Interactive effects of acute exercise and carbohydrate-energy replacement on insulin sensitivity in healthy adults.

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ABSTRACT

This study investigated whether carbohydrate-energy replacement immediately after prolonged endurance exercise attenuates insulin sensitivity the following morning, and whether exercise improves insulin sensitivity the following morning *independent* of an exercise-induced carbohydrate deficit.

Oral glucose tolerance and whole-body insulin sensitivity were compared the morning after three evening conditions, involving: (1) treadmill exercise followed by a carbohydrate replacement drink (200 or 150 g maltodextrin for males and females, respectively; *CHO-replace*); (2) treadmill exercise followed by a non-caloric, taste-matched placebo (*CHO-deficit*); or (3) seated rest with no drink provided (*Rest*). Treadmill exercise involved 90 minutes at ~80% age-predicted maximum heart rate. Seven males and two females (aged 23 ± 1 years; body mass index 24.0 ± 2.7 $kg \cdot m^{-2}$) completed all conditions in a randomized order.

Matsuda index improved by 22% (2.2 [0.3, 4.0] au, $p = .03$) and HOMA2-IR improved by 10% (-0.04 [-0.08, 0.00] au, $p = .04$) in *CHO-deficit* versus *CHO-replace*, without corresponding changes in postprandial glycemia. Outcomes were similar between *Rest* and other conditions. These data suggest that improvements to insulin sensitivity in healthy populations following acute moderate/vigorous intensity endurance exercise may be dependent on the presence of a carbohydrate-energy deficit.

NOVELTY

- Restoration of carbohydrate balance following acute endurance exercise attenuated whole-body insulin sensitivity
- Exercise *per se* failed to enhance whole-body insulin sensitivity
- Maximizing or prolonging the post-exercise carbohydrate deficit may enhance acute benefits to insulin sensitivity

KEYWORDS

Glycemic, glycaemic, glycaemia, insulin resistance, refeeding, physical activity, metabolism, energy, deficit, carbohydrate availability

INTRODUCTION

Physical activity and exercise reduce glycemic exposure in both clinical and healthy populations (Schwingshackl *et al.*, 2014; Cavero-Redondo *et al.*, 2018), with these benefits occurring largely through improvements in insulin sensitivity (Way *et al.*, 2016). Regular exercise enhances insulin sensitivity not only through morphological and molecular adaptations, such as increased skeletal muscle capillarization or glucose transporter content, but also via a transient improvement in peripheral insulin sensitivity following each individual exercise bout (Mikines *et al.*, 1988; Sylow and Richter, 2019). While these benefits are well documented, less is understood about the key physiological signals (i.e. mediators) that initiate and maintain these effects as a result of repeated skeletal-muscle contractions (Cartee, 2015; Bird and Hawley, 2017). Likely mediators include molecular signalling events and/or alterations in substrate availability (Cartee, 2015). Moreover, nutrition has the potential to amplify or attenuate exercise-induced effects, likely via interactions with these mediators (Hearris *et al.*, 2018). Therefore, the deliberate manipulation of cellular signalling or substrate availability, through exercise or dietary strategies, may enable the enhancement of exercise-induced benefits to insulin sensitivity (Taylor *et al.*, 2018; Edinburgh *et al.*, 2020). Progress in identifying such strategies may enable the improvement of diet and exercise guidelines for promoting metabolic health.

Carbohydrate is likely a key substrate in this regard, and the negative carbohydrate balance (the sum of carbohydrate ingestion and synthesis, minus carbohydrate utilization) induced by acute endurance exercise may mediate the transient improvements in insulin sensitivity that follow. Supporting this hypothesis, studies using intravenous glucose administrations have demonstrated that post-exercise improvements in insulin sensitivity are attenuated when the carbohydrate expended during exercise is subsequently replaced, restoring carbohydrate balance (Bogardus *et al.*, 1983; Black *et al.*, 2005; Holtz *et al.*, 2008; Newsom *et*

al., 2010). Whilst intravenous glucose/insulin administration is considered the 'gold standard' method to assess insulin sensitivity, it diminishes the importance of several key factors that affect glucose appearance and disappearance rates when ingested orally. These include the incretin response, gastric emptying, intestinal glucose absorption and hepatic and renal extraction of plasma glucose (Kahn, 2001). The ability of acute exercise to accelerate both hepatic glucose disposal (Galassetti *et al.*, 1999) and oral glucose appearance in the blood (Knudsen *et al.*, 2014) highlights the necessity to investigate the role of carbohydrate balance in post-exercise improvements in insulin sensitivity under normal physiological conditions.

Taylor *et al*. (2018) recently began to address this issue, demonstrating that the oral glucose tolerance and whole-body insulin sensitivity of healthy participants are impaired by ~20% and ~25%, respectively, if the carbohydrate expended during exercise is replaced immediately post-exercise, versus when the carbohydrate deficit is maintained. However, due to the lack of a non-exercise control condition in that investigation, it remains unclear whether carbohydrate balance *fully* or *partially* mediates transient post-exercise improvements to insulin sensitivity. It is critical to understand whether carbohydrate balance is the key mediator of these benefits, as this may have vital implications regarding how nutrition and/or exercise may be structured to optimize metabolic health, potentially informing future guidelines. Therefore, the present study aimed to investigate, firstly, whether immediate post-exercise replacement of expended carbohydrate attenuates insulin sensitivity the following morning, and secondly, whether exercise improved insulin sensitivity the following morning *independent* of the negative exercise-induced carbohydrate balance.

METHODS

Approach to Problem

The present study provides particular novel insight due to the inclusion of a non-exercise control (*Rest*) in addition to exercise conditions, with (*CHO-replace*) and without (*CHO-deficit*) carbohydrate replacement. Comparison of these three conditions therefore isolates the independent effects of both exercise *per se* and an exercise-induced carbohydrate deficit: the former through comparison of *Rest* versus *CHO-replace* (whereby carbohydrate balance is maintained in both conditions by replenishing expended carbohydrate following exercise); the latter through comparison of *CHO-deficit* versus *CHO-replace* (whereby both conditions involve sustained skeletal muscle contraction but differ in carbohydrate replenishment post-exercise). The relative importance of carbohydrate balance in transient exercise-induced improvements in insulin sensitivity can then be inferred by simultaneously considering these comparisons.

Evening exercise was selected to enable a sufficient duration between exercise and assessment of glucose tolerance/insulin sensitivity, whilst minimizing the number of meals in that time. Glucose tolerance is often impaired when assessed immediately (1-2 hours) post-exercise, and the precise duration of this effect is unknown (Knudsen *et al.*, 2014). So, an appropriate duration was needed to ensure that such effects could dissipate, whereas fewer meals minimized the opportunity to restore carbohydrate balance independent of carbohydrate treatment. For this same reason, a standard low-carbohydrate dinner was provided following each condition and was the only energy-containing food or drink consumed other than the post-exercise carbohydrate treatment. Prolonged moderate/vigorous intensity exercise was used to achieve a substantial carbohydrate deficit and therefore achieve adequate differences in carbohydrate balance between exercise conditions. Further study controls and design considerations were enacted in line with the PRESENT 2020 checklist, aimed at ensuring appropriate standards of evidence reporting for sport and exercise nutrition trials were met (Betts *et al.*, 2020).

Participants and Sample Size Estimations

Seven males and two females without obesity and who self-reported as healthy were recruited from the Bath area (UK). All participants provided written informed consent for their participation. Mean ± SD age, body mass and body mass index (BMI) of participants were 23 ± 1 years, 72.6 ± 9.4 kg and 24.0 ± 2.7 $kg \cdot m^{-2}$, respectively. Exclusion criteria were: aged <18 years, habitual smoker within the past five years, any history of metabolic or respiratory disease and BMI >30 or <18.5 $kg \cdot m^{-2}$. The mean range in individual participants' body masses across all conditions was 0.8 ± 0.6 kg (1.1% ± 0.8% of mean body mass), suggesting that participants were weight stable. *A priori* sample size estimations were calculated using G*Power (Version 3.1, Department of Psychology, Germany) and data from Taylor *et al*. (2018). Taylor *et al*. (2018) observed an effect size of 1.05 when comparing the Matsuda Insulin Sensitivity Index following exercise with and without carbohydrate replacement. Thus, a sample size of 10 was estimated as appropriate for detecting this magnitude of difference ($\alpha = 0.05$; $\beta = 0.2$).

Study Design

The study protocol is summarized in **Figure 1**. Participants completed three conditions in a repeated measures design, each separated by a washout period of one to five weeks. Simple randomization, determined by a random number generator and performed by the lead researcher, was used to determine condition order. Conditions included an evening component (beginning 16:00-17:00) and an oral glucose tolerance test (OGTT) the following morning (beginning 08:00-09:00). The evening component of two conditions (*CHO-deficit* and *CHO-replace*) involved 90 minutes of treadmill exercise, followed by ingestion of a drink (900 mL water; 100 mL orange cordial) containing either maltodextrin (*CHO-replace*; 150 or 200 g for females or males, respectively) or two zero-calorie artificial sweetener tablets (Canderel,

London, UK) as a placebo (*CHO-deficit*). Orange cordial (1.1 g carbohydrate [all sugars], 0.5 g fat and 0.5 g protein; Co-Op, Manchester, UK) was used to taste-match drinks, blinding participants to maltodextrin (Myprotein, Cheshire, UK) treatment. No participant was able to identify in which trial they received either drink, which was confirmed using an exit questionnaire. The evening component of the resting condition (*Rest*) involved supervised, seated rest with no drink provided. Ethical approval was provided by the Research Ethics Approval Committee for Health, University of Bath (MSES 18/19-004) and the work was conducted in accordance with the Declaration of Helsinki.

Pre-Trial Standardizations

Participants were instructed to refrain from moderate or vigorous-intensity physical activity for the day of and the day prior to evening components, separating such activity (not including treadmill exercise) from the OGTT for a minimum of ~56 hours. For this same timeframe preceding the first condition, participants were instructed to complete a food diary to be replicated before the following two conditions. The food diary required participants to weigh any energy-containing foods or drinks consumed or used in the preparation of meals, and to record the time that foods/drinks were consumed. This ensured that both the timing and content of meals could be replicated precisely. Participants were instructed that pre-packaged items need not be weighed but that any leftovers should be. Participants were also instructed to abstain from caffeine and alcohol on the day of evening components (within ~24 hours of the OGTT) and not to consume food or drink (except water) within two hours of evening components. Compliance to pre-trial standardizations were confirmed using a 'yes'/'no' questionnaire, with space to provide details of any discrepancies between dietary timing or content. All participants reported being fully compliant with these standardizations.

Protocol – Evening Components

Height and body mass were measured using a stadiometer (Holtain Ltd, Pembrokeshire, UK) and electronic weighing scales (BC-543 Monitor, Tanita, Tokyo, Japan), respectively. Participants then completed a 'yes'/'no' questionnaire to verify compliance to pre-trial standardizations, and the International Physical Activity Questionnaire (short form) to confirm that habitual physical activity did not differ between conditions in the week preceding trials. Thereafter, participants underwent 90 minutes of treadmill exercise or seated rest according to treatment allocation.

Treadmill exercise (HP Cosmos Saturn 250/100r, HaB International Ltd, Warwickshire, UK) began with a maximum of five three-minute stages, progressing from an initial speed of 4-5 $km \cdot h^{-1}$ until 75-80% age-predicted maximum heart rate (HR_{max}; 220 – age in years; Fox and Naughton, 1972) was achieved. The speed that elicited 75-80% HR_{max} was maintained until 90 minutes of total exercise was completed. Heart rate (HR; Polar RS400, Kempele, Finland) and rating of perceived exertion (RPE; Borg 1973) were recorded during the final 30 seconds of each incremental stage. If HR increased beyond 80% HR_{max} during the three-minute stages of the first exercise condition, treadmill speed was reduced accordingly. Two participants verbally communicated (45 and 60 minutes into exercise) that they could not continue at the given intensity. Treadmill speed was therefore reduced to an intensity that the participants felt they could sustain (-11% for both), enabling them to complete the 90 minutes. Treadmill speeds were matched exactly during the subsequent exercise condition, to standardize exercise intensity within participants. The relative intensity selected (75-80% HR_{max}) approximated a similar protocol used by Taylor *et al*. (2018), which involved treadmill exercise for 90 minutes at 70% $\dot{V}O_2peak$ (see Garber *et al*. [2011] for approximate $\%HR_{max}$ to $\dot{V}O_2peak$ conversion). One-minute expired gas samples were obtained using the Douglas bag technique at 29-30, 44-45, 59-60, 74-75 and 89-90 minutes during exercise, to estimate

substrate (carbohydrate and lipid) oxidation and energy expenditure. HR and RPE were also obtained during the final 30 seconds of each gas sample. During *Rest*, four-minute expired gas samples were obtained at 26-30, 56-60 and 86-90 minutes. Exercise summary statistics and substrate/energy utilizations during evening components are shown in **Table 1**.

Participants consumed prescribed drinks within 30 minutes of exercise cessation. Maltodextrin doses were selected to approximately match the carbohydrate expended during exercise; less was therefore provided to female participants to account for their relatively lower treadmill speed and body mass, as is consistent with prescribed energy requirements for males and females (British Nutrition Foundation, 2019). Following each evening component, participants were provided with a standard dinner to take away (feta cheese, walnut, tomato, carrot and mixed leaf salad; 677 kcal, carbohydrate 14 g, fat 49 g, protein 45 g), which was prepared by the lead researcher. Participants were instructed to consume dinners by 20:30 and to consume nothing else but water (*ad libitum*) until completion of the OGTT the following morning. Adherence to these instructions was confirmed the following morning using a 'yes'/'no' questionnaire. Participants were also asked to aim for at least eight hours sleep on the night between evening component and OGTT.

Protocol - OGTT

Upon arrival, participants assumed a seated position for ~15 minutes before undergoing a fasted, baseline four-minute expired gas sample. Following this, a forearm vein was cannulated and a baseline blood sample drawn. A 75 g glucose solution (Polycal, Nutricia, Wiltshire, UK) was then ingested within five minutes. Blood was drawn 15, 30, 45, 60, 90 and 120 minutes after ingestion. Due to issues with a cannula, insulin data from *CHO-deficit* were not available for one participant; glucose concentration at 45 minutes was unavailable and concentrations at 60, 90 and 120 minutes were obtained from fingertip capillary blood samples using a handheld

glucose analyzer (FreeStyle Optium, Abbott, Dublin, Ireland). Therefore, this participant's insulin and insulin sensitivity data were not included in between-conditions comparisons or in means on figures (where n = 8 is specified). Further four-minute expired gas samples were collected at 24-28, 54-58, 84-88 and 114-118 minutes after drink ingestion, to estimate energy and substrate utilization.

Gas and Blood Sampling and Analyses

Expired gas samples were collected according to best practice using Douglas bags (Compher *et al.*, 2006) then analyzed for O_2 and CO_2 concentrations via paramagnetic and infrared transducers, respectively (Mini MP 5200, Servomex Group Ltd., Surrey, UK). Ambient air was sampled proximally to participants during each expired gas sample to adjust for inspired O_2 and CO_2 concentrations (Betts and Thompson, 2012). Volumes and temperatures of expired gas samples were measured using a dry gas meter (Harvard Apparatus, Kent, UK) and thermometer (CheckTemp1C, Hanna Instruments, Rhode Island, USA), respectively. Substrate oxidation and energy expenditure were estimated using indirect calorimetry and stoichiometric equations during exercise (Jeukendrup and Wallis, 2005) and at rest (Frayn, 1983).

Blood samples (4 mL) were immediately transferred into a tube containing ethylenediaminetetraacetic acid (BD, Oxford, UK) and centrifuged (5810, Eppendorf, Munich, Germany) for ten minutes (4000·gravity at 4ºC). Plasma was aliquoted, frozen on dry ice and stored at -80ºC. Plasma insulin was analyzed in duplicate using enzyme-linked immunosorbent assays (Mercodia AB, Uppsala, Sweden; intra-assay coefficient of variation [CV] = 5.4%). Plasma glucose was analyzed based on absorbance using a glucose oxidase assay and automated spectrophotometric analyzer (Randox Daytona, Randox Laboratories Ltd., County Atrim, UK; inter-assay CV = 2.7%).

Data Handling and Statistical Analyses

Data collation and calculations were performed in Microsoft Excel (v1902, WA, USA) unless stated otherwise. Total substrate oxidation and energy expenditure during evening components were estimated by multiplying mean utilization rates by 90 (minutes). As no expired gases were collected during the initial 15 minutes of exercise (during the incremental stages), an estimation was made for this period assuming a linear relationship between metabolic cost and treadmill speed (Mayhew, 1977). The influences of *CHO-deficit* and *CHO-replace* interventions on substrate (carbohydrate and fat) and energy balances were calculated relative to baseline conditions, i.e., *Rest*. Therefore, substrate and energy utilizations during *CHO-deficit* and *CHO-replace* conditions were subtracted from substrate and energy utilizations during *Rest*, then substrate and energy content of post-exercise drinks (from orange cordial and maltodextrin) were added to these values. Plasma glucose and insulin iAUCs were calculated using the Time Series Response Analyser (Narang *et al.*, 2020). The Matsuda index was calculated as described by Matsuda and DeFronzo (1999) and homeostatic model of insulin resistance-2 (HOMA2-IR; Levy, Matthews and Hermans, 1998) was calculated using the University of Oxford HOMA2 calculator (downloaded on 18 January 2021 from: https://www.dtu.ox.ac.uk/homacalculator/download.php).

Statistical analyses were performed in SPSS 26 (IBM, New York, USA). Mean differences between conditions are presented with 95% confidence intervals as: 'mean difference (lower bound, upper bound)'. Outcomes were tested for significant ($p \leq 0.05$) main effects of condition using one-way within-subjects ANOVAs, after which, *post hoc* comparisons were made between the three conditions using paired t-tests (Fisher's LSD). Data were interpreted using the 95% confidence intervals surrounding mean differences and relevant *p* values. A Greenhouse-Geisser correction was applied to hypothesis tests where sphericity was violated (epsilon < .75) and Huynh-Feldt *p* values used otherwise. No univariate outliers

(± 3.29 z-score; Tabachnick and Fidell 2012) were present for paired differences between conditions. Paired differences between conditions were normally distributed unless stated otherwise, as confirmed by the Shapiro-Wilk test (significance at $p \leq 0.05$).

Error bars on figures represent 95% normalized confidence intervals (nCIs; Masson and Loftus, 2003) unless stated otherwise. Normalized CIs are corrected for interindividual variation (i.e., the correction removes between-subjects differences) using the specific error term from the pairwise contrast at each timepoint. Thus, nCIs provide a visual representation of contrast between means, rather than interindividual variance around the mean (as would a standard deviation; Loftus and Masson, 1994). Using this approach, error bars that overlap by no more than half one side of an interval would typically generate a p value ≤ 0.05 if using a paired t-test (Masson and Loftus, 2003). Normalized CIs on line graphs were calculated using the omnibus error term from a one-way ANOVA rather than pairwise comparisons.

Matsuda index, HOMA2-IR, glucose iAUC and insulin iAUC were tested for main effects of trial order using one-way ANOVAs. These outcomes were also tested for a condition by trial order interaction effect using two-way ANOVAs, with trial order as a between-subjects factor. As there were six possible trial orders and nine participants, trial order was taken as the first trial performed by each participant, leaving three discrete options. No significant main effects of trial order or interaction effects were found.

RESULTS

Substrate and Energy Balances

Substrate and energy balances (in kcal) relative to *Rest* are presented in **Figure 2**. Mean carbohydrate balances (± SD) were -168 ± 47 g for *CHO-deficit* and 12 ± 48 g for *CHO-replace*, while fat balances were -12 ± 17 g for *CHO-deficit* and -7 ± 13 g for *CHO-replace*. Carbohydrate balance (95% CIs) was 179 (165, 194) g more negative in *CHO-deficit* than

CHO-replace ($p < .001$), whereas fat balance was similar (5 [-2, 11] g) between the two ($p = .10$).

Glucose/Insulin Responses and Insulin Sensitivity

Glycemic and insulinemic responses during OGTTs, along with iAUCs, are presented in **Figure 3**. Summary statistics for glycemic and insulinemic responses can be seen in **Table 2** and insulin sensitivity indices in **Figure 4**. Neither fasted plasma glucose nor glucose iAUCs were significantly different between conditions ($p = .23$ and .66 for ANOVA main effects, respectively). However, peak plasma glucose concentration was reduced by 8% in *CHO-deficit* (-0.86 [-1.56, -0.15] $mmol \cdot L^{-1}$, $p = .02$) versus *CHO-replace*, and by 9% (-0.77 [-1.22, -0.32] $mmol \cdot L^{-1}$; $p < .01$) in *CHO-deficit* versus *Rest* ($p = .01$ for ANOVA main effect). Peak glucose was similar between *CHO-replace* and *Rest* (mean difference: 0.09 [-0.53, 0.71] $mmol \cdot L^{-1}$, $p = .78$). Insulinemic responses were similar in both magnitude and shape between conditions when examining both mean and median values (**Table 2** and **Figure 3)**.

Both Matsuda index and HOMA2-IR showed significant ANOVA main effects; $p = .04$ and .05, respectively. Matsuda index and HOMA2-IR were improved in *CHO-deficit* versus *CHO-replace*, by 22% (2.2 [0.3, 4.0] au, $p = .03$) and 10% (-0.04 [-0.08, 0.00] au, $p = .04$), respectively. Differences in Matsuda index (1.6 [-0.4, 3.7] au) and HOMA2-IR (-0.03 [-0.07, 0.02] au) between *CHO-deficit* versus *Rest* were not statistically significant ($p = .12$ and .18). Both Matsuda index and HOMA2-IR were similar between *CHO-replace* and *Rest*, with mean differences of -0.6 (-2.1, 0.9) au ($p = .26$) and 0.02 (0.00, 0.04) au ($p = .10$), respectively.

Substrate Utilization During OGTTs

Total carbohydrate utilizations during OGTTs were 76 ± 31, 89 ± 24 and 93 ± 32 kcal for *CHO-deficit*, *CHO-replace* and *Rest*, respectively. Total fat utilizations during OGTTs were 93 ± 39,

82 ± 31 and 76 ± 32 kcal for *CHO-deficit*, *CHO-replace* and *Rest*, respectively. There were no significant differences between conditions in total carbohydrate ($p = .28$), fat ($p = .30$) or energy ($p = .96$) utilizations.

DISCUSSION

Fasted and postprandial indices of whole-body insulin sensitivity were improved by 10% (0.04 au) and 22% (2.2 au), respectively, when the carbohydrate deficit following prolonged treadmill exercise was maintained, compared to when carbohydrate balance was restored. In contrast, exercise alone did not improve insulin sensitivity versus seated rest, suggesting that a carbohydrate/energy deficit may be required to elicit transient improvements in insulin sensitivity following acute exercise in healthy individuals.

The 22% (2.2 au) and 10% (0.04 au) improvements in Matsuda index and HOMA2-IR following post-exercise carbohydrate replacement were consistent with the 25% (2 au) and 16% (0.1 au) changes reported by Taylor *et al*. (2018) under similar conditions. These authors' comparisons were made the morning after 90 minutes running at 70% $\dot{V}O_2$peak in a similar study population, with or without replacement of carbohydrate (maltodextrin) to precisely match that expended during exercise. This supports the hypothesis that post-exercise insulin sensitivity is mediated by carbohydrate balance.

Due to the energy content of carbohydrate, manipulation of carbohydrate balance in the present study also altered energy balance. However, studies that have replaced energy using carbohydrate following acute endurance exercise have consistently attenuated insulin sensitivity or glycemic control (Bogardus *et al.*, 1983; Black *et al.*, 2005; Stephens *et al.*, 2007; Holtz *et al.*, 2008; Newsom *et al.*, 2010; Taylor *et al.*, 2018; Schleh *et al.*, 2020), whereas most studies that have replaced expended energy with fat have shown no such effect (Fox, Kaufman and Horowitz, 2004; Schenk *et al.*, 2005; Newsom *et al.*, 2010). This suggests that

carbohydrate rather than energy balance may be critical in this context. In contrast, one recent study (Areta *et al.*, 2020) found that both glycemic and insulinemic responses to a recovery drink (1.20 and 0.38 g·kg[fat free mass]$^{-1}$ maltodextrin and whey protein isolate, respectively) were increased by 13% (13 au) and 35% (190 au), respectively, the morning after evening exercise followed by energy replacement using fat, versus when the energy deficit was maintained. Reasons for this discrepancy are unclear, although some characteristics that are unique to Areta *et al*. (2020) versus the previously mentioned studies are the use of 'well-trained' ($\dot{V}O_2$peak 66 mL·kg^{-1}·min^{-1}) as opposed to 'healthy' participants, and the measurement of glycemic and insulinemic responses 30 minutes after a 75-minute intermittent high-intensity training session, rather than under rested conditions. The findings of Areta *et al*. (2020) highlight that energy balance may be a confounding factor, although notably there was no carbohydrate-energy replacement condition as a comparison in that study. Based on the available evidence it seems that carbohydrate-energy replacement mediates transient changes to insulin sensitivity more potently than fat-energy replacement (Newsom *et al.*, 2010), implying that carbohydrate balance does exert mediatory effects beyond concurrent changes in energy balance.

The present study expands on the findings of Taylor *et al*. (2018) with the inclusion of the *Rest* condition, whereby similar insulin sensitivity following seated rest and exercise followed by carbohydrate replacement may suggest that endurance exercise does not improve insulin sensitivity independent of a negative carbohydrate/energy balance. This concurs with Venables *et al*. (2007), who showed no improvement in glucose/insulin responses versus rest when a 200:50 g carbohydrate:protein beverage was ingested following one hour of cycling at 75% peak power output. Other authors have demonstrated an 11% (31 mU·L^{-1}·h) exercise-induced reduction to postprandial insulinemia, despite post-exercise energy replacement to match that expended during exercise (Burton *et al.*, 2008). However, these authors only

replaced ~75% of expended carbohydrate on average (~89 of 115 g), which may explain why some benefits persisted despite energy replacement. Conversely, this discrepancy could stem from Burton *et al*. (2008) studying middle-aged individuals with overweight or obesity rather than lean young adults, as there may be separate mechanisms involving inflammatory cytokines or intracellular lipid intermediates that contribute to post-exercise improvements in insulin sensitivity in such populations (Cartee, 2015).

Importantly, the present data do not suggest that exercise is not beneficial for glycemic control or insulin sensitivity, should carbohydrate be consumed post-exercise. Although *CHO-deficit* failed to improve insulin sensitivity indices versus *Rest*, the acute changes in insulin sensitivity following individual exercise bouts must be viewed as separate and additive to the positive effects of *chronic* exercise training. Chronic exercise contributes to preventing excess adiposity (Catenacci and Wyatt, 2007) and elicits beneficial adaptations that contribute to improved glucose control and insulin sensitivity (Sylow and Richter, 2019), as well as numerous other predictors of cardiometabolic health (Pinckard, Baskin and Stanford, 2019).

Improvements in whole-body insulin sensitivity derived from oral glucose ingestion may indicate either: reduced rates of dietary glucose appearance, enhanced suppression of endogenous glucose output, and/or greater glucose disposal per-unit insulin (Abdul-Ghani, Tripathy and DeFronzo, 2006). As carbohydrate oxidation rates were not different during OGTTs, improvements in insulin sensitivity were likely due to greater non-oxidative glucose disposal (i.e., glycogen storage; Mikines *et al*. 1988). Variation in the postprandial glycemic control of healthy populations is determined primarily by rates of glucose disposal rather than endogenous glucose suppression (Ferrannini *et al.*, 1985), which may explain why relative improvements in the Matsuda index (a postprandial measure) were greater than the HOMA2-IR (a fasted measure).

The present study did not confirm the ~20% (68 mmol·L^{-1}·120min) reduction in glucose tolerance shown by Taylor and colleagues (2018) when carbohydrate was replaced immediately post-exercise, despite improvements in insulin sensitivity. This is consistent with many investigations of postprandial glucose control in healthy populations, whereby glucose concentrations are fairly stable while insulin concentrations fluctuate more dramatically (Brestoff *et al.*, 2009; Hengist *et al.*, 2020). Despite this, Schleh *et al.* (2020) demonstrated that energy/carbohydrate replacement following cycling at 65% $\dot{V}O_2$peak increased interstitial glycemia (+1 mmol·L^{-1}·h^{-1} per 3 h) at breakfast the following morning, supporting findings of Taylor and colleagues (2018). Differences in physiological traits or lifestyle factors between study participants, such as training status (Steenberg *et al.*, 2019), could explain why changes in glucose tolerance differed between studies. It is also possible that the exercise prescribed by Taylor *et al*. (2018) elicited a greater relative stimulus than in the present study, due to the ramping up of exercise intensity over the first 15 minutes in the present study, and because mean %HR$_{max}$ could be exaggerated due to cardiovascular drift (Wingo, Ganio and Cureton, 2012). Despite the lack of notable reductions in glucose iAUC, peak glucose concentrations were reduced in *CHO-deficit* versus the other conditions. Although some data suggest that hyperglycemic spikes may better predict endothelial damage than total glycemic exposure (Hanefeld *et al.*, 1999), it is unclear whether the relatively small magnitude of differences observed in peak glucose (< 1 mmol·L^{-1}) would be clinically meaningful.

With the available data suggesting that a negative carbohydrate/energy balance may be necessary for transient post-exercise improvements in insulin sensitivity, this could have implications for both training and nutrition strategies aimed at improving metabolic health. Previous research has sought to establish appropriate exercise doses that elicit improvements in insulin sensitivity based on the energy cost of exercise (Magkos *et al.*, 2008), and indeed the transient benefits of acute exercise do increase with greater energetic expenditure at a given

intensity (Ding *et al.*, 2019). However, the apparent improvements with greater energy expenditure in that context may be largely explained by concomitantly greater carbohydrate utilization. Should carbohydrate balance mediate these benefits, this may explain (in part) why small doses of exercise that are highly reliant on carbohydrate rather than lipid energy sources, such as brief sprints, can be as beneficial as greater doses of lower intensity exercise for acutely improving glycemic control (Metcalfe *et al.*, 2018). Thus, future investigations may seek to determine whether changes in carbohydrate or energy balance better predict improvements in insulin sensitivity.

Regarding nutritional implications, the present data suggest that transient post-exercise benefits in insulin sensitivity may only persist until carbohydrate balance is restored. Notably, the duration for these transient benefits is generally reported as 'up to 48 hours' in healthy populations (Mikines *et al.*, 1988; Sylow and Richter, 2019), which maps onto the time-course for muscle glycogen restoration following prolonged endurance exercise (consuming 8.6 $g \cdot kg[body\ mass]^{-1} \cdot day^{-1}$ carbohydrate on average; Piehl, 1974). In support of this, a study using continual glucose monitoring recently demonstrated that glucose control was improved only at breakfast, not lunch and dinner, the day after 350 kcal cycling exercise (Schleh *et al.*, 2020). This seems to support that benefits were attenuated following restoration of carbohydrate balance, i.e., after breakfast. Therefore, purposefully delaying or minimizing carbohydrate replacement after exercise, thus potentially extending or enhancing these transient benefits, may be beneficial if preceding occasions when an exaggerated metabolic response to feeding could be expected, such as acute overeating (Hengist *et al.*, 2020). However, such nutritional approaches would need to be tested experimentally over longer study durations to establish whether they prove beneficial for cardiometabolic outcomes.

Carbohydrate balance necessarily equates to fluctuations in whole-body carbohydrate availability (i.e., endogenous carbohydrate available for energy production as blood glucose,

liver glycogen and skeletal-muscle glycogen). Therefore, alterations in endogenous glycogen concentrations provide a potential mechanism through which carbohydrate balance could mediate insulin sensitivity. Glycogen has been theorized to regulate glucose uptake via glycogen synthase activity or potential interactions with adenosine monophosphate-activated protein kinase (AMPK), glucose transporter translocation and insulin signalling (Bogardus *et al.*, 1983; Richter, Derave and Wojtaszewski, 2001; Jensen *et al.*, 2011). As tissue glycogen concentrations were not measured in the present study, the patterns of glycogen depletion and repletion across skeletal muscle and liver with the experimental treatments is unclear. However, potential differences in glycogen restoration patterns with different carbohydrate types, timings or multi-macronutrient combinations may warrant further investigation using different carbohydrate replacement strategies (Stephens *et al.*, 2007; Gonzalez *et al.*, 2017).

In the present study, participants were not provided the precise mass of carbohydrate expended during *CHO-replace*, which resulted in some variation in carbohydrate balance between participants in this condition. This decision was made on a practical basis, but had expended carbohydrate been replaced precisely, there may have been a more consistent response in outcomes between *CHO-balance* and *Rest*. However, given the lack of differences observed between these conditions, this is unlikely to have affected overall results. Additionally, while carbohydrate balances were carefully manipulated and recorded during lab sessions, it was not possible to obtain valid/reliable estimates of substrate and energy expenditure outside of lab settings. Therefore, participants' overall substrate/energy status across the entire study period was unknown. This could contribute to variation *between* participants in responses to interventions, but because precautions were undertaken to standardize substrate/energy balances *within* participants, it should not have influenced between-conditions comparisons of primary outcomes. While it is possible that random or systematic variation in carbohydrate utilization, oxidation or synthesis in-between evening

components and OGTTs could have undermined these standardizations to some degree, random variation would not be expected to bias any one condition, and any systematic changes in carbohydrate balance following exercise would likely counteract the carbohydrate deficit achieved in *CHO-deficit*. Therefore, any systematic bias would likely tend toward the null. Possible systematic changes include: reduced carbohydrate oxidation at the expense of fat (Mulla, Simonsen and Bülow, 2000), greater gluconeogenesis due to increased glycerol availability from lipolysis (Bortz *et al.*, 1972; Magkos *et al.*, 2008), as well as compensatory reductions in non-exercise physical activity thermogenesis (Washburn *et al.*, 2014). Additionally, whilst both males and females were recruited, the present study is not powered to analyze sex differences in responses.

In summary, the present study revealed that post-exercise carbohydrate replacement attenuated fasted and postprandial whole-body insulin sensitivity following acute moderate/vigorous intensity endurance exercise, whereas exercise *per se* did not enhance these outcomes. Therefore, these data suggest that the negative carbohydrate balance achieved during exercise may be critical to elicit transient exercise-induced improvements in insulin sensitivity, which could have implications for both diet and exercise strategies aimed at improving metabolic health.

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TABLES

Table 1. Summary statistics for the treadmill exercise bouts during *CHO-deficit* and *CHO-replace*, as well as substrate/energy utilizations during all evening components.

	CHO-deficit	*CHO-replace*	*Rest*
Treadmill speed ($km \cdot h^{-1}$)	8.6 ± 2.2	8.6 ± 2.2	–
$\%HR_{max}$	79 ± 4	79 ± 6	–
RPE (Borg scale)	15 ± 1	15 ± 1	–
Oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$)	32.0 ± 7.5	32.2 ± 7.3	–
CHO utilization (g)	185 ± 44	194 ± 58	16 ± 7
Lipid utilization (g)	29 ± 12	24 ± 8	7 ± 3
Energy utilization (kcal)	1022 ± 242	1015 ± 258	126 ± 12

All values are mean ± SD; CHO = carbohydrate; carbohydrate oxidation at rest is assumed as 100% glucose; carbohydrate oxidation during exercise is assumed as a 1:4 glucose:glycogen ratio (Jeukendrup and Wallis 2005); energy contents per g are: glycogen 4.15 kcal, glucose 3.73 kcal and lipid (palmoyl-stearol-oleoyl glycerol) 9.42 kcal (Frayn 1983); mean carbohydrate content of the drinks provided were 190 g in *CHO-replace* and 1 g in *CHO-deficit*.

Table 2. Plasma glucose and insulin summary statistics during OGTTs.

	CHO-deficit	*CHO-replace*	*Rest*	ANOVA main effect (*p*)
Fasted glucose ($mmol \cdot L^{-1}$)	4.47 ± 0.34	4.78 ± 0.48	4.85 ± 0.65	.23
Peak glucose ($mmol \cdot L^{-1}$)	8.70 ± 1.41	9.56 ± 2.06	9.47 ± 1.83	.01*
ToP glucose (minutes)	38 ± 16	35 ± 13	42 ± 13	.44
Fasted insulin ($pmol \cdot L^{-1}$)	20.0 ± 1.0	21.8 ± 3.9	20.8 ± 3.2	-
Peak insulin ($pmol \cdot L^{-1}$)	254.9 ± 127.6	284.8 ± 104.8	307.5 ± 140.5	.14
ToP insulin (minutes)	54 ± 8	47 ± 10	53 ± 11	.21

All values are mean ± SD; ToP = time of peak; * denotes $p < .05$; n = 8 for insulin values; n = 9 for glucose values; the majority of fasted insulin concentrations fell below the minimum detection limit so were assigned the minimum value (19.62 pmol/L; n = 7/8 *CHO-deficit*; n = 5/9 *CHO-replace*; n = 8/9 *Rest*), hence the ANOVA was not applied in this comparison.

FIGURES

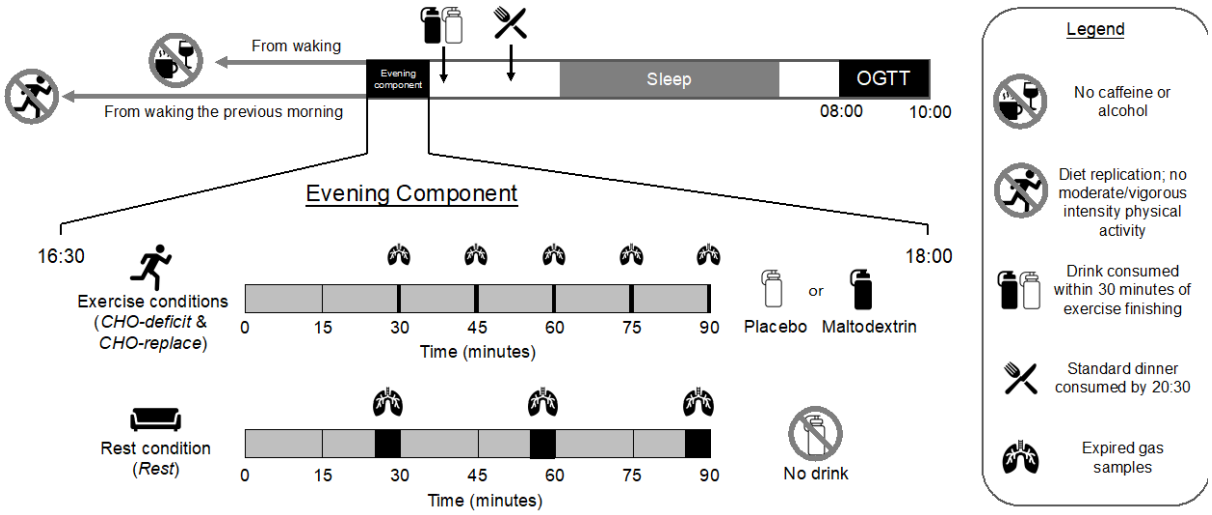

Figure 1. Study protocol schematic; start times for evening components and OGTTs (oral glucose tolerance tests) are approximate (within 30 minutes) but their durations were consistent; each participant (n = 9) completed all conditions in a randomized order.

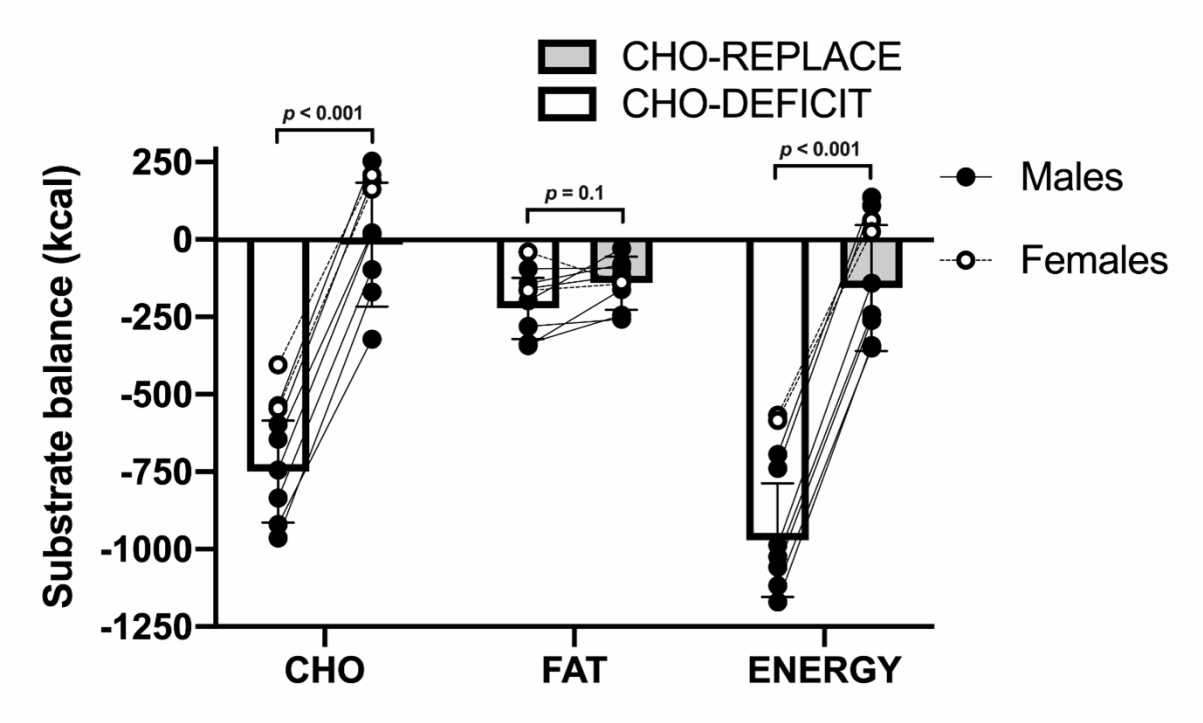

Figure 2. Substrate and energy balances relative to *Rest*; error bars are SD; CHO = carbohydrate; individual data for males and females are represented by solid and dashed lines with filled and open markers, respectively.

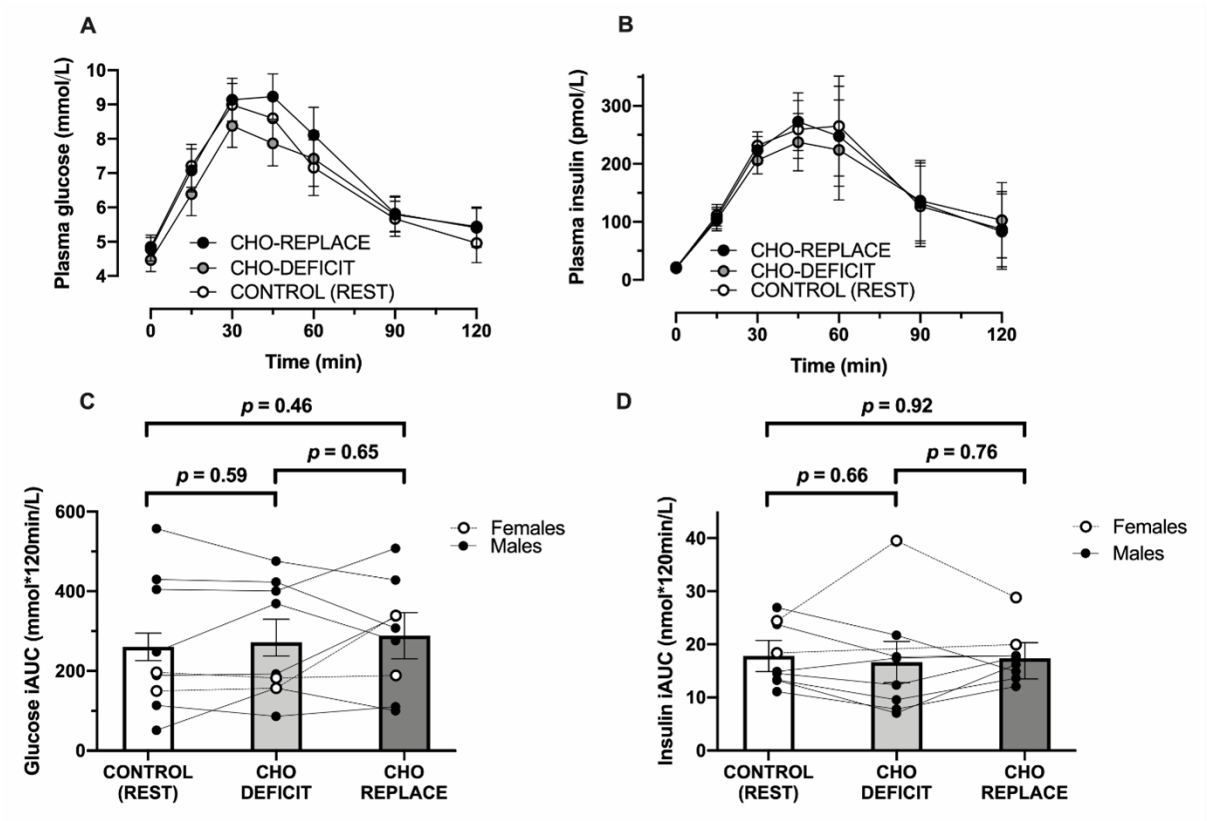

Figure 3. Panels A and B illustrate time-course responses for plasma glucose and insulin during OGTTs; panels C and D show the corresponding plasma glucose and insulin iAUCs; all error bars are 95% normalized confidence intervals; error bars on panels C and D are asymmetrical (upper error bars correspond to the greater of the other two conditions and lower error bars to the lesser of the other two conditions); n = 9 for mean glucose values; n = 8 for mean insulin values; individual data for males and females are represented by solid and dashed lines with filled and open markers, respectively.

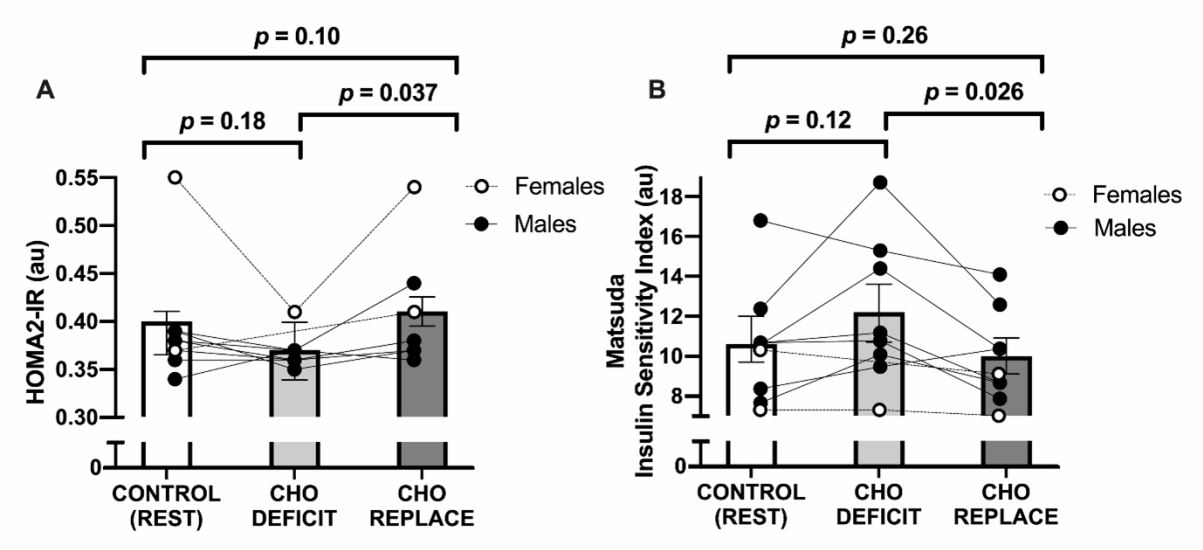

Figure 4. Insulin sensitivity indices; error bars are 95% normalized confidence intervals; error bars are asymmetrical (upper error bars correspond to the greater of the other two conditions and lower error bars to the lesser of the other two conditions); n = 8 for mean values; individual data for males and females are represented by solid and dashed lines with filled and open markers, respectively.

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COMPETING INTERESTS

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AUTHOR CONTRIBUTIONS

Conceptualization – DJ-B, JAB, JTG and HT; study design – DJ-B and JAB; data collection – DJ-B, BJN, HAS, AH, JGW, JTG and J-PW; biochemical analyses – DJ-B and RD; data handling – DJ-B; writing (original draft) – DJ-B and JAB; writing (reviewing and editing) – DJ-B, JAB, BJN, HAS, AH, JGW, JTG, RD, J-PW and HT; creation of figures – JTG; project administration – DJ-B; project supervision – JAB.

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