** Area under the curve (AUC) for overall appetite perceptions across all time points
889 during each experimental session. **C)** Absolute energy intake across all days surrounding each
890 experimental session. ¥ denotes differences between CTRL vs MICT at specific time points; $
891 denotes differences between CTRL vs SIT at specific time points; # denotes differences between MICT
892 vs SIT at specific time points. * denotes energy intake differences compared to the day of the
893 experimental session. CTRL, no-exercise control; MICT, moderate-intensity continuous training;
894 SIT, sprint interval training.
895
896 **Figure 5. A)** Correlation of absolute Δ glucose compared to absolute Δ acylated ghrelin across all
897 participants and sessions. **B)** Correlation of absolute Δ glucose compared to acylated ghrelin AUC
898 across all participants and sessions. **C)** Correlation of absolute Δ glucose compared to absolute Δ
899 active GLP-1 across all participants and sessions. **D)** Correlation of absolute Δ glucose compared
900 to active GLP-1 AUC across all participants and sessions. **E)** Correlation of absolute Δ glucose
901 compared to absolute Δ overall appetite perceptions across all participants and sessions. **F)**
902 Correlation of absolute Δ glucose compared to overall appetite perceptions AUC across all
903 participants and sessions. Change (Δ) in glucose, acylated ghrelin, GLP-1, and overall appetite
904 perceptions were calculated by subtracting pre-exercise values from immediately post-exercise
905 values.

Figure 6. A) Correlation of absolute Δ insulin compared to absolute Δ acylated ghrelin across all participants and sessions. **B)** Correlation of absolute Δ insulin compared to acylated ghrelin AUC across all participants and sessions. **C)** Correlation of absolute Δ insulin compared to absolute Δ active GLP-1 across all participants and sessions. **D)** Correlation of absolute Δ insulin compared to active GLP-1 AUC across all participants and sessions. **E)** Correlation of absolute Δ insulin compared to absolute Δ overall appetite perceptions across all participants and sessions. **F)** Correlation of absolute Δ insulin compared to overall appetite perceptions AUC across all participants and sessions. Change (Δ) in insulin, acylated ghrelin, GLP-1, and overall appetite perceptions were calculated by subtracting pre-exercise values from immediately post-exercise values.

Table 1. Participant Characteristics. Values presented as Mean±SD. Range presented in brackets.

	***n*=12**	**8 M**	**4 F**
Age (y)	26±5	25±6 (19-34)	28±4 (24-33)
Height (cm)	172±12	175±9 (163-192)	159±3 (156-163)
Body Mass (kg)	68.0±11.9	74.1±9.2 (63-92)	55.9±5.1 (49-61)
BMI ($kg \cdot m^{-2}$)	23.2±2.1	23.8±2.1 (19.6-25.6)	22.2±1.7 (19.6-25.6)
$\dot{V}O_{2max}$ ($mL \cdot kg \cdot min^{-1}$)	48.08±7.01	51.35±3.21 (45.80-55.60)	41.05±8.76 (33.20-51.00)
%$\dot{V}O_2$ during MICT	68.7±2.0	68.1±1.8 (65.0-70.3)	69.8±2.8 (66.3-72.0)
Female menstrual cycle day of session	3±1	N/A	3±1 (1-5)
Weekly Moderate-Vigorous PA (min)	179±55	174±61 (120-270)	187±50 (120-225)

Note: BMI, body mass index; MICT, moderate intensity continuous; PA, physical activity.

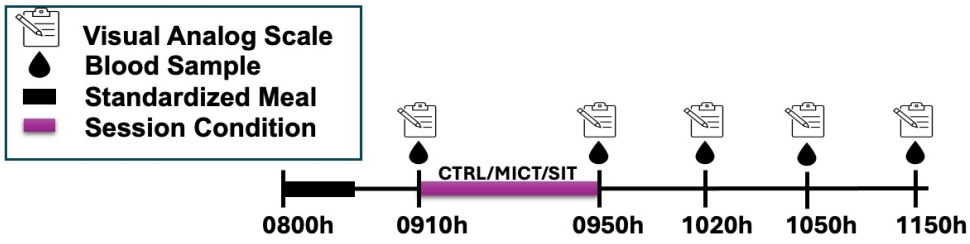

Figure 1. Experimental timeline. CTRL, no-exercise control; MICT, moderate-intensity continuous training; SIT, sprint interval training.

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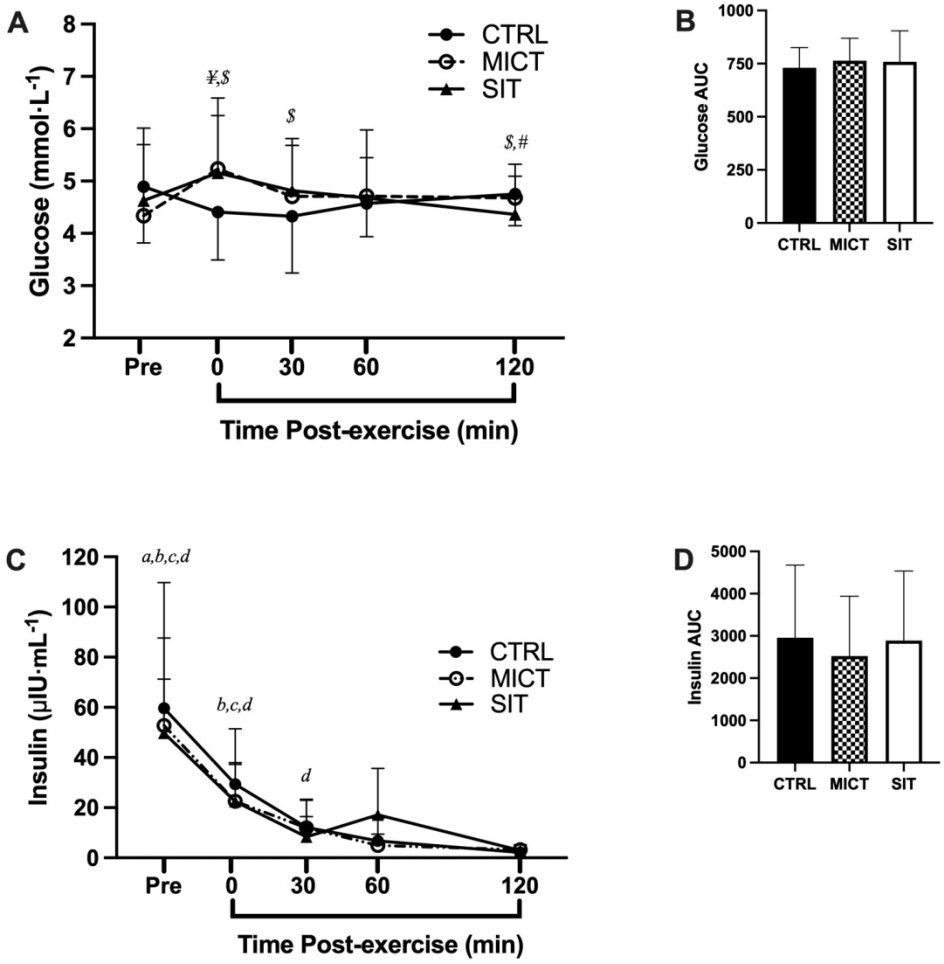

Figure 2. A) Absolute plasma glucose concentrations across all time points during each experimental session. B) Area under the curve (AUC) for plasma glucose across all time points during each experimental session. C) Absolute insulin concentrations across all time points during each experimental session. D) AUC for insulin concentrations across all time points during each experimental session. ¥ denotes differences between CTRL vs MICT at specific time points; $ denotes differences between CTRL vs SIT at specific time points; # denotes differences between MICT vs SIT at specific time points. Specific time point differences within sessions denoted by a – vs 0-min post-exercise; b – vs 30-min post-exercise; c– vs 60-min post-exercise; d – vs 120-min post-exercise. CTRL, no-exercise control; MICT, moderate-intensity continuous training; SIT, sprint interval training.

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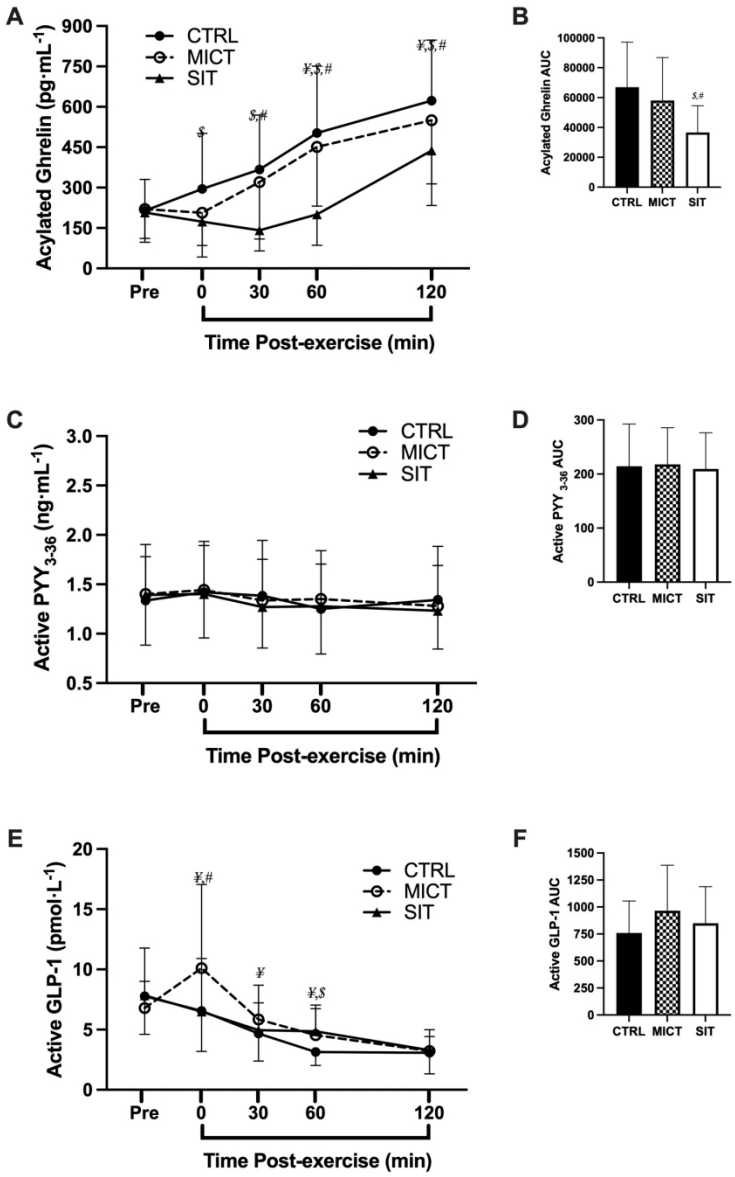

Fig 3. A) Absolute acylated ghrelin concentrations across all time points during each experimental session. B) Area under the curve (AUC) for acylated ghrelin across all time points during each experimental session. C) Absolute active PYY concentrations across all time points during each experimental session. D) AUC for active PYY concentrations across all time points during each experimental session. E) Absolute active GLP-1 concentrations across all time points during each experimental session. F) AUC for active GLP-1 concentrations across all time points during each experimental session. ¥ denotes differences between CTRL vs MICT at specific time points; $ denotes differences between CTRL vs SIT at specific time points; # denotes differences between MICT vs SIT at specific time points. CTRL, no-exercise control; MICT, moderate-intensity continuous training; SIT, sprint interval training.

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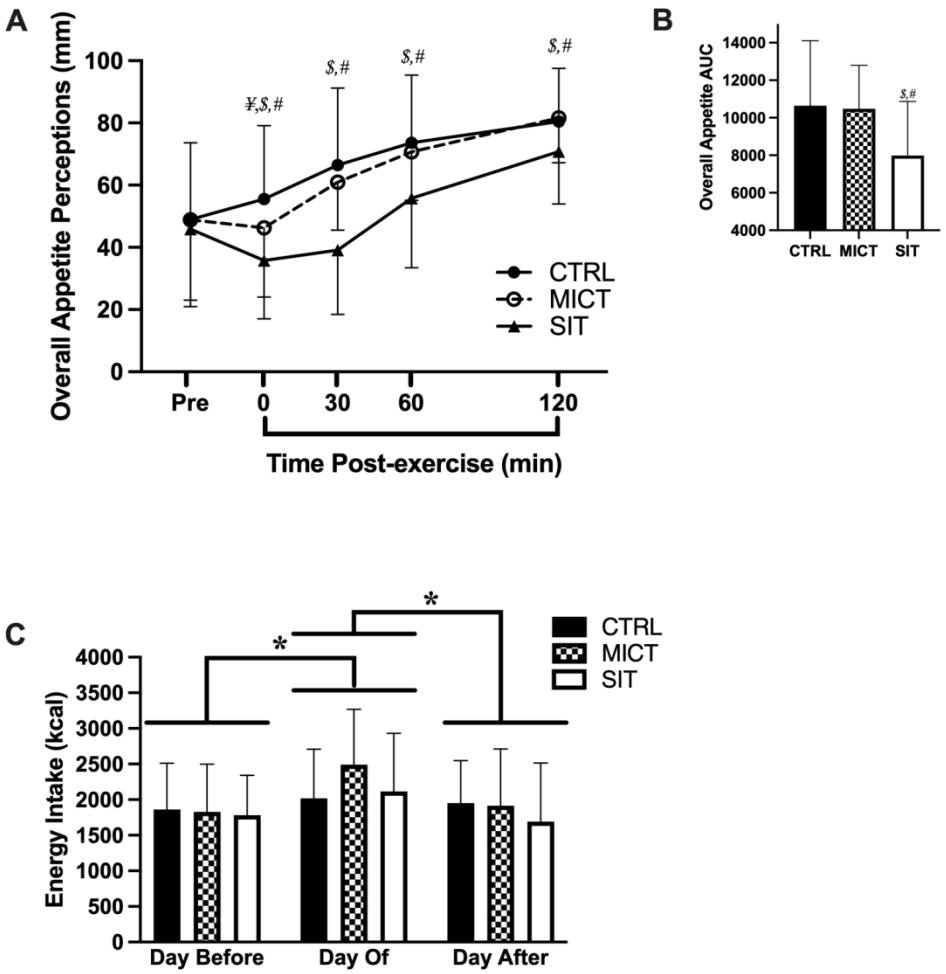

Figure 4. A) Absolute overall appetite perceptions across all time points during each experimental session. B) Area under the curve (AUC) for overall appetite perceptions across all time points during each experimental session. C) Absolute energy intake across all days surrounding each experimental session. ¥ denotes differences between CTRL vs MICT at specific time points; $ denotes differences between CTRL vs SIT at specific time points; # denotes differences between MICT vs SIT at specific time points. * denotes energy intake differences compared to the day of the experimental session. CTRL, no-exercise control; MICT, moderate-intensity continuous training; SIT, sprint interval training.

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