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RESEARCH ARTICLE

Exercise Metabolism

Application of ^{13}C MRS demonstrates carbohydrate feeding spares muscle but not liver glycogen utilization during high-intensity interval exercise

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Abstract

We examined liver and muscle glycogen utilization during high-intensity interval cycling, and the impact of carbohydrate (CHO) feeding, using noninvasive ^{13}C magnetic resonance spectroscopy (MRS). Following 24 h of standardized dietary intake, nine male cyclists completed 8 × 5-min intervals (1-min recovery), ingesting either placebo (PLA), 60 g maltodextrin (CHO), or 60 g maltodextrin plus caffeine, taurine, L-theanine, L-citrulline, and citicoline (CHO$^+$) in a randomized crossover design. ^{13}C MRS and ^{1}H imaging were performed pre- and postexercise to determine liver and muscle glycogen and liver volume, respectively. Liver glycogen utilization was not significantly different between trials ($P = 0.101$) despite lower postexercise plasma glucagon concentrations in CHO and CHO$^+$ ($P = 0.001$). In contrast, muscle glycogen utilization was significantly lower (~40%) with CHO feeding compared with PLA ($P = 0.006$), yet this sparing effect was not evident with CHO$^+$ ($P = 0.073$) in accordance with a higher mean power output during the late intervals (+2.8%, $P = 0.046$). Plasma glucose was comparable between trials ($P = 0.175$), whereas plasma lactate was higher in CHO$^+$ versus CHO ($P = 0.003$), alongside lower blood bicarbonate ($P = 0.005$), base excess ($P < 0.001$), and total CO_2 ($P = 0.004$). These findings demonstrate preferential use of skeletal muscle glycogen during high-intensity interval training (HIIT), which is attenuated under conditions of CHO feeding. This sparing effect is, however, not evident with the coingestion of a caffeine-containing multi-ingredient blend, potentially due to an increased capacity to sustain higher power outputs resulting in greater glycogen utilization.

NEW & NOTEWORTHY Using ^{13}C MRS, we provide data demonstrating preferential use of skeletal muscle glycogen during HIIT. Furthermore, data show muscle glycogen utilization is attenuated with CHO feeding, yet sparing is not evident when coingesting a caffeine-containing formulation, potentially reflecting increased capacity to perform more total work rather than a direct metabolic effect of caffeine. In contrast, liver glycogen utilization was not significantly different with CHO feeding despite a modest reduction of ~5 g versus placebo.

^{13}C magnetic resonance spectroscopy; exercise; glycogen; HIIT; liver

INTRODUCTION

Both muscle and liver glycogen provide important substrates for energy production during prolonged moderate-to-high intensity endurance exercise. Although these stores only represent <5% of total energy stored, muscle glycogen contributes >50% toward total energy requirements during moderate-to-high intensity exercise (1, 2). Despite its comparatively limited capacity, liver glycogen plays an essential role in the maintenance of euglycemia and rates of whole body carbohydrate (CHO) oxidation, accounting for ~18% of total energy expenditure (2, 3). Moreover, the contribution of liver glycogen toward energy expenditure appears to increase during the latter stages of prolonged exercise once muscle glycogen becomes depleted, whereby liver-derived glucose can almost exclusively sustain the high rates of CHO oxidation needed to maintain exercise intensity (4). Accordingly, endogenous glucose production (EGP) has been

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shown to progressively increase during prolonged endurance exercise (5, 6), reflecting the increasing reliance on liver glycogen to maintain energy production.

Alongside both muscle and liver glycogen, exogenous CHO provides an alternative fuel source during prolonged exercise, allowing for the maintenance of blood glucose concentrations and rates of whole body CHO oxidation (4), thereby delaying the crossover point where fat becomes the predominant fuel (7, 8). In this way, CHO feeding during exercise attenuates the oxidation of endogenous CHO stores (9). Although this effect appears to be predominantly related to the liver, given that EGP can be completely suppressed with rates of CHO ingestion of 175 $g \cdot h^{-1}$ (3), muscle glycogen sparing may also contribute to a lesser extent (10). To date, only one study has directly quantified the impact of CHO feeding on liver glycogen utilization in response to prolonged endurance exercise using ^{13}C magnetic resonance spectroscopy (MRS), demonstrating the exercise-induced decline in liver glycogen content is abolished when CHO is ingested at a rate of 102 $g \cdot h^{-1}$ (11). Thus, it remains unclear whether such sparing effects are maintained during more strenuous training protocols where metabolic demands are further increased.

Despite the clear physiological importance of liver glycogen during exercise, much of our understanding of its metabolism is based on steady-state endurance exercise, largely due to methodological constraints of using stable isotope tracers to assess EGP during high-intensity intermittent exercise and a lack of accessibility to directly measure liver glycogen content. Consequently, little is known about liver glycogen utilization during high-intensity interval training (HIIT), despite its growing popularity, perceived enjoyment and substantial contribution to endurance athletes' overall training volume in recent decades (12–14). However, the use of ^{13}C MRS now permits the direct noninvasive measurement of liver glycogen content, allowing for the assessment of its utilization in response to high-intensity exercise. Although not interval-based, some evidence does suggest that CHO feeding during higher-intensity steady-state exercise (80% $\dot{V}O_{2peak}$) also attenuates EGP, yet the rate of glucose appearance from the gut may be insufficient to fully suppress EGP at such intensities (6). This attenuated suppression may stem from a combination of reduced gastric emptying rates at high intensities and a potent sympathoadrenal "feed-forward" drive (i.e., circulating catecholamines) for hepatic glycogenolysis, effectively overriding the inhibitory feedback signals usually provided by pancreatic hormones (i.e., insulin and glucagon) and blood glucose (6). Furthermore, given that only 26% of ingested CHO has been reported to reach systemic circulation during exercise (6), it is feasible that some may undergo first-pass hepatic uptake, potentially contributing to hepatic glycogen resynthesis during exercise. Together, these observations suggest that CHO ingestion during high-intensity interval exercise may attenuate the decline in liver glycogen (via reduced EGP or rapid resynthesis from first-pass uptake), yet this hypothesis has not been directly tested.

In addition to CHO, caffeine is also widely recognized for its ergogenic properties during exercise (15–17) and is often consumed as part of multi-ingredient formulations with purported ergogenic properties targeted to enhance exercise performance and selected components of cognitive function. Caffeine ingestion [3–9 $mg \cdot kg^{-1}$ body mass (BM)] can also increase circulating concentrations of adrenaline during exercise, particularly at higher doses (18, 19), which may subsequently alter hepatic glucose output. Indeed, previous studies have shown physiological adrenaline infusion (resulting in plasma adrenaline concentrations below those observed in response to caffeine intake) can modestly increase EGP during low-intensity exercise (20), reflective of accelerated liver glycogenolysis. These findings raise the possibility that caffeine-induced changes in catecholamine concentrations could potentially influence liver glycogen utilization during high-intensity exercise, warranting further investigation. Furthermore, given the effects of caffeine on self-selected power output during HIIT (16), it is also possible that the coingestion of caffeine may accelerate muscle glycogen use.

Accordingly, the primary aim of the present study was to assess liver and muscle glycogen utilization during a prolonged bout of high-intensity interval cycling using noninvasive ^{13}C MRS. To evaluate the effects of exogenous CHO on both liver and muscle glycogen utilization, participants also completed a separate trial in conditions of CHO feeding. Finally, considering the potential for caffeine to enhance high-intensity exercise performance and alter endogenous glycogen metabolism, participants completed an additional CHO feeding trial that included a caffeine-containing multi-ingredient formulation. This experimental prototype was designed to reflect the proprietary formulations of supplemental "pre-workout" blends (21) commonly used by athletes before high-intensity exercise. To achieve our aims, nine male cyclists completed 8 × 5 min intervals at maximal self-selected intensity with 1-min recovery periods in a randomized crossover design. We hypothesized that *1*) CHO feeding would attenuate endogenous glycogen utilization compared with placebo and *2*) the coingestion of a caffeine-containing multi-ingredient formulation would facilitate higher power outputs and subsequently augment endogenous glycogen utilization when compared with CHO feeding in isolation.

METHODOLOGY

Ethical Approval

Before participation, all participants were fully informed of the experimental procedures and potential risks associated with the study before providing written informed consent. All trials were conducted at the Manchester Metropolitan University Institute of Sport (Manchester, UK) following approval from the Faculty of Science and Engineering Research Ethics and Governance Committee (EthOS ID: 70456) in accordance with the latest revision of the Declaration of Helsinki (apart from registration in a publicly accessible database).

Participants

Nine amateur male cyclists (means ± SD: age 29 ± 8 yr; body mass 77.2 ± 14.1 kg; stature 180.5 ± 8.2 cm) volunteered to participate in the study. Mean maximal oxygen consumption ($\dot{V}O_{2max}$) and peak power output (PPO) were 54.6 ± 10.2

$mL \cdot kg^{-1} \cdot min^{-1}$ and 323 ± 50 W, respectively. Participants were defined as trained (*tier 2*) in accordance with the criteria specified by McKay et al. (22). Sample size was determined a priori and utilized previous liver glycogen data by Gonzalez et al. (11). Based on this data, the expected effect size was calculated from the difference in liver glycogen utilization (per hour) with the ingestion of CHO (−6.7 ± 31.7 $mmol \cdot L^{-1}$) versus placebo (57.0 ± 42.2 $mmol \cdot L^{-1}$). These data provide an effect size of $f = 0.661$ (converted from Cohen's $d_z = 1.32$), deeming a sample size of seven participants sufficient to provide statistical power above 0.90 with an α-level of 0.05. This calculation is based on an F test for repeated-measures ANOVA (within factors) with one group (reflecting the repeated-measures design), three measurements (corresponding to the 3 experimental treatments), a correlation among repeated measures of 0.5, and a nonsphericity correction (ε) of 1.0 (G*Power, version .3.1, Düsseldorf, Germany).

Experimental Overview

In a repeated-measures, counterbalanced, randomized crossover design, participants completed a bout of high-intensity interval exercise (8 × 5-min repetitions at maximal self-selected intensity with 1-min recovery) while ingesting gels as either placebo (PLA), 60 g maltodextrin (CHO), or a multi-ingredient formulation of maltodextrin (60 g) with caffeine, L-theanine, L-citrulline, taurine, and citicoline (CHO^{+}), before and during exercise in a double-blind manner. ^{1}H magnetic resonance imaging (MRI) and ^{13}C spectroscopy (MRS) were acquired immediately pre- and postexercise to establish liver volume and both liver and muscle glycogen concentrations. Blood samples were obtained during each recovery period, and expired gas was collected during *intervals 2*, *4*, *6*, and *8*. Participants also completed a series of computerized cognitive assessments pre- and postexercise to assess various components of cognitive function. Each experimental trial commenced after 24 h of standardized dietary intake. Figure 1 provides an overview of the experimental design.

Preliminary Testing

At least 1 wk before the first experimental trial, participants completed an incremental test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Lode BV, Groningen, The Netherlands) to determine maximal oxygen uptake and PPO. In brief, participants commenced the test with a 5-min warm-up at 100 W before starting the incremental cycling test at 150 W, which increased by 25 W every 2.5 min until exhaustion. $\dot{V}O_{2max}$ was established from the highest average 30-s period of oxygen consumption captured (Vyntus CPX Vyaire Medical GmbH, Höchberg, Germany). The final power output and time at exhaustion enabled PPO to be calculated with the following equation (23).

$$PPO = W_{final} + (t/150) \times PI,$$

where W_{final} is the final power output, t is the time spent in the final uncompleted stage (seconds), 150 is the duration of each stage (seconds), and PI is the increase in power output (W) between stages.

Familiarization

After completing the maximal test, participants rested for ~20 min before undertaking familiarization to both the cognitive assessment testing battery and the high-intensity interval session. During familiarization, participants were instructed to select the highest power output they believed they could sustain for 8 × 5 min work bouts. In circumstances where participants failed to repeatedly produce power outputs >70% PPO, they were required to return to the laboratory to undertake a further familiarization trial until the principal investigator deemed the participant capable of reproducing consistent power outputs during the subsequent experimental trials. In addition, participants were fully familiarized with all cognitive assessment tasks.

Preexperimental Controls

Participants were provided with a prepackaged standardized diet (3 meals, snacks, and drinks), consisting of 8 $g \cdot kg^{-1}$ carbohydrate, 2 $g \cdot kg^{-1}$ protein, and 0.7 $g \cdot kg^{-1}$ fat to consume 24 h before each experimental trial. Participants were also asked to refrain from alcohol, caffeine, and physical activity during this 24-h period to ensure appropriate standardization between trials.

Experimental Protocol

Participants reported to the laboratory at 08:00 following a 12-h overnight fast and underwent baseline MRI and MRS measurements to establish liver volume and both liver and muscle glycogen concentrations (see *Acquisition and Measurement of Liver and Muscle Glycogen Concentrations* for more details). Following scans, participants completed a computerized cognitive assessment battery (see *Cognitive Testing Battery* for more details) before an indwelling cannula (BD Nexiva closed IV catheter system; Becton Dickinson Infusion Therapy Systems Inc., Utah) was inserted into the antecubital vein, and a resting blood sample was collected. After the collection of resting measures, participants ingested one of three nutritional treatments (see *Nutritional Treatments* for more details) immediately before commencing a 10-min standardized warm-up. Immediately afterward, participants completed 8 × 5-min repetitions at maximal self-selected intensity on an electronically braked cycle ergometer, interspersed with standardized 1-min recovery periods at 80 W between repetitions. The ergometer was set to linear mode (linear factor = power/rpm^2), with the linear factor adjusted to elicit 80% PPO at the participants' preferred cadence, in accordance with previous literature using this model (16, 24). Power output, cadence, and heart rate were monitored continuously throughout exercise, whereas rating of perceived exertion (RPE) (25) was recorded, and a venous blood sample was collected at the completion of each interval. Expired gas was collected for a period of 2.5 min during *intervals 2*, *4*, *6*, and *8*. Throughout the trials, participants received no external feedback or motivational cues beyond the display of elapsed time during each interval and the number of intervals remaining and were cooled with a floor fan to minimize thermal stress. All exercise trials were performed at the same time of day and under normal laboratory conditions (18.6 ± 1.5°C; 42 ± 8% humidity). Following exercise, participants completed the computerized cognitive assessment battery before repeating MRI and MRS measurements.

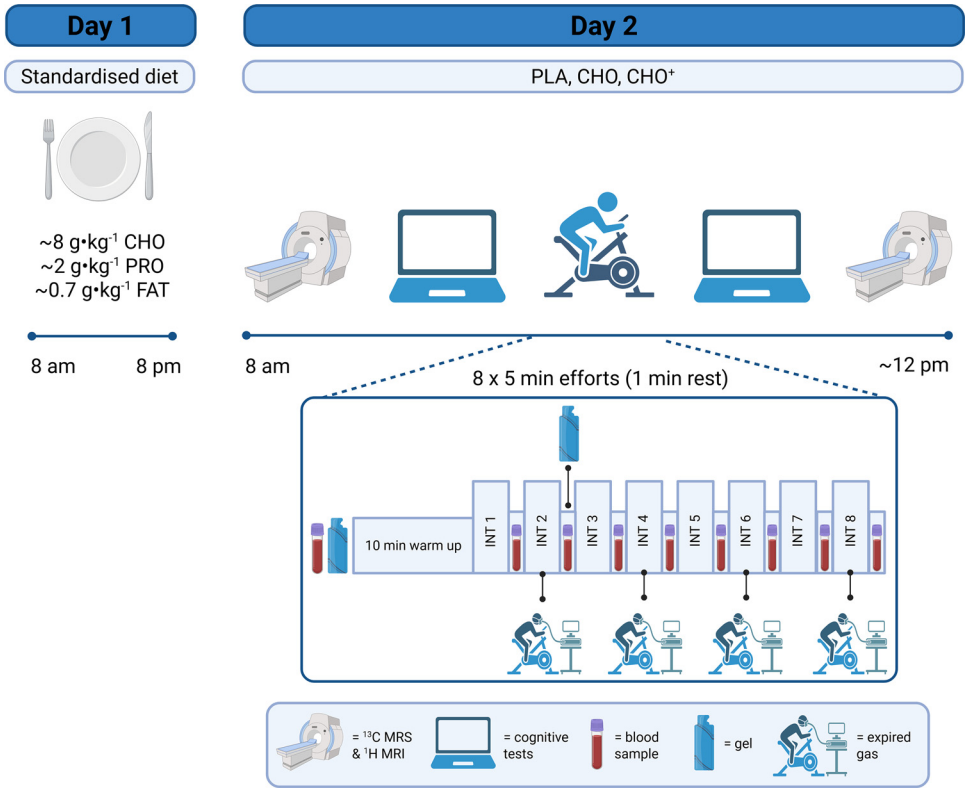

Figure 1. Schematic overview of the experimental protocol for each trial. Following 24 h of dietary standardization, participants arrived fasted for a pre-exercise MRS/MRI scan, followed by a battery of cognitive assessments (Stroop, running memory span, and number-letter in a randomized order). Participants then completed 8 × 5-min bouts at self-selected maximal-intensity cycling, ingesting either placebo (PLA), maltodextrin (CHO), or maltodextrin plus caffeine, L-theanine, L-citrulline, taurine, and citicoline (CHO^+). Postexercise cognitive assessments and MRS/MRI were then repeated. CHO, carbohydrate; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy.

Nutritional Treatments

Immediately before commencing the 10-min warm-up, participants ingested a 50-mL gel containing either placebo (PLA; water, gellan gum, xanthan gum, sucralose, and flavorings), 30 g maltodextrin (CHO), or 30 g maltodextrin plus 150 mg caffeine, 330 mg L-theanine, 1 g taurine, 3 g L-citrulline, and 250 mg citicoline (CHO^+). The CHO^+ gel was developed as an experimental prototype to reflect the ingredient profile of commercially available caffeine-containing multi-ingredient formulations used by athletes before high-intensity exercise (21). Total CHO intake equated to ~60 $g \cdot h^{-1}$. All gels were developed in a commercial laboratory to ensure appropriate matching of flavor and texture and were packaged in opaque foils to ensure double blinding of treatments. Following the completion of two high-intensity intervals (i.e., during the 1-min recovery period), participants ingested another 50 mL gel. Ad libitum water intake in the initial trial was recorded and replicated in all subsequent trials.

Acquisition and Measurement of Liver and Muscle Glycogen Concentrations

Glycogen concentrations were determined from the natural abundance signal of the C-1 carbon of glycogen at a chemical shift of ~100 ppm using a Siemens MAGNETOM Vida 3T MR system (Siemens Healthcare GmbH, Erlangen, Germany). A dual-channel $^1H/^{13}C$ transmit-receive flexible surface coil (01365; Rapid Biomedical GmbH, Rimpar, Germany) was positioned over the right lobe of the liver for liver glycogen measurements and the widest part of the vastus lateralis muscle for muscle glycogen assessment. Automated shimming was used to ensure magnetic field uniformity over the active coil volume, whereas the B_1 profile of the ^{13}C surface coil localized the MR signal to the targeted tissue. Scout images were acquired to confirm reproducible coil placement across measurements. ^{13}C magnetic resonance spectroscopy (MRS) was acquired using a standard pulse-acquire sequence. For liver measurements, the sequence was ^1H coupled (TR 200 ms, TE 0.18 ms, bandwidth 8,000 Hz, 4,096 averages, and spectral points 2,048), whereas muscle data were acquired with partial ^1H decoupling to improve spectral resolution while minimizing SAR and hardware demands [repetition time (TR) 610 ms, TE 0.18 ms, bandwidth 8,000 Hz, 2,048 averages, and spectral points 2,048]. This simple pulse-acquired sequence was selected to maintain compatibility across different MR systems, with the spectral window centered on the glycogen C-1 resonance (~100 ppm). Data acquisition times were ~13 min for the liver and 20 min for the muscle.

Spectra were processed in MATLAB (R2024a; The MathWorks, Natick, MA) and fitted with Gaussian functions using nonlinear least squares to quantify the glycogen C-1 peak. Absolute concentrations were derived by normalizing the area under the curve to a 50-mM acetate fiducial and a 100-mM glycogen phantom, expressed as mM glycosyl units, and used to calculate total liver glycogen from individual liver volume. For additional methodological details, please refer to Hannon et al. (26).

Measurement of Liver Volume

Liver volume was assessed using ^1H MRI scans as previously described (26). A Siemens Spine 32 and Body 18 array

coil was used to acquire high-resolution three-dimensional (3-D) T_1-weighted axial and T_2-weighted coronal images of the liver. Liver scans were collected during breath holds in the inhalation phase. Volumetric segmentation of the liver was performed using 3-D Slicer software with an artificial intelligence-based tool (TotalSegmentator) for precise volume extraction. The intraindividual coefficient of variation of duplicate liver volume measurements using TotalSegmentator was 1.5%.

Calculation of Liver Glycogen Content

Total liver glycogen content (LGC) was calculated from the following equation:

$$\mathrm{LGC(g)} = \frac{162 \times \mathrm{V_L} \times [\mathrm{Glyc}]}{1,000},$$

where 162 represents the molar mass of a glycosyl unit ($g \cdot mol^{-1}$), V_L is liver volume (L), and Glyc is glycogen concentration (mM). Although glycogen concentrations are presented as mmol of glycosyl units [previously described by Hannon et al. (26)], LGC represents the total mass of glycogen in grams and is irrespective of changes in glycogen particle size.

Blood Sampling and Analysis

Venous blood samples were collected into K_2 EDTA and serum separation Vacutainers (BD Biosciences, Plymouth, UK). The K_2 EDTA tubes were stored on ice before centrifugation at 1,500 g for 10 min at 4°C. Plasma was aliquoted and stored at −80°C for subsequent analysis. Plasma samples were analyzed for glucose, lactate (Randox Laboratories Ltd, Crumlin, UK), nonesterified fatty acids (NEFA), and glycerol (Horiba UK Ltd, Northampton, UK) using commercially available enzymatic spectrophotometric assays. Intra-assay coefficients of variation (CVs) were 0.5%, 0.5%, 2.2%, and 3.2% for glucose, lactate, NEFA, and glycerol, respectively. Blood pH, bicarbonate, base excess, and total CO_2 in the extracellular fluid were measured from the serum separation Vacutainers in real-time using a portable gas analyzer (i-STAT 1; Abbott, Illinois). Plasma adrenaline (ab287788, Abcam, Cambridge, UK) and glucagon (Mercodia Glucagon ELISA, Mercodia AB, Uppsala, Sweden) concentrations were determined via ELISA kits, with intra-assay CVs of 6.9% and 7.5%, respectively. These assays were performed using commercially available kits in duplicate according to the manufacturer's instructions. Plasma caffeine and paraxanthine concentrations were quantified via high-performance liquid chromatography (HPLC) as previously described (27). All time points and visits for each subject were run in a single batch to decrease between-run variability, with a series of known standards run at the start and end of the run, respectively.

Cognitive Testing Battery

In considering that caffeine may also exert its ergogenic effects through central nervous system modulation, we also assessed three core components of cognition in attentional switching, working memory updating, and inhibitory control as proposed in accepted models of executive functioning (28). Participants completed the tasks on a laptop running Inquisit v7 (Millisecond, Seattle). Participants completed a short practice version of each task before the main task. The cognitive tests were completed in a randomized order to further reduce any systematic learning or order effects.

Running Memory Span Task (Working Memory Updating)

In this task, adapted from Morris and Jones (29), participants were presented with a randomly generated series of letters, one at a time, for 300 ms (with an interstimulus interval of 200 ms) on the screen. The length of each series varied unpredictably between three and eight letters, and at the end of each sequence, participants were required to recall the most recent *n* letters in the correct order. For example, if the sequence J-Q-S-R-K was shown and participants were instructed to recall the last two letters, the correct response would be R-K. After the sequence, a response screen appeared displaying all possible letters, and participants were required to select the presented letters in the correct order. If they could not recall one or more letters, they had the option to click "BLANK." If they made an error and wished to restart their response, they could press "CLEAR" to reset their input. Thirty-six trials were completed in total, and the running span score was the total number of letters recalled in the correct serial position over the whole task.

Number-Letter Task (Attentional Switching)

Adapted from Miyake et al. (28), the number-letter task requires participants to complete three blocks where they are presented with a number-letter pair in one of four quadrants on a computer screen (e.g., B2). In *block 1*, all stimuli are presented in the two quadrants at the top of the screen, and participants have to respond "E" if the letter is a consonant, and "I" if the letter is a vowel. In the second block, all stimuli are presented in the bottom two quadrants, and participants have to respond "E" if the number is even, and "I" if the number is odd. In the third block, sequential stimuli travel clockwise around the screen, and participants have to internally switch between making a letter judgment (top 2 quadrants) or a number judgment (bottom 2 quadrants) every two trials. The switch cost (the additional time it takes the brain to switch between making the 2 types of judgment) is calculated by subtracting the average time taken for *blocks 1* and *2* from the total time taken for *block 3*.

Stroop Task (Inhibitory Control)

The Stroop task (30) is a widely used method for examining selective attention and response inhibition. In this computerized color-word Stroop test, participants were presented either the word "RED," "GREEN," "BLUE," or "BLACK" in either red, green, blue, or black font. In congruent trials, the ink matched the word (e.g., GREEN in green ink), and in incongruent trials, the word was printed in different colored ink (e.g., GREEN in red ink). Participants were instructed to state the color and not read the word in every trial; thus, incongruent trials were more difficult as they required inhibition of the automatic response to read the word. Participants responded by clicking a corresponding key on the keyboard for each color. In addition to the congruent and incongruent trials, control trials were included where a colored rectangle appeared on the screen and

participants had to press the corresponding color key. In total, seven trials for each color in each condition were implemented ($7 \times 4 \times 3 = 84$ trials). The dependent measures were the proportion correct and the difference in reaction time between the congruent and incongruent trials (ms).

Statistical Analysis

All statistical analyses were performed using SPSS Statistics version 29 (IBM). For outcomes with randomly missing datapoints (metabolic and hormonal responses and heart rate), linear mixed-effects models were used, with two models fitted for each outcome: *1*) a model including a random intercept, and *2*) a model including both a random intercept and a random slope for time. The optimal model for each outcome was selected based on the lowest Akaike information criterion (AIC) and Bayesian information criterion (BIC). Fixed effects included treatment, time, and treatment × time interaction to analyze differences over time (e.g., intervals) and between treatments. For outcomes with complete datasets (muscle and liver glycogen, cognitive outcomes, power output, respiratory outcomes, and RPE), repeated-measures ANOVA was used. Power output and oxygen consumption during exercise were categorized into early (i.e., *intervals 1–4*) and late (i.e., *intervals 5–8*) intervals to increase the resolution of these outcomes over time in accordance with the hypothesized increase in plasma caffeine during the late phase of exercise. Pairwise comparisons were analyzed using post hoc least significant difference (LSD) tests to locate specific differences (31) where a significant main effect or interaction was observed. Data in text and figures are presented as raw means ± SD with P values ≤ 0.05 indicating statistical significance.

RESULTS

Effects of CHO and Caffeine Availability on Liver and Muscle Glycogen Use during HIIT

Muscle and liver glycogen concentrations and liver glycogen content are presented in Fig. 2, *A–F*. Muscle glycogen concentrations were significantly reduced following exercise (time effect, $P < 0.001$) and displayed a different pattern of utilization between conditions (interaction effect, $P = 0.007$). Specifically, muscle glycogen utilization was significantly lower in the CHO trial when compared with the PLA trial ($P = 0.006$) with no differences between CHO$^+$ and PLA ($P = 0.073$) or between CHO and CHO$^+$ ($P = 0.120$) trials (Fig. 2*B*). Liver glycogen concentrations and content were also significantly reduced following exercise (time effect, $P = 0.002$ and $P < 0.001$, respectively) but demonstrated a similar pattern of utilization between conditions (interaction effect, $P = 0.147$ and $P = 0.075$, respectively) (Fig. 2, *C* and *E*). Liver glycogen concentration and content utilization (Fig. 2, *D* and *F*) showed no significant difference between trials ($P = 0.150$ and $P = 0.101$, respectively). Liver volume was also significantly reduced following exercise (time effect, $P < 0.001$), although the magnitude of decline was not significantly different between treatments (interaction effect, $P = 0.233$). Taken together, these data demonstrate that CHO feeding spares muscle glycogen utilization during HIIT, though this sparing effect was not evident when CHO was coingested with a caffeine-containing multi-ingredient formulation.

Effects of CHO and Caffeine Availability on Self-Selected Exercise Intensity and Performance during HIIT

In accordance with the lack of a glycogen-sparing effect in the CHO$^+$ trial, we also observed changes in self-selected exercise intensity. Indeed, although power output was not significantly different between trials during the early intervals (1–4; treatment effect, $P = 0.639$) (Fig. 3*A*), a significant main effect of treatment ($P = 0.021$) was observed during the late intervals (5–8) (Fig. 3*B*). Specifically, power output was significantly higher in the CHO$^+$ trial when compared with the PLA ($P = 0.046$; 2.8% improvement) and CHO trials ($P = 0.046$; 3.8% improvement). Similarly, when expressed as % PPO, power output during the early intervals was not significantly different between treatments ($P = 0.685$), whereas during the late intervals, % PPO was significantly higher (treatment effect, $P = 0.019$) in the CHO$^+$ trial when compared with both PLA and CHO trials ($P = 0.045$ and $P = 0.042$, respectively) (Fig. 3, *C* and *D*).

Physiological Responses to HIIT

Both heart rate and RPE increased during exercise (time effect, $P < 0.001$ and $P = 0.007$, respectively) but were not significantly different between trials (treatment effect, $P = 0.413$ and $P = 0.245$, respectively; interaction, $P = 0.277$ and $P = 0.633$, respectively). There was no difference in $\dot{V}O_2$ between treatments (treatment effect, $P = 0.288$), during the early intervals. However, there was a significant difference between trials during the late intervals ($P < 0.001$), whereby $\dot{V}O_2$ was significantly higher in the CHO$^+$ trial compared with the PLA ($P = 0.001$) and CHO ($P = 0.003$) trials (Fig. 3*E*). Accordingly, exercise intensity expressed as $\%\dot{V}O_{2max}$ did not differ between treatments during the early intervals (treatment effect, $P = 0.554$). However, a significant treatment effect was observed during the late intervals ($P < 0.001$), whereby $\%\dot{V}O_{2max}$ was significantly higher in the CHO$^+$ trial compared with both PLA ($P = 0.003$) and CHO ($P < 0.001$) trials (Fig. 3, *G* and *H*). Finally, $\dot{V}CO_2$ showed no difference between trials (treatment effect, $P = 0.287$) during the early intervals, whereas during the late intervals, there was a significant main effect of treatment ($P = 0.005$), whereby $\dot{V}CO_2$ was significantly higher in CHO$^+$ when compared with PLA and CHO ($P = 0.025$ and $P = 0.012$). Taken together, these data demonstrate that CHO$^+$ improved exercise performance in the late intervals during HIIT, with greater power output and oxygen uptake compared to PLA and CHO trials.

Effects of CHO and Caffeine Availability on Circulating Metabolites and Hormonal Responses to HIIT

To explore the potential mechanisms underlying the performance benefits observed in response to CHO and caffeine coingestion, a comparison of circulating metabolic metabolites, hormones, and caffeine concentrations are presented in Figs. 4, 5, 6, and 7.

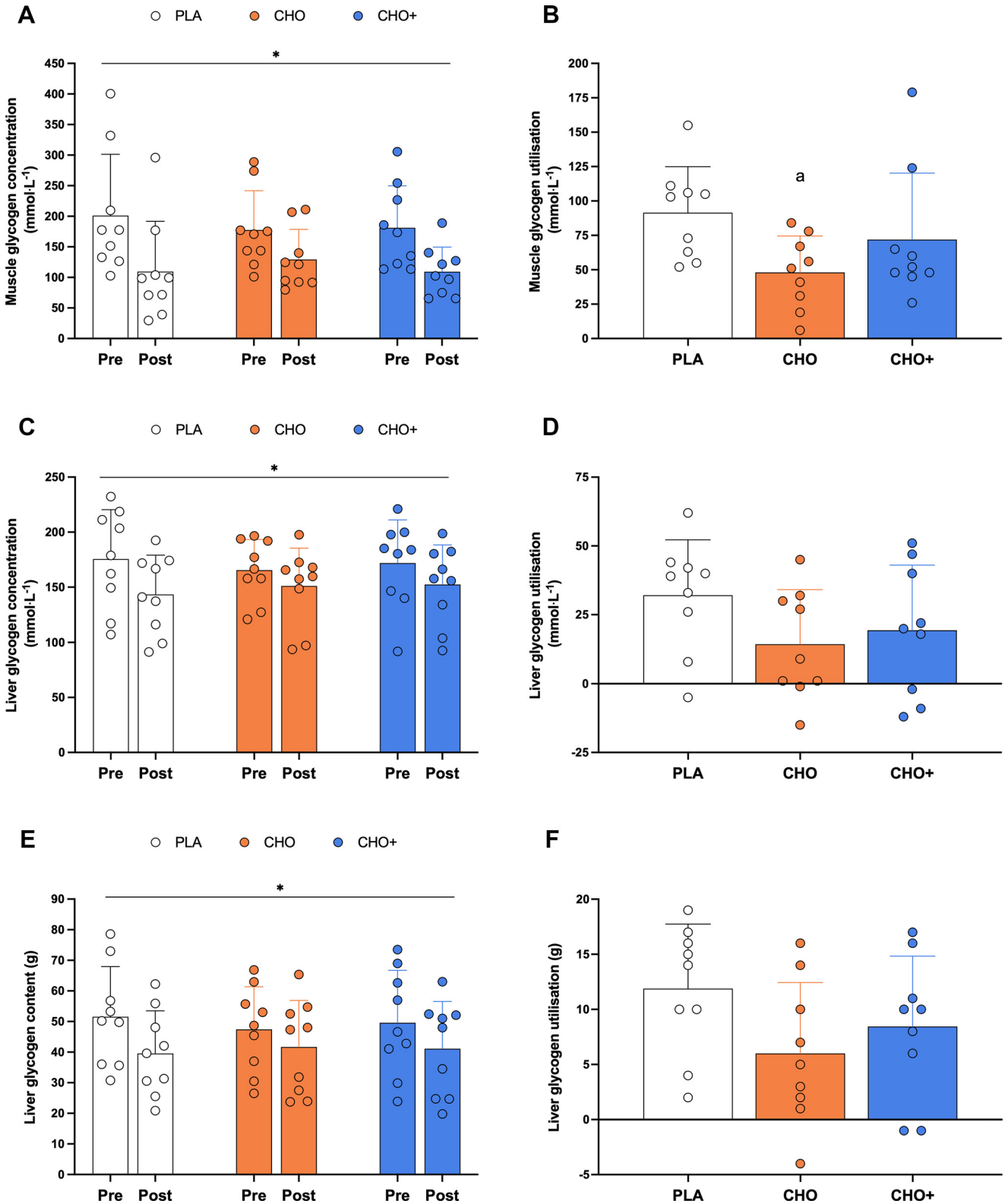

Figure 2. Quadriceps muscle (*A*) and liver (*C*) glycogen concentrations and liver glycogen content (*E*) in response to PLA, CHO, and CHO^{+} trials pre- and postexercise. Glycogen utilization in quadriceps muscle (*B*) and liver, expressed as both concentrations (*D*) and total content (*F*). $n = 9$. *Time effect; [a]CHO and PLA significantly different. CHO, carbohydrate; PLA, placebo.

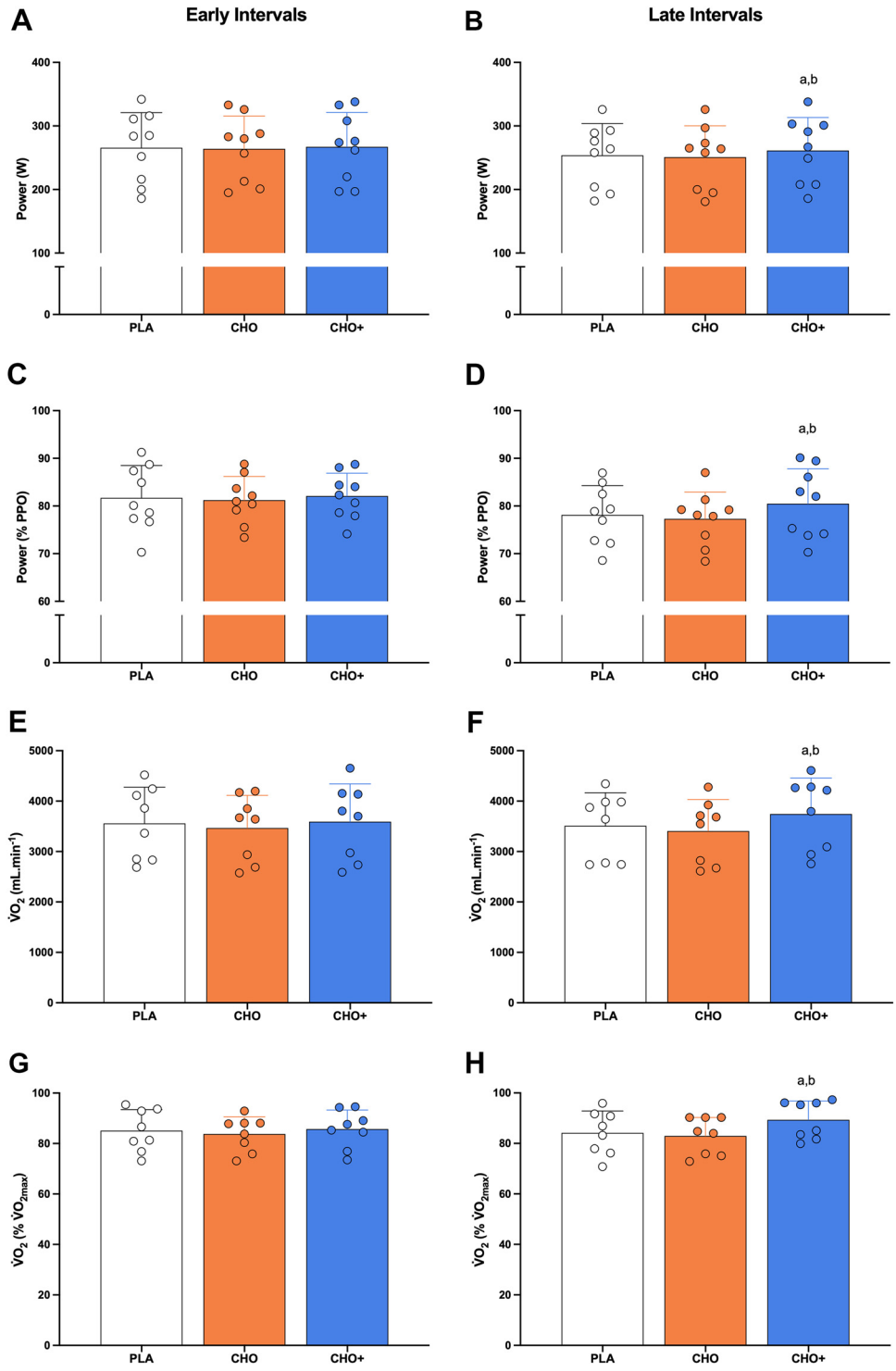

Figure 3. Mean power output, % peak power output, $\dot{V}O_2$, and $\%\dot{V}O_{2max}$ during both early (*A*, *C*, *E*, and *G*) and late (*B*, *D*, *F*, and *H*) intervals in response to PLA, CHO, and CHO^+ trials. $n = 9$ for power output; $n = 8$ for $\dot{V}O_2$ due to technical issues with the metabolic cart. $^{a}CHO^+$ and PLA significantly different; $^{b}CHO^+$ and CHO significantly different. CHO, carbohydrate; PLA, placebo; PPO, peak power output.

Metabolic Responses

Plasma glucose, lactate, and glycerol concentrations all progressively increased during exercise (time effect, $P < 0.001$ for all) (Fig. 4, *A*, *B*, and *D*, respectively). Although plasma glucose and glycerol concentrations did not display any significant differences between trials (treatment effect for glucose, $P = 0.175$ and glycerol, $P = 0.901$) or interaction effects (interaction effect $P = 0.826$ and $P = 0.898$, respectively), plasma lactate was significantly lower (treatment effect, $P = 0.010$) in the CHO trial when compared with the CHO^+ ($P = 0.003$) and PLA trials ($P = 0.017$) with no interaction ($P = 0.639$) (Fig. 4*B*). Plasma NEFA concentrations were reduced at the onset of exercise and remained below resting values throughout the duration of exercise (time effect, $P < 0.001$) but did not display any significant differences between trials (treatment effect, $P = 0.690$) or interaction ($P = 1.000$) (Fig. 4*C*).

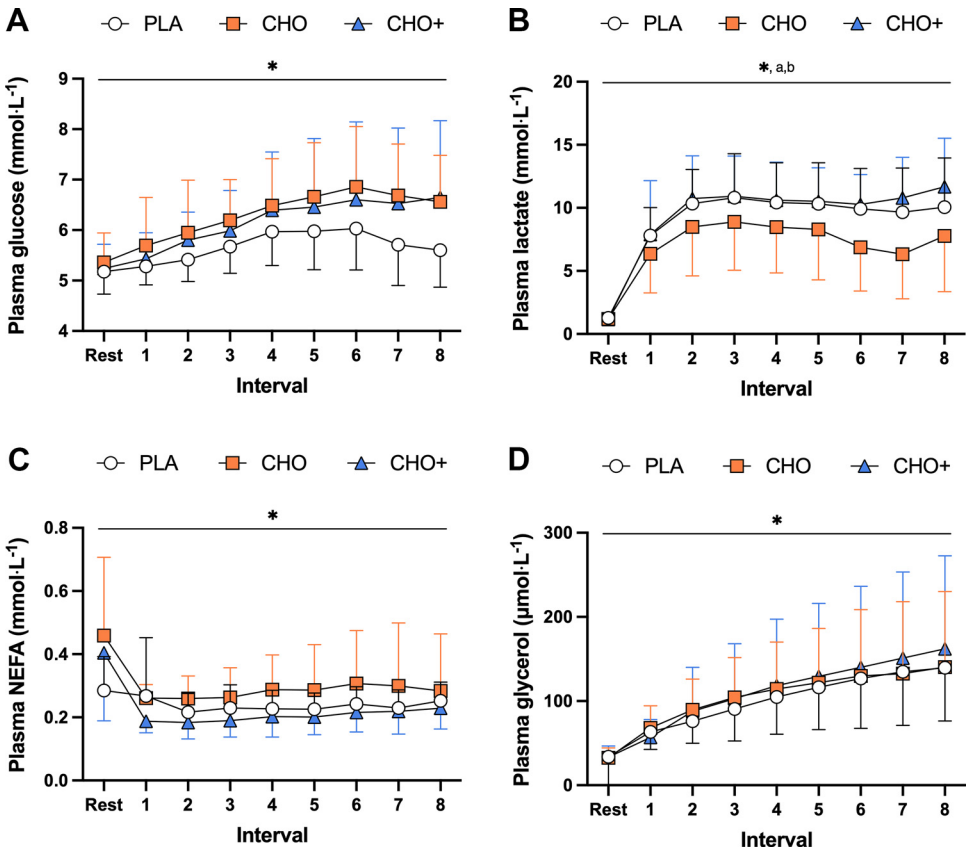

Figure 4. Plasma glucose (*A*), lactate (*B*), NEFA (*C*), and glycerol (*D*) in response to high-intensity interval exercise. $n = 9$ for glucose, NEFA, and glycerol, except for the CHO trial where $n = 8$ due to failed cannulation. $n = 8$ for lactate due to technical issues with sample measurement, except for the CHO trial where $n = 7$ due to failed cannulation. *Time effect; [a]CHO^+ and CHO significantly different; [b]CHO and PLA significantly different. CHO, carbohydrate; PLA, placebo.

Blood bicarbonate (time effect, $P < 0.001$), base excess (time effect, $P < 0.001$), blood pH (time effect, $P = 0.042$), and total CO_2 (time effect, $P < 0.001$), all reduced during exercise (Fig. 5, *A–D*). A significant main effect of treatment was observed for blood bicarbonate (treatment effect, $P = 0.016$) whereby concentrations in the CHO^+ trial were significantly lower when compared with CHO ($P = 0.005$) but not PLA ($P = 0.067$) (Fig. 5*A*). A significant time × treatment interaction was observed for base excess (interaction effect, $P = 0.020$), indicating that the reduction in base excess during exercise differed between treatments. Indeed, base excess was significantly lower in the CHO^+ trial compared with the CHO trial after *intervals 6* ($P = 0.017$) and *8* ($P < 0.001$) and compared with the PLA trial after *interval 8* ($P = 0.001$). In addition, base excess was significantly lower in the PLA trial when compared with CHO trial at *interval 6* ($P = 0.026$) (Fig. 5*B*). A significant main effect of treatment was also observed for total blood CO_2 (treatment effect, $P = 0.012$) whereby total blood CO_2 concentrations were significantly lower in the CHO^+ trial when compared with both PLA ($P = 0.049$) and CHO ($P = 0.004$) trials, respectively (Fig. 5*D*). Accordingly, a significant time × treatment interaction was observed for blood pH, whereby blood pH was significantly lower in the CHO^+ trial when compared with both PLA and CHO trials after the completion of *interval 8* ($P = 0.011$ and $P = 0.003$) (Fig. 5*C*).

Hormonal Responses

Plasma glucagon and adrenaline concentrations progressively increased during exercise (time effect, $P = 0.004$ and $P < 0.001$, respectively) (Fig. 6, *A* and *B*). A significant time × treatment interaction was observed for plasma glucagon (interaction effect, $P = 0.003$) indicating that the exercise and feeding induced increase differed between treatments. Indeed, glucagon concentrations were significantly higher in the PLA trial when compared with CHO and CHO^+ trials after the completion of *interval 8* (both $P = 0.001$) (Fig. 6*A*). No significant differences in plasma adrenaline were observed between treatments (treatment effect, $P = 0.460$; interaction effect, $P = 0.345$) (Fig. 6*B*).

Plasma Caffeine Responses

Mean total caffeine intake was 3.85 $mg \cdot kg^{-1}$ body mass (BM), ranging between 3.20 and 6.24 $mg \cdot kg^{-1}$ BM. A significant time × treatment interaction was observed for plasma caffeine (interaction effect, $P < 0.001$) whereby concentrations were significantly higher in the CHO^+ trial when compared with PLA and CHO trials at both *interval 4* and *interval 8* ($P < 0.001$ for all comparisons) (Fig. 7*A*). A significant time × treatment interaction was also observed for plasma paraxanthine (interaction effect, $P < 0.001$) whereby concentrations were significantly higher in the CHO^+ trial when compared with PLA and CHO at *interval 8* ($P = 0.002$ for both) (Fig. 7*B*).

Effects of CHO and Caffeine Availability on Cognitive Function

Cognitive responses to the cognitive testing battery are displayed in Fig. 8, *A–D*. The number of correct responses during the Stroop task was not significantly different

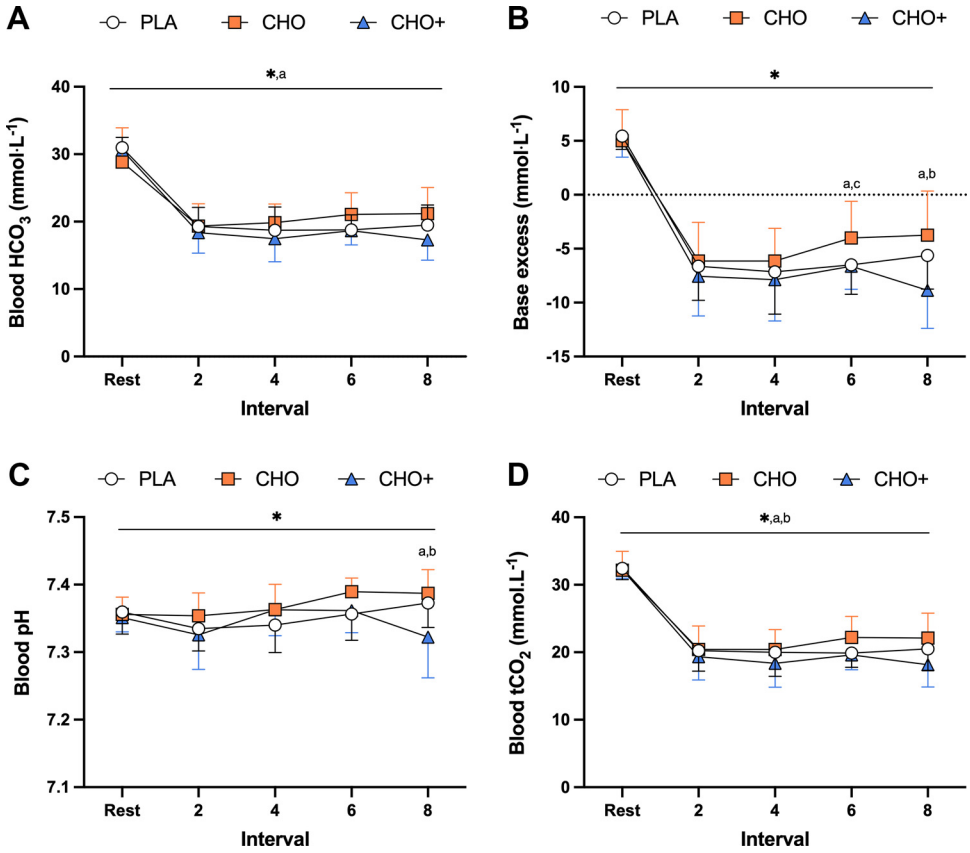

Figure 5. Blood HCO_3 (*A*), base excess in the extracellular fluid (*B*), pH (*C*), and total CO_2 (*D*) in response to high-intensity interval exercise. $n = 9$, except for the CHO trial where $n = 8$ due to failed cannulation. *Time effect; [a]CHO^+ and CHO significantly different; [b]CHO^+ and PLA significantly different; [c]CHO and PLA significantly different. CHO, carbohydrate; PLA, placebo.

between conditions (treatment effect, $P = 0.101$) but was across time (time effect, $P = 0.039$) (Fig. 8*A*). Stroop test reaction time was also faster postexercise (time effect, $P = 0.024$) and demonstrated a trend toward a significant time × treatment interaction (interaction effect, $P = 0.080$). Although the interaction did not reach statistical significance, forced exploratory post hoc analysis demonstrated that postexercise reaction time was significantly faster with CHO^+ when compared with PLA ($P = 0.029$) (Fig. 8*B*). Switch cost during the number-letter task was also significantly lower postexercise (time effect, $P = 0.029$) and differed between treatments (treatment effect, $P = 0.028$) but the change from baseline was not significantly different between treatments (interaction effect, $P = 0.104$). Post hoc analysis for the treatment effect revealed that, irrespective of time point, switch cost was significantly lower (indicating superior task-switching performance) in the CHO^+ trial when compared with CHO ($P = 0.011$). There were no significant differences observed between PLA and CHO^+ ($P = 0.545$) or CHO ($P = 0.094$) trials (Fig. 8*C*). Span score during the Running Memory task was not significantly different between conditions (treatment effect, $P = 0.840$) or time points (time effect, $P = 0.400$) (Fig. 8*D*).

DISCUSSION

Using noninvasive ^{13}C magnetic resonance spectroscopy, we provide the first report to simultaneously characterize

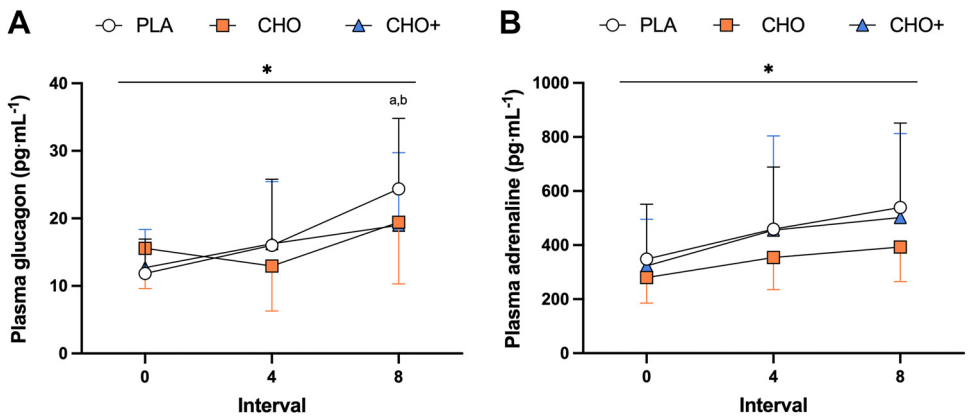

Figure 6. Plasma glucagon (*A*) and adrenaline (*B*) concentrations in response to high-intensity interval exercise. $n = 9$, except for the CHO trial where $n = 8$ due to failed cannulation. *Time effect; [a]CHO^+ and PLA significantly different, [b]CHO and PLA significantly different. CHO, carbohydrate; PLA, placebo.

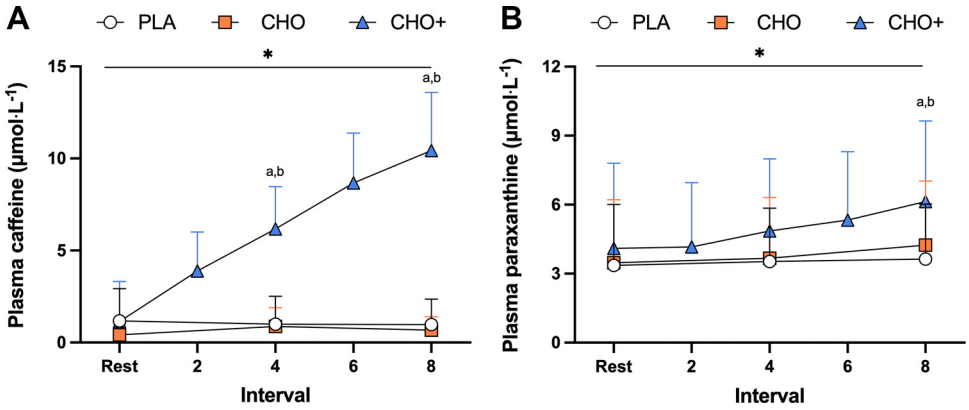

Figure 7. Plasma caffeine (*A*) and paraxanthine (*B*) concentrations in response to high-intensity interval exercise. $n = 9$, except for the CHO trial where $n = 8$ due to failed cannulation. *Time effect; ^{a}CHO^{+} and CHO significantly different; ^{b}CHO + and PLA significantly different. CHO, carbohydrate; PLA, placebo.

liver and muscle glycogen utilization in response to an acute bout of high-intensity interval training. In contrast to prolonged endurance exercise, these data demonstrate that liver glycogen utilization during high-intensity exercise is relatively small whereby muscle glycogen serves as the predominant energy substrate (Fig. 2). In assessing the impact of CHO availability on substrate use, we also observed that CHO ingestion spares muscle glycogen utilization. However, this effect was not evident with the coingestion of a caffeine-containing multi-ingredient formulation, likely due to an increased capacity to perform more total work rather than a direct metabolic effect of caffeine. Although the pattern of glycogen utilization observed here is specific to the chosen exercise protocol, these data further our understanding of

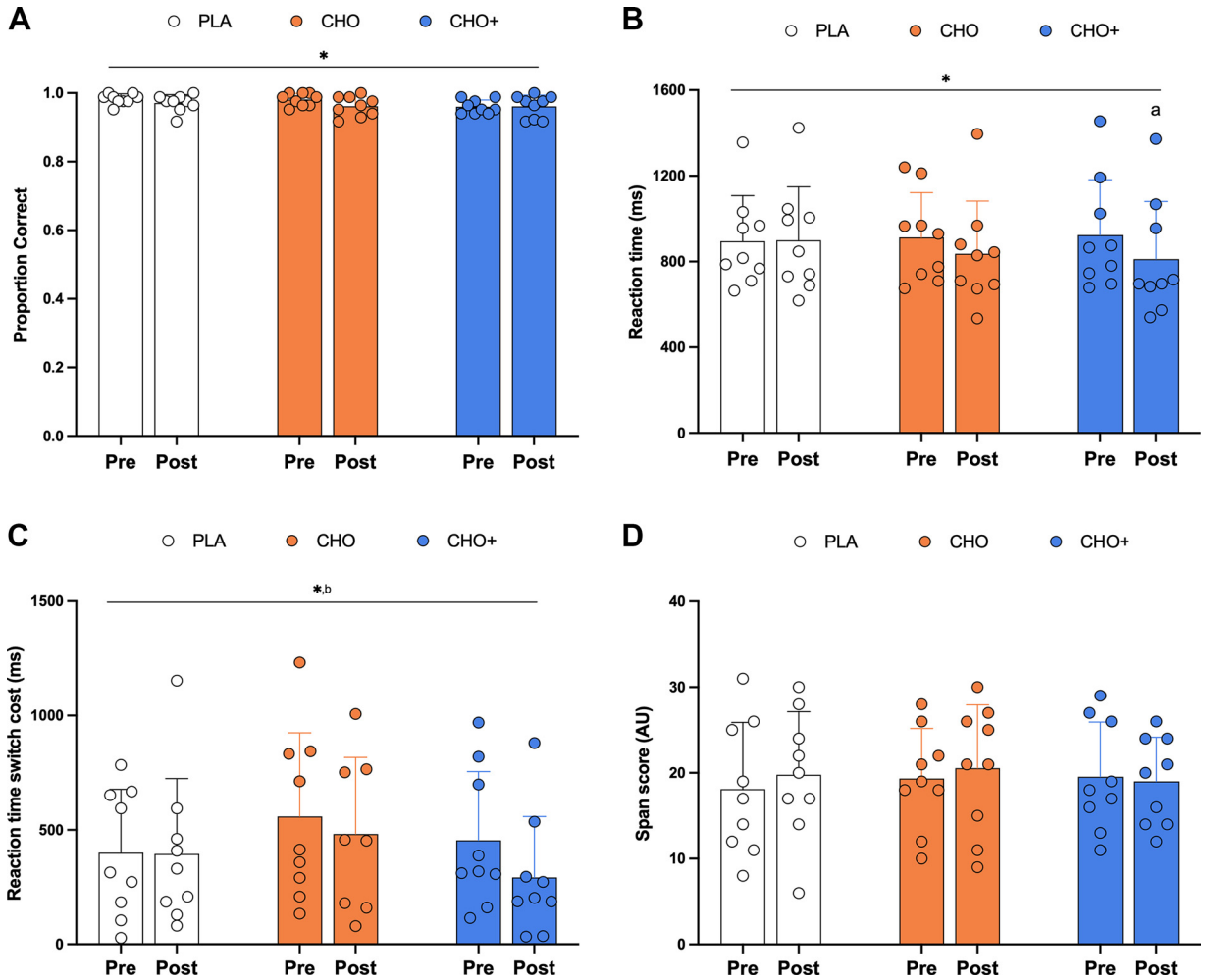

Figure 8. Proportion correct during Stroop test (*A*), Stroop test reaction time (*B*), reaction time switch cost (*C*), and span score (*D*) in response to PLA, CHO, and CHO^{+} trials pre- and postexercise. $n = 9$. *Time effect; ^{a}CHO^{+} and PLA significantly different postexercise; ^{b}CHO^{+} and CHO significantly different. AU, arbitrary units; CHO, carbohydrate; PLA, placebo.

the glycogen cost of high-intensity interval exercise and may inform practical fueling strategies to promote training intensity.

In considering the absolute glycogen cost of our chosen exercise model, our data indicate that liver glycogen utilization during high-intensity interval cycling is relatively small (~12 g) in comparison to previous reports during prolonged endurance cycling. For instance, estimations of liver glycogen utilization from stable isotope infusion studies during prolonged (up to 2 h) cycling demonstrate rates of EGP equivalent to ~25–30 $g \cdot h^{-1}$ (3, 6). Similarly, direct measurements of liver glycogen using ^{13}C MRS demonstrate liver glycogen utilization of 171 ± 73 $mmol \cdot L^{-1}$ in response to 3 h of steady-state cycling and are comparably higher than the 30 ± 20 $mmol \cdot L^{-1}$ reduction observed across the 48 min of exercise in the present study (11). Such discrepancies between studies are likely underpinned by differences in exercise intensity and duration, which are known to have profound effects on substrate utilization during exercise (2, 32). In relation to the latter, EGP has been shown to continually rise throughout prolonged exercise (6, 33) to support the maintenance of whole body carbohydrate oxidation in the face of reduced muscle glycogen availability (4, 34) and likely explains the higher utilization during prolonged exercise models.

In contrast to prolonged exercise protocols, we also observed that liver glycogen utilization was not significantly altered by CHO feeding, despite reduced glucagonemia in response to both CHO feeding trials (Fig. 6*A*). Nonetheless, liver glycogen utilization was ~40% lower in the CHO trial, equating to an absolute difference of ~5 g (Fig. 2, *D* and *F*), and may represent a physiologically meaningful difference. However, given that liver glycogen utilization in the present study was markedly lower than previous data used for our a priori sample size calculation (11), our ability to detect statistically significant between-treatment differences may have been limited. The apparent dissociation between lower glucagon concentrations and the absence of a statistically significant attenuation in liver glycogen use is consistent with the notion that pancreatic hormones play a minor role in the regulation of EGP during high-intensity exercise, where EGP may be more closely regulated by catecholamine concentrations (6). Although plasma adrenaline concentrations did not differ between trials, the exercise-induced increase in plasma adrenaline observed across all trials suggests that catecholamines may still play a role in the overall regulation of EGP under these high-intensity conditions (Fig. 6*B*). Although previous studies have demonstrated CHO feeding can reduce EGP by 50%–70% and completely prevent declines in liver glycogen concentrations when fed at doses of up to ~100 $g \cdot h^{-1}$ (3, 33, 35), this appears to be primarily relevant to prolonged exercise where liver glycogen utilization is relatively high. For example, rates of EGP measured in the aforementioned studies in the absence of CHO feeding equate to ~25 to ~35 $g \cdot h^{-1}$ (~30–45 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and appear comparatively higher than the total absolute glycogen utilization of 12 g measured in the present study. Although CHO feeding has been shown to reduce EGP during short duration, high-intensity exercise (80% $\dot{V}O_{2max}$) (6), rates of EGP (~30–40 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) were comparable to those observed during prolonged exercise at lower intensities and are likely to explain such discrepancies. Nonetheless, considering that the suppression of EGP may be dose dependent up to ingestion rates of ~90 $g \cdot h^{-1}$, at which point EGP becomes negligible (36), a higher CHO dose may have produced clearer effects on liver glycogen sparing in the present study.

In the absence of CHO feeding, our exercise protocol also induced a ~50% decrease in skeletal muscle glycogen concentrations, a magnitude of utilization that is consistent with previous reports using this exercise model in highly trained male cyclists (24). Our data also indicate that the magnitude of glycogen utilization is reduced with CHO feeding at a rate of ~60 $g \cdot h^{-1}$ and provide support to recent meta-analysis data which demonstrate a glycogen-sparing effect of CHO feeding (10). This effect is also consistent with the notion that glycogen sparing observed with CHO feeding occurs during the initial stages of exercise, when the contribution of muscle glycogen to energy production is greatest. In accordance with this, Stellingwerff et al. (37) previously demonstrated that CHO ingestion at a rate of ~50 $g \cdot h^{-1}$ reduces muscle glycogen utilization during the first hour of prolonged cycling (with no differences in the average rate of glycogen use between CHO and placebo treatments during the final 2 h of exercise). Although data on high-intensity cycling are limited, findings from 60 min of continuous running at 70% $\dot{V}O_{2max}$ support this notion, demonstrating muscle glycogen sparing when fed CHO at a rate of 50 $g \cdot h^{-1}$ (38).

The glycogen sparing effect observed with CHO feeding may also be partly explained by a proportion of the ingested CHO being taken up by skeletal muscle and stored as glycogen during the recovery period between intervals. Indeed, previous observations report that only ~55%–65% of glucose clearance from the circulation [measured by rate of glucose disappearance (R_d), using stable isotope approaches] is oxidized when infused at a rate of 1 $g \cdot min^{-1}$ during a 1-h cycling time trial (39). These data suggest that some of the nonoxidized glucose may be stored within inactive (or less active) skeletal muscle, which may contribute to the glycogen sparing effect observed in the present study. Indeed, the maximal self-selected intensity of the intervals in the present study may have elicited a metabolic milieu similar to that reported by Katz et al. (40), where rapid glycogenolysis during maximal cycling increased glucose-6-phosphate (G6P) concentrations sufficiently to inhibit hexokinase, thereby limiting glucose oxidation at the phosphorylation step. This inhibition may prevent the immediate oxidation of blood glucose during the work bout, leading to intracellular glucose accumulation. Consequently, the 1-min recovery periods in the present protocol may have provided critical windows when metabolic demand decreased, relieving the inhibition on hexokinase, whereas glucose availability remained high, facilitating the diversion of surplus glucose toward glycogen resynthesis. In support of this, muscle glycogen synthesis has previously been reported during 3 h of low-intensity exercise when glycogen concentrations are depleted by prior exercise (41).

Despite the muscle glycogen-sparing effect of CHO feeding in the present study, this sparing effect was not evident when CHO was coingested with a caffeine-containing, multi-ingredient formulation. Although caffeine ingestion has been demonstrated to increase plasma

adrenaline concentrations and may subsequently enhance muscle glycogenolysis (42, 43), we observed no statistically significant differences in plasma adrenaline concentrations between trials (Fig. 6*B*). Although mean postexercise plasma adrenaline concentrations were ~100 pg·mL^{-1} lower in the CHO trial compared with both PLA and CHO^{+} trials (Fig. 6*B*), such differences are markedly smaller those reported in previous adrenaline infusion studies shown to increase skeletal muscle glycogenolysis (e.g., mean differences of ~250–700 pg·mL^{-1}) (42, 43). Furthermore, given that elevating plasma adrenaline to concentrations equivalent to those observed in the CHO trial (~366 pg·mL^{-1}) has been previously shown to increase muscle glycogenolysis (42), additional adrenaline may not further enhance muscle glycogen use. Thus, the attenuation of glycogen sparing in the CHO^{+} trial is unlikely to be primarily attributed to a caffeine-induced increase in systemic adrenergic stimulation. Instead, the absence of a glycogen sparing effect in the CHO^{+} trial may potentially be explained by the higher self-selected power outputs achieved during the latter stages of exercise (Fig. 3*B*) and are consistent with previous data from Lane et al. (16) in response to a comparable dose of caffeine (equivalent to 3 mg·kg^{-1} BM). Consequently, participants performed more total work in the CHO^{+} trial, likely resulting in increased glycolytic flux, evidenced by the observed increases in plasma lactate (Fig. 4*B*) and concomitant reduction in blood bicarbonate, base excess, and pH during the latter stages of exercise (Fig. 5, *A*–*C*). Together, these data suggest that caffeine's effect on muscle glycogen utilization may be indirect and mediated by the increase in power output.

Beyond the observed physical benefits, exploratory analyses suggested that CHO^{+} may have improved aspects of selective attention, as evidenced by faster reaction times during the Stroop and number-letter tasks (Fig. 8, *B* and *C*). However, given the lack of a statistically significant treatment × time interaction, these findings should be interpreted as exploratory and warrant confirmation in adequately powered studies specifically designed to examine the effects of caffeine-containing formulations on cognitive performance. Nonetheless, although it is inherently difficult to ascertain the specific contributions of each individual component that underpins the observed effects within multi-ingredient formulations, our data are consistent with the well-documented effects of caffeine on selective attention (44–46) and the marked increase in plasma caffeine concentrations during the latter half of the trial (Fig. 7*A*). Furthermore, combining caffeine with L-theanine is known to provide additive cognitive enhancing effects, whilst also reducing negative subjective mood effects of caffeine alone (47, 48). In relation to selective attention, this combination appears to improve performance by increasing the allocation of attention-related neural resources to process target stimuli while simultaneously reducing such neural resources to distractor stimuli, such as those observed in the Stroop task (49). For a cyclist, this improvement in "inhibitory control" may have practical relevance in the context of a high-intensity race or interval session whereby the ability to filter out internal "noise" (e.g., perception of fatigue) to focus on external cues (e.g., an opponent's breakaway or technical road positioning) may provide a distinct competitive advantage. Moreover, faster reaction time is critical for safety and tactical execution, potentially aiding in crash avoidance or more rapid responses to gear changes and tactical maneuvers under high levels of physiological stress. Although the present design is unable to determine whether the higher power output observed in the CHO^{+} trial can be partly attributed to improvements in selective attention, it could be speculated that increased allocation of neural resources to the exercise task (i.e., maintaining power output) and reduced neural allocation to distractor stimuli (e.g., perception of fatigue) may provide performance benefits.

From a practical perspective, the present data demonstrate that the high metabolic demands of interval cycling dictate a predominant reliance on skeletal muscle glycogen, with a comparatively smaller contribution from hepatic glycogen stores and suggest that nutritional strategies before high-intensity interval training should prioritize high muscle glycogen availability. Although the observed sparing of muscle glycogen with CHO feeding did not enhance self-selected training intensity, this nutritional strategy may be of relevance for athletes who are required to complete subsequent training sessions in close proximity, where the time to replete muscle glycogen is limited and/or where the glycogen cost of the subsequent training session is high. In considering the well-documented effects of muscle glycogen availability on exercise performance (50–53), the sparing of muscle glycogen observed here would offer a metabolic advantage by allowing subsequent exercise bouts to be commenced with higher glycogen availability. Although this glycogen-sparing effect was not evident with the coingestion of a caffeine-containing multi-ingredient formulation, this strategy promoted higher power outputs during the latter stages of exercise at a similar glycogen cost to that observed in the placebo trial. Although these observations are specific to the exercise model and nutritional treatments studied here, these data may contribute to a better understanding of the glycogen cost associated with the high workloads sustained during interval cycling and inform fueling strategies to promote training intensity.

Despite the practical relevance of our data, several limitations should be acknowledged. First, as participants were able to self-select exercise intensity, the CHO^{+} trial resulted in a different metabolic stimulus when compared with PLA and CHO trials. Accordingly, between-condition differences in glycogen utilization, circulating metabolites and hormonal responses should also be interpreted in the context of the greater total work achieved in this condition. Second, although the CHO^{+} trial is representative of the caffeine-containing multi-ingredient formulations commonly used by athletes (21), this design does not allow the independent effect of caffeine to be isolated from the other ingredients without the inclusion of a caffeine-only comparator. Finally, although the study was powered using available liver glycogen data (11), those data were derived from prolonged endurance exercise that displayed a larger magnitude of liver glycogen utilization than observed in the present study. As such, our ability to detect between-treatment differences in liver glycogen utilization may have been limited.

In conclusion, our data provide the first report to simultaneously characterize liver and muscle glycogen utilization during HIIT, demonstrating the preferential use of skeletal

muscle glycogen toward energy production. Importantly, our data also show that CHO ingestion spares muscle glycogen utilization, yet this sparing effect is not evident when coingested with a caffeine-containing multi-ingredient formulation, in accordance with the observed increase in self-selected exercise intensity. These findings highlight a trade-off between preserving glycogen and maximizing training intensity, suggesting that although CHO feeding may attenuate muscle glycogen utilization, the addition of caffeine enhances self-selected exercise intensity and subsequently increases glycogen utilization.

DATA AVAILABILITY

Glycogen data for this study are openly available at https://doi.org/10.17605/OSF.IO/THZAX.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

S.C.H., J.V., C.M., J.P.M., and M.A.H. conceived and designed research; S.C.H., L.H., J.V., and G.F.P. performed experiments; S.C.H., J.V., C.M., G.F.P., A.J.K., and M.A.H. analyzed data; S.C.H., J.V., J.P.M., and M.A.H. interpreted results of experiments; S.C.H., J.V., and M.A.H. prepared figures; S.C.H., J.V., J.P.M., and M.A.H. drafted manuscript; S.C.H., L.H., C.M., G.F.P., F.B.S., B.T.W., N.H., A.J.K., and M.A.H. edited and revised manuscript; S.C.H., L.H., J.V., C.M., G.F.P., F.B.S., B.T.W., N.H., J.P.M., A.J.K., and M.A.H. approved final version of manuscript.

REFERENCES

1. **Romijn JA**, **Coyle EF**, **Sidossis LS**, **Gastaldelli A**, **Horowitz JF**, **Endert E**, **Wolfe RR.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Physiol* 265: E380–E391, 1993. doi:10.1152/ajpendo.1993.265.3.E380.
2. **van Loon LJ**, **Greenhaff PL**, **Constantin-Teodosiu D**, **Saris WH**, **Wagenmakers AJ.** The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol* 536: 295–304, 2001. doi:10.1111/j.1469-7793.2001.00295.x.
3. **Jeukendrup AE**, **Wagenmakers AJ**, **Stegen JH**, **Gijsen AP**, **Brouns F**, **Saris WH.** Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. *Am J Physiol Endocrinol Physiol* 276: E672–E683, 1999. doi:10.1152/ajpendo.1999.276.4.E672.
4. **Coyle EF**, **Coggan AR**, **Hemmert MK**, **Ivy JL.** Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol (1985)* 61: 165–172, 1986. doi:10.1152/jappl.1986.61.1.165.
5. **Coggan AR**, **Swanson SC**, **Mendenhall LA**, **Habash DL**, **Kien CL.** Effect of endurance training on hepatic glycogenolysis and gluconeogenesis during prolonged exercise in men. *Am J Physiol Endocrinol Physiol* 268: E375–E383, 1995. doi:10.1152/ajpendo.1995.268.3.E375.
6. **McConell GK**, **Canny BJ**, **Daddo MC**, **Nance MJ**, **Snow RJ.** Effect of carbohydrate ingestion on glucose kinetics and muscle metabolism during intense endurance exercise. *J Appl Physiol (1985)* 89: 1690–1698, 2000. doi:10.1152/jappl.2000.89.5.1690.
7. **Fell JM**, **Hearris MA**, **Ellis DG**, **Moran JEP**, **Jevons EFP**, **Owens DJ**, **Strauss JA**, **Cocks M**, **Louis JB**, **Shepherd SO**, **Morton JP.** Carbohydrate improves exercise capacity but does not affect subcellular lipid droplet morphology, AMPK and p53 signalling in human skeletal muscle. *J Physiol* 599: 2823–2849, 2021 [Erratum in *J Physiol* 604: 660, 2026]. doi:10.1113/JP281127.
8. **Hearris MA**, **Pugh JN**, **Langan-Evans C**, **Mann SJ**, **Burke L**, **Stellingwerff T**, **Gonzalez JT**, **Morton JP.** ^{13}C-glucose-fructose labeling reveals comparable exogenous CHO oxidation during exercise when consuming 120 g/h in fluid, gel, jelly chew, or coingestion. *J Appl Physiol (1985)* 132: 1394–1406, 2022. doi:10.1152/japplphysiol.00091.2022.
9. **Jeukendrup AE**, **Saris WH**, **Brouns F**, **Halliday D**, **Wagenmakers JM.** Effects of carbohydrate (CHO) and fat supplementation on CHO metabolism during prolonged exercise. *Metabolism* 45: 915–921, 1996. doi:10.1016/s0026-0495(96)90169-9.
10. **Rothschild JA**, **Dudley-Rode H**, **Carpenter H**, **Smith ASM**, **Plews DJ**, **Maunder E.** Carbohydrate ingestion during prolonged exercise and net skeletal muscle glycogen utilization: a meta-analysis. *J Appl Physiol (1985)* 140: 76–87, 2026. doi:10.1152/japplphysiol.00861.2025.
11. **Gonzalez JT**, **Fuchs CJ**, **Smith FE**, **Thelwall PE**, **Taylor R**, **Stevenson EJ**, **Trenell MI**, **Cermak NM**, **van Loon LJ.** Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *Am J Physiol Endocrinol Physiol* 309: E1032–E1039, 2015. doi:10.1152/ajpendo.00376.2015.
12. **Coates AM**, **Joyner MJ**, **Little JP**, **Jones AM**, **Gibala MJ.** A perspective on high-intensity interval training for performance and health. *Sports Med* 53: 85–96, 2023. doi:10.1007/s40279-023-01938-6.
13. **Leo P**, **Spragg J**, **Simon D**, **Lawley JS**, **Mujika I.** Training characteristics and power profile of professional U23 cyclists throughout a competitive season. *Sports (Basel)* 8: 167, 2020. doi:10.3390/sports8120167.
14. **Bartlett JD**, **Close GL**, **MacLaren DP**, **Gregson W**, **Drust B**, **Morton JP.** High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. *J Sports Sci* 29: 547–553, 2011. doi:10.1080/02640414.2010.545427.
15. **Hogervorst E**, **Bandelow S**, **Schmitt J**, **Jentjens R**, **Oliveira M**, **Allgrove J**, **Carter T**, **Gleeson M.** Caffeine improves physical and cognitive performance during exhaustive exercise. *Med Sci Sports Exerc* 40: 1841–1851, 2008. doi:10.1249/MSS.0b013e31817bb8b7.
16. **Lane SC**, **Areta JL**, **Bird SR**, **Coffey VG**, **Burke LM**, **Desbrow B**, **Karagounis LG**, **Hawley JA.** Caffeine ingestion and cycling power output in a low or normal muscle glycogen state. *Med Sci Sports Exerc* 45: 1577–1584, 2013. doi:10.1249/MSS.0b013e31828af183.
17. **Peeling P**, **Binnie MJ**, **Goods PSR**, **Sim M**, **Burke LM.** Evidence-based supplements for the enhancement of athletic performance. *Int J Sport Nutr Exerc Metab* 28: 178–187, 2018. doi:10.1123/ijsnem.2017-0343.
18. **Graham TE**, **Spriet LL.** Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *J Appl Physiol (1985)* 78: 867–874, 1995. doi:10.1152/jappl.1995.78.3.867.
19. **Jackman M**, **Wendling P**, **Friars D**, **Graham TE.** Metabolic catecholamine, and endurance responses to caffeine during intense exercise. *J Appl Physiol (1985)* 81: 1658–1663, 1996. doi:10.1152/jappl.1996.81.4.1658.
20. **Howlett K**, **Galbo H**, **Lorentsen J**, **Bergeron R**, **Zimmerman-Belsing T**, **Bülow J**, **Feldt-Rasmussen U**, **Kjaer M.** Effect of adrenaline on glucose kinetics during exercise in adrenalectomised humans. *J Physiol* 519: 911–921, 1999. doi:10.1111/j.1469-7793.1999.0911n.x.
21. **Jagim AR**, **Harty PS**, **Camic CL.** Common ingredient profiles of multi-ingredient pre-workout supplements. *Nutrients* 11: 254, 2019. doi:10.3390/nu11020254.
22. **McKay AKA**, **Stellingwerff T**, **Smith ES**, **Martin DT**, **Mujika I**, **Goosey-Tolfrey VL**, **Sheppard J**, **Burke LM.** Defining training and performance caliber: a participant classification framework. *Int J*

Sports Physiol Perform 17: 317–331, 2022. doi:10.1123/ijspp.2021-0451.
23. **Kuipers H**, **Verstappen FT**, **Keizer HA**, **Geurten P**, **van Kranenburg G.** Variability of aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med* 6: 197–201, 1985. doi:10.1055/s-2008-1025839.
24. **Stepto NK**, **Martin DT**, **Fallon KE**, **Hawley JA.** Metabolic demands of intense aerobic interval training in competitive cyclists. *Med Sci Sports Exerc* 33: 303–310, 2001. doi:10.1097/00005768-200102000-00021.
25. **Borg GA.** Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377–381, 1982.
26. **Hannon SC**, **McStravick J**, **Henthorn L**, **Bawden SJ**, **Tang JCY**, **Dunn R**, **Makino R**, **Smith K**, **Gonzalez JT**, **Hodson N**, **Morton JP**, **Kennerley AJ**, **Hearris MA.** Co-ingesting whey protein with dual-source carbohydrate enhances amino acid availability without compromising post-exercise liver glycogen resynthesis. *J Physiol*, 2025. doi:10.1113/JP288473.
27. **Holland DT**, **Godfredsen KA**, **Page T**, **Connor JD.** Simple high-performance liquid chromatography method for the simultaneous determination of serum caffeine and paraxanthine following rapid sample preparation. *J Chromatogr B Biomed Sci Appl* 707: 105–110, 1998. doi:10.1016/s0378-4347(97)00590-2.
28. **Miyake A**, **Emerson MJ**, **Friedman NP.** Assessment of executive functions in clinical settings: problems and recommendations. *Semin Speech Lang* 21: 169–183, 2000. doi:10.1055/s-2000-7563.
29. **Morris N**, **Jones DM.** Memory updating in working memory: the role of the central executive. *Br J Psychol* 81: 111–121, 1990. doi:10.1111/j.2044-8295.1990.tb02349.x.
30. **Stroop JR.** Studies of interference in serial verbal reactions. *J Exp Psychol* 18: 643–662, 1935. doi:10.1037/h0054651.
31. **Parker RA**, **Weir CJ.** Non-adjustment for multiple testing in multi-arm trials of distinct treatments: rationale and justification. *Clin Trials* 17: 562–566, 2020. doi:10.1177/1740774520941419.
32. **Watt MJ**, **Heigenhauser GJ**, **Dyck DJ**, **Spriet LL.** Intramuscular triacylglycerol, glycogen and acetyl group metabolism during 4 h of moderate exercise in man. *J Physiol* 541: 969–978, 2002. doi:10.1113/jphysiol.2002.018820.
33. **McConell G**, **Fabris S**, **Proietto J**, **Hargreaves M.** Effect of carbohydrate ingestion on glucose kinetics during exercise. *J Appl Physiol (1985)* 77: 1537–1541, 1994. doi:10.1152/jappl.1994.77.3.1537.
34. **Gollnick PD**, **Pernow B**, **Essen B**, **Jansson E**, **Saltin B.** Availability of glycogen and plasma FFA for substrate utilization in leg muscle of man during exercise. *Clin Physiol* 1: 27–42, 1981. doi:10.1111/j.1475-097X.1981.tb00872.x.
35. **Bosch AN**, **Dennis SC**, **Noakes TD.** Influence of carbohydrate ingestion on fuel substrate turnover and oxidation during prolonged exercise. *J Appl Physiol (1985)* 76: 2364–2372, 1994. doi:10.1152/jappl.1994.76.6.2364.
36. **Rollo I**, **Gonzalez JT**, **Fuchs CJ**, **van Loon LJC**, **Williams C.** Primary, secondary, and tertiary effects of carbohydrate ingestion during exercise. *Sports Med* 50: 1863–1871, 2020 [Erratum in *Sports Med* 51: 2671, 2021]. doi:10.1007/s40279-020-01343-3.
37. **Stellingwerff T**, **Boon H**, **Gijsen AP**, **Stegen JH**, **Kuipers H**, **van Loon LJ.** Carbohydrate supplementation during prolonged cycling exercise spares muscle glycogen but does not affect intramyocellular lipid use. *Pflugers Arch* 454: 635–647, 2007. doi:10.1007/s00424-007-0236-0.
38. **Tsintzas OK**, **Williams C**, **Boobis L**, **Greenhaff P.** Carbohydrate ingestion and glycogen utilization in different muscle fibre types in man. *J Physiol* 489: 243–250, 1995. doi:10.1113/jphysiol.1995.sp021046.
39. **Carter JM**, **Jeukendrup AE**, **Mann CH**, **Jones DA.** The effect of glucose infusion on glucose kinetics during a 1-h time trial. *Med Sci Sports Exerc* 36: 1543–1550, 2004. doi:10.1249/01.mss.0000139892.69410.d8.
40. **Katz A**, **Broberg S**, **Sahlin K**, **Wahren J.** Leg glucose uptake during maximal dynamic exercise in humans. *Am J Physiol Endocrinol Physiol* 251: E65–E70, 1986. doi:10.1152/ajpendo.1986.251.1.E65.
41. **Kuipers H**, **Keizer HA**, **Brouns F**, **Saris WH.** Carbohydrate feeding and glycogen synthesis during exercise in man. *Pflugers Arch* 410: 652–656, 1987. doi:10.1007/BF00581327.
42. **Febbraio MA**, **Lambert DL**, **Starkie RL**, **Proietto J**, **Hargreaves M.** Effect of epinephrine on muscle glycogenolysis during exercise in trained men. *J Appl Physiol (1985)* 84: 465–470, 1998. doi:10.1152/jappl.1998.84.2.465.
43. **Watt MJ**, **Howlett KF**, **Febbraio MA**, **Spriet LL**, **Hargreaves M.** Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans. *J Physiol* 534: 269–278, 2001. doi:10.1111/j.1469-7793.2001.t01-1-00269.x.
44. **Dodd FL**, **Kennedy DO**, **Riby LM**, **Haskell-Ramsay CF.** A double-blind, placebo-controlled study evaluating the effects of caffeine and L-theanine both alone and in combination on cerebral blood flow, cognition and mood. *Psychopharmacology (Berl)* 232: 2563–2576, 2015. doi:10.1007/s00213-015-3895-0.
45. **Scholey A**, **Savage K**, **O'Neill BV**, **Owen L**, **Stough C**, **Priestley C**, **Wetherell M.** Effects of two doses of glucose and a caffeine-glucose combination on cognitive performance and mood during multi-tasking. *Hum Psychopharmacol* 29: 434–445, 2014. doi:10.1002/hup.2417.
46. **Wu SH**, **Chen YC**, **Chen CH**, **Liu HS**, **Liu ZX**, **Chiu CH.** Caffeine supplementation improves the cognitive abilities and shooting performance of elite e-sports players: a crossover trial. *Sci Rep* 14: 2074, 2024. doi:10.1038/s41598-024-52599-y.
47. **Kahathuduwa CN**, **Dassanayake TL**, **Amarakoon AMT**, **Weerasinghe VS.** Acute effects of theanine, caffeine and theanine–caffeine combination on attention. *Nutr Neurosci* 20: 369–377, 2017. doi:10.1080/1028415X.2016.1144845.
48. **Haskell CF**, **Kennedy DO**, **Milne AL**, **Wesnes KA**, **Scholey AB.** The effects of L-theanine, caffeine and their combination on cognition and mood. *Biol Psychol* 77: 113–122, 2008. doi:10.1016/j.biopsycho.2007.09.008.
49. **Kahathuduwa CN**, **Dhanasekara CS**, **Chin SH**, **Davis T**, **Weerasinghe VS**, **Dassanayake TL**, **Binks M.** L-theanine and caffeine improve target-specific attention to visual stimuli by decreasing mind wandering: a human functional magnetic resonance imaging study. *Nutr Res* 49: 67–78, 2018. doi:10.1016/j.nutres.2017.11.002.
50. **Bergström J**, **Hermansen L**, **Hultman E**, **Saltin B.** Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140–150, 1967. doi:10.1111/j.1748-1716.1967.tb03720.x.
51. **Hawley JA**, **Schabort EJ**, **Noakes TD**, **Dennis SC.** Carbohydrate-loading and exercise performance. An update. *Sports Med* 24: 73–81, 1997. doi:10.2165/00007256-199724020-00001.
52. **Hearris MA**, **Hammond KM**, **Seaborne RA**, **Stocks B**, **Shepherd SO**, **Philp A**, **Sharples AP**, **Morton JP**, **Louis JB.** Graded reductions in preexercise muscle glycogen impair exercise capacity but do not augment skeletal muscle cell signaling: implications for CHO periodization. *J Appl Physiol (1985)* 126: 1587–1597, 2019. doi:10.1152/japplphysiol.00913.2018.
53. **Widrick JJ**, **Costill DL**, **Fink WJ**, **Hickey MS**, **McConell GK**, **Tanaka H.** Carbohydrate feedings and exercise performance: effect of initial muscle glycogen concentration. *J Appl Physiol (1985)* 74: 2998–3005, 1993. doi:10.1152/jappl.1993.74.6.2998.