

ORIGINAL INVESTIGATION

Title:

Caffeine, cycling performance & exogenous CHO oxidation: a dose-response study

Running Title:

Caffeine, cycling performance & CHO oxidation

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Abstract

Purpose: This study investigated the effects of a low and moderate caffeine dose on exogenous CHO oxidation and endurance exercise performance.

Method: 9 trained and familiarised male cyclists [29.4±4.5 y, 81.3±10.8 kg BW, 183.8±8.2 cm, $VO_{2\text{ peak}}$ 61.7±4.8 mL·kg⁻¹·min⁻¹; values are mean±SD] undertook 3 trials, with training and high CHO diet being controlled. One hour prior to exercise subjects ingested capsules containing placebo, 1.5 or 3 mg·kg⁻¹ BW of caffeine using a double blind administration protocol. Trials consisted of 120 min steady-state (SS) cycling at ~70% $VO_{2\text{ peak}}$, immediately followed by a 7 kJkg⁻¹ BW time trial (TT). During exercise subjects were provided with fluids containing ¹⁴C glucose every 20min to determine exogenous CHO oxidation.

Results: No significant TT performance improvements were observed during caffeine containing trials (Placebo 30:25±3:10, 1.5 mg·kg⁻¹ BW 30:42±3:41, 3 mg·kg⁻¹ BW 29:51±3:38 TT Time±SD (min:ss)). Furthermore, caffeine failed to significantly alter maximal exogenous CHO oxidation (maximal oxidation rates Placebo 0.95±0.2, 1.5 mg·kg⁻¹ BW 0.92±0.2, 3 mg·kg⁻¹ BW 0.96±0.2 g·min⁻¹).

Conclusion: Low and moderate doses of caffeine have failed to improve endurance performance in fed, trained subjects.

Key Words: Endurance, Substrate, Metabolism, Glucose

Introduction

Paragraph Number 1 The ergogenic effect of caffeine on endurance-exercise performance is well recognised (15). However, the dose of caffeine required to elicit optimal performance during long-duration (>60 min), endurance exercise remains unclear (15). The only reported study to specifically investigate a dose-dependent response to caffeine ingestion on a task of this duration failed to include a dose <5 mg·kg⁻¹ body weight (BW; 6). Investigating the smallest caffeine dose required to elicit ergogenic benefits when performing long-duration exercise has implications for the safe use of the supplement and is relevant in the sporting context; the consumption of caffeine by endurance athletes is typically lower (≤5 mg·kg⁻¹ BW) than those doses often used in laboratory research (12).

Paragraph Number 2 Numerous studies have investigated the effect of low-dose (i.e., <3 mg·kg⁻¹ BW) caffeine ingestion on exercise performance, (4, 10, 14, 17, 19, 20, 24, 29, 33), often without including higher doses for comparison. Only one study (10) has demonstrated the effect of low-dose caffeine ingestion on continuous endurance-exercise lasting more than 60min. Findings from the study by Cox et al (10) suggested that very low doses of caffeine (≤3 mg·kg⁻¹ BW) have the potential to improve endurance-exercise performance lasting longer than 60min to the same extent as higher caffeine doses (e.g., 6 mg·kg⁻¹ BW).

Paragraph Number 3 Cox and co-workers (10) demonstrated positive effects of caffeine on performance when providing a cola beverage ($\sim 1.5 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$) to fed subjects during the later stages of a cycling protocol that lasted almost 2.5h. A second, but related investigation that looked at the cola beverage components independently, demonstrated that the performance enhancement was the result of the combined effects of the additional carbohydrate (CHO) and caffeine contained within the cola beverage (10). This finding suggests that long-duration, endurance-exercise performance can be enhanced by low doses of caffeine when taken in combination with CHO.

Paragraph Number 4 One possible explanation for ergogenic benefit seen when caffeine and CHO are coingested during prolonged endurance-exercise may relate to an effect seen at the gastrointestinal level. The gastrointestinal absorption of glucose was enhanced in fasted subjects when a low caffeine dose ($1.35 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$) was co-ingested with CHO during a 90min exercise task (37). Recently, Yeo and colleagues (40) demonstrated that a larger caffeine dose ($5 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}\cdot\text{h}^{-1}$) increased exogenous CHO oxidation rates during a 2h cycling protocol in previously fasted subjects. The authors suggested that caffeine's impact on glucose gastrointestinal absorption (rather than effects on hepatic glucose output or increased muscle glucose uptake) was the most likely cause for the elevated exogenous substrate use as CHO uptake appears to be the limiting step to maximising exogenous CHO oxidation (26). To date the impact of a low caffeine dose on exogenous CHO oxidation and its subsequent impact on endurance performance has not been examined in fed subjects.

Paragraph Number 5 The aim of the present study is to investigate the effects of a low and moderate caffeine dose on CHO oxidation during exercise in fed subjects, and subsequently to determine if there is a dose-response ergogenic effect of caffeine on endurance cycling performance lasting >2 h. By concurrently investigating dose responses to a metabolic and ergogenic variable, we aim to clarify the impact of the caffeine on performance and the degree to which any performance changes relate to changes seen in exogenous CHO oxidation. It is hypothesised that the caffeine will increase exogenous CHO oxidation and endurance cycling performance in fed subjects, independent of caffeine dose, compared to a placebo.

Methods

Subjects

Paragraph Number 6 Nine trained male cyclists or triathletes [29.4±4.5 y, 81.3±10.8 kg BW, 183.8±8.2 cm, VO_2 peak 61.7±4.8 mL·kg⁻¹·min⁻¹; values are mean±SD] who were cycling ≥200 km ·wk⁻¹ volunteered to participate as subjects in the present study. The reported habitual average caffeine intake of subjects ranged from 70-395 mg·d⁻¹. All subjects were fully informed of the nature and possible risks of the study before giving their written informed consent. The investigation was approved by the Human Research Ethics Committee of Griffith University.

Experimental Design

Paragraph Number 7 Each subject visited the laboratory on at least six occasions. The first visit was preliminary testing to confirm participants' maximal exercise capacity. This was followed by a minimum of two familiarisation visits using the experimental protocol. Once familiar with the protocol, each subject then undertook three experimental trials, with the subject's regular cycling training and diet being controlled before each trial. One hour before commencing an experimental trial, subjects were provided with capsules containing either a placebo (metamucil[®]), 1.5 or 3 mg·kg⁻¹ BW of caffeine (provided as 3 and 6 mg·kg⁻¹ BW of caffeine citrate PCCA, Texas, USA) using a double-blind administration protocol. The three experimental treatments were randomised via an incomplete Latin square design.

Each experimental trial consisted of 120min of SS cycling performed at ~70% VO_2 peak immediately followed by a $7 \text{ kJ}\cdot\text{kg}^{-1}$ BW TT (Figure 1). Throughout the exercise measures of perceived exertion, HR and rates of whole body CHO, fat and exogenous CHO oxidation were collected as dependent variables.

Preliminary Testing

Paragraph Number 8 Each subject performed an incremental test to exhaustion (VO_2 peak test) on an electromagnetically braked cycle ergometer (Lode Instruments, Groningen, The Netherlands) to determine VO_2 peak and Peak Sustainable Power Output (PPO). The VO_2 peak test protocol and the methods used for determining VO_2 peak and PPO have been previously described (13). Briefly, each test began at 100W and increased in 50W increments every 5min until exhaustion. During the VO_2 peak test, which typically lasted between 30 and 35min, each subjects' expired air was continuously analysed by a calibrated metabolic measurement system (MedGraphics, Minnesota, USA).

Familiarisation

Paragraph Number 9 Subjects performed at least two familiarisation rides over the protocol to establish their tolerance to the chosen SS intensity (initially 65%PPO) and subsequent linear factor (initially 82%PPO at a cadence of 110rpm) used during the TT. Three subjects had their SS intensity lowered (~60%PPO) during familiarisation to ensure they were able to satisfactorily complete the entire protocol.

The SS intensity and linear factor then remained consistent for each subject during all subsequent experimental trials.

Training and Dietary Standardisation

Paragraph Number 10 Experimental trials were separated by at least 7d and were conducted at the same time of the day in a stable laboratory environment ($19\pm 2^{\circ}$ C, 55% relative humidity). Subjects refrained from consuming caffeine-containing substances (i.e., coffee, chocolate and soft drinks) for 24h before each experiment. Subjects were asked to refrain from heavy training 24h prior to each trial and any light training was to be completed by 1200h the day before the experimental trials. During the 24h period immediately preceding each trial, subjects were provided with a prepacked standard diet with an energy content of approximately $200 \text{ kJ}\cdot\text{kg}^{-1}$ BW, composed of 64% CHO ($8 \text{ g}\cdot\text{kg}^{-1}$ BW), 24% fat, and 12% protein. Subjects were also given an additional meal to be consumed on the morning of the trial that provided $2 \text{ g}\cdot\text{kg}^{-1}$ BW CHO and included a 600mL commercial sports drink (Gatorade[®]), fruit bread, jam and a Power Bar[®]. Food and exercise diaries were used to examine compliance. All dietary preparation and analysis was performed by a qualified dietitian using Foodworks[®] Version 5.1, 2007, (Xyris Software, Australia) dietary analysis software.

Experimental Protocol

Paragraph Number 11 On the morning of an experimental trial, subjects reported to the laboratory at approximately 0600h having already consumed their prepacked breakfast between 0500 and 0515h. Within 10min of arriving at the laboratory, a blood sample was taken and the subjects were then asked to ingest the trial capsules with a small amount of water. Subjects rested in the laboratory before mounting the cycle ergometer at 0700h. A 2min resting sample of expired gas was collected for whole-body substrate analysis and a ~1L expired breath sample was collected to determine background ^{14}C -glucose oxidation. A second blood sample was taken immediately after the 2min rest period on the cycle ergometer and a bottle containing $8 \text{ mL}\cdot\text{kg}^{-1}$ BW of 6% glucose solution and trace amounts ($\sim 0.1 \text{ mL}\cdot\text{L}^{-1}$) of isotopic ^{14}C -glucose (GE Healthcare Biosciences, UK) was provided to the subject with instructions indicating that it was to be consumed within the first 2-3 min of exercise. Subjects then began cycling at the predetermined SS workload (average 236 ± 44 W).

Paragraph Number 12 Following 15min of SS exercise, expired gas was collected for 5min followed by a further ~1L breath sample. Subjects were then presented with a new bottle ($5 \text{ mL}\cdot\text{kg}^{-1}$ BW) of glucose drink. This pattern of gas exchange, expired breath samples and presentation of new drink continued on a 20min rotation for 120min of SS. After 120min of SS exercise, subjects dismounted and the cycle ergometer was adjusted into a pedaling rate-dependent (linear) mode as described by Cox et al (10). After approximately 3min of rest, subjects commenced a $7 \text{ kJ}\cdot\text{kg}^{-1}$ BW TT that typically lasted 25-30 min. During the first experimental trial, subjects were provided with $5 \text{ mL}\cdot\text{kg}^{-1}$ BW of CHO solution and instructed to consume fluids

ad libitum throughout the TT. The volume consumed was then recorded and that amount of CHO solution was provided in the subsequent trials where subjects were instructed to consume the entire volume. Subjects were instructed to complete the TT “as fast as possible,” and a financial incentive was provided to all subjects to produce the fastest average TT time. The same researcher supervised each TT and provided standardised feedback to each subject. Subjects were able to view their HR, cadence and power output for the first 10% of the TT only. After completion of the first 10% the only information available to subjects was elapsed work as a percentage of the final work; furthermore, subjects were given the results of their TT only after the entire study was completed. No gas exchange data or blood samples were collected during the TT. Subjective ratings of perceived exertion (RPE) and heart-rate values (Polar Electro, Kempele, Finland) were recorded every 20min during SS exercise and at each 10% of the TT.

Paragraph Number 13 At the conclusion of all experimental exercise trials, subjects completed a questionnaire which asked them to identify the order of treatments received during the study and nominate which treatment and trial they perceived was associated with their best TT performance.

Blood Sampling and Analysis

Paragraph Number 14 A 5mL blood sample was collected via venipuncture on arrival. This sample was placed in a tube containing lithium heparin and centrifuged at 3000rpm for 10min. The resultant plasma was stored at -80°C for subsequent analysis. In addition, 6mL blood samples were collected immediately before exercise

as well as after 60 and 120 min of SS exercise. The 6mL blood samples were divided into 5mL and 1mL aliquots for the subsequent determination of caffeine and glucose, respectively. The caffeine sample was treated as previously mentioned. The glucose sample was added to a fluoridated tube and was centrifuged as above with the resultant plasma stored at -80°C for later analysis.

Plasma Caffeine Analysis

Paragraph Number 15 The quantitative analysis of caffeine was performed using an automated high-performance liquid chromatography (HPLC) system, consisting of a Varian Prostar 240 Quaternary Solvent Delivery Module, Varian Prostar Photodiode Array Detector, Varian Prostar Autosampler, equipped with a Varian Pursuit C18 reverse phase column (250 x 4.6 mm, 5µm particle size). The column was thermostated at 30°C and eluted caffeine was detected at wavelengths of 210 and 273nm. The HPLC conditions used were modified from reported methods described previously (12) and chromatography was carried out using an isocratic method in which solvent A was 0.05M NaH₂PO₄ (adjusted to pH 5.5 using triethylamine) and solvent B methanol. A mobile phase of 75% solvent A: 25% solvent B at a flow rate of 1 mLmin⁻¹ eluted caffeine at a retention time (R_t) of 8.7min. The identity and purity of caffeine peaks were achieved by spectral purity analysis and comparison to pure caffeine verification samples scattered throughout the run. The reverse phase, isocratic method allows for the analysis of caffeine with limits of quantification (LOQ) ≥1.7 µM (signal-to-noise ratio of 10-to-1) and limits of detection ≥1 µM

(signal-to-noise ratio of 3-to-1). The calculated standard curves for caffeine were linear in the range from 12.5 to 50 μM and relative standard deviations of $\leq 5\%$ were obtained for both repeatability and intermediate precision studies.

Sample preparation. Serum proteins were precipitated before the sample could be injected onto the chromatographic system. Protein precipitation was achieved using the following method: 100 μL serum + 200 μL methanol were mixed on a Vortex for 1 min and centrifuged at 14000rpm for 10min. 20 μL of supernatant were injected into the HPLC system in triplicate. The concentration of caffeine in samples was calculated by extrapolation from the aforementioned standard curve run immediately prior to sample analysis.

Plasma Glucose Analysis

Paragraph Number 16 The quantitative analysis of plasma glucose was performed on a automated Cobas-Mira (Roche, Switzerland) diagnostic system using an Infinity™ (Thermo Electron Corp, France) glucose oxidase liquid stable reagent. Manufacturers recommended procedures were followed which included a sample:reagent ratio of 1:150, a 5min incubation period and photometric detection of the red quinoneimine dye at a 500nm wavelength. Known glucose standards confirmed the accuracy of the method.

Rates of whole body CHO, Fat and Exogenous Glucose Oxidation

Paragraph Number 17 Total CHO (CHO Ox) and Fat oxidation (Fat Ox) rates ($\text{g}\cdot\text{min}^{-1}$) during SS were determined using respiratory gas data ($\text{L}\cdot\text{min}^{-1}$) and the following stoichiometric equations (28).

$$\text{CHO Ox} = 4.21\cdot\text{VCO}_2 - 2.962\cdot\text{VO}_2$$

$$\text{Fat Ox} = 1.695\cdot\text{VO}_2 - 1.701\cdot\text{VCO}_2$$

Exogenous (ingested) glucose oxidation was determined following the method described by Carey and colleagues (5). That is, on the initiation of exercise subjects began ingesting a glucose solution containing trace amounts of isotopic ^{14}C -glucose. All beverages were prepared 24h before each trial by one of the authors (BD). Two 1mL samples were taken from each drink to determine the ^{14}C enrichment.

Paragraph Number 18 Prior to SS a sample of $\sim 1\text{L}$ of expired air was collected into a rubber anaesthetic bag via a two-way Hans Rudolph valve and passed through a solution containing 1mL hyamine hydroxide in methanol, 1mL 96% ethanol, and two drops of 1% phenolphthalein indicator. The air was bubbled through this mixture for 1-2 min until the phenolphthalein turned from pink to clear, at which point 1 mM of CO_2 had been absorbed (34). Liquid scintillation cocktail (10 mL; Ready Gel, Beckmann) was then added to the solution. This sample collection protocol was repeated every 20min during the SS period. On the completion of each trial $^{14}\text{CO}_2$ disintegrations $\cdot\text{mmol}^{-1}\cdot\text{min}^{-1}$ were counted for each breath sample and the drink standards in a scintillation counter (Packard 2100TR Tri-Carb, Downers Grove, IL) within 5d of collection.

Paragraph Number 19 The rates of total exogenous glucose oxidation were calculated from the following equation

$$\text{EXO}_{\text{ox}} = (\text{SA CO}_2 / \text{SA Drink}) \cdot \text{VCO}_2$$

where EXO_{ox} is the rate of exogenous glucose oxidation in $\text{mmol} \cdot \text{min}^{-1}$, later converted to $\text{g} \cdot \text{min}^{-1}$; SA CO_2 is the specific (radio)activity of expired $^{14}\text{CO}_2$ in disintegrations $\cdot \text{min}^{-1} \cdot \text{mmol}^{-1}$; SA Drink is the mean corresponding specific (radio)activity of the drink standards in disintegrations $\cdot \text{min}^{-1} \cdot \text{mmol}^{-1}$; and VCO_2 is the volume of expired CO_2 in $\text{mmol} \cdot \text{min}^{-1}$, calculated from the $\text{L} \cdot \text{min}^{-1} \text{VCO}_2$ value and the $22.4 \text{ mL} \cdot \text{mmol}^{-1}$ gas volume.

Statistical Analyses

Paragraph Number 20 All data was coded and entered into Microsoft Office Excel (Microsoft Corporation. 2003). Differences in TT performance, heart rate and RPE along with SS plasma glucose and caffeine levels across time were compared using paired *t*-tests generated by a spreadsheet for fully controlled crossovers (Sports science Web site. Hopkins 2005). Effect size and 95% confidence intervals were also calculated for TT performance data. The effect of trial order was assessed using one-way ANOVA. Significant differences were accepted when $P \leq 0.05$. All data are reported as means \pm SD.

6.4 Results

Pretrial standardisation.

Paragraph Number 21 The subjects' habitual caffeine intake was estimated as 232 ± 129 (range 74–395) $\text{mg} \cdot \text{day}^{-1}$. No exercise was reported during the 18h period before each trial, and complete caffeine withdrawal was reported for 24h before each trial. Pretrial mean plasma caffeine levels later revealed that one subject failed to comply with the requested 24h caffeine abstinence in the $1.5 \text{ mg} \cdot \text{kg}^{-1}$ BW trial (subject 6). On removal of this subject's data the final CHO oxidation and performance results were unaffected and as only a small amount of plasma caffeine was present within this subject on this trial the data has been included within the final analysis. Mean pretrial plasma caffeine values were $0, 2.1 \pm 4.5$ and $0.2 \pm 0.4 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ for the 0, 1.5 and $3 \text{ mg} \cdot \text{kg}^{-1}$ BW trials respectively, confirming the reports of minimal caffeine for most subjects. There were no differences among treatments for reported intakes of CHO and energy during the 24h pretrial period. Average intakes throughout this period were CHO $8.2 \pm 1.1 \text{ g} \cdot \text{kg}^{-1}$ BW and Energy $210 \pm 13 \text{ kJ} \cdot \text{kg}^{-1}$ BW.

Drinks consumed during exercise.

Paragraph Number 22 Subjects complied with the consumption of their drinks during each treatment. Drink volumes varied marginally between individuals based on ad libitum consumption of the initial 120min drink. The SS fluid ingestion

protocol provided a total of $1.98 \text{ g}\cdot\text{kg}^{-1}$ BW of CHO and $33 \text{ mL}\cdot\text{kg}^{-1}$ BW of fluid during exercise. Athletes then consumed a further $10\pm 8 \text{ g}$ (range 0-22g) of CHO immediately prior to or throughout the TT.

SS Data

Plasma Caffeine & Glucose.

Paragraph Number 23 Figure 2 summarises concentrations of plasma caffeine taken prior to and throughout SS exercise for each trial. Plasma caffeine concentrations during caffeinated trials differed from placebo at the initiation of exercise and remained different throughout SS ($p<0.05$). Initial differences between the 1.5 and $3 \text{ mg}\cdot\text{kg}^{-1}$ BW caffeine doses were observed following 60min of exercise and these differences remained throughout the remainder of SS. No differences were observed in plasma glucose, heart rate or RPE between treatments during the 120min of SS ($p>0.05$).

Rates of whole body CHO, Fat and Exogenous Glucose Oxidation

Paragraph Number 24 Rates of whole body CHO, fat (Figure 3) and exogenous glucose oxidation (Figure 4) were all unaffected by caffeine administration. Due to technical difficulties exogenous glucose oxidation values could only be calculated for eight subjects. Peak exogenous glucose oxidation rates (average of 80-120 min of SS) were 0.95 ± 0.2 , 0.92 ± 0.2 , $0.96\pm 0.2 \text{ g}\cdot\text{min}^{-1}$ for the 0, 1.5 and $3 \text{ mg}\cdot\text{kg}^{-1}$ BW doses, respectively.

TT Data

Performance and post trial feedback.

Paragraph Number 25 Mean TT results are summarised in Table 1 including calculations of effect sizes and confidence intervals. Individual TT data is displayed in Figure 5. Despite a trend toward improved TT performance with the 3 mg·kg⁻¹ BW dose, no statistically significant differences were found for either the 1.5 or 3 mg·kg⁻¹ BW caffeine dose. The trial order did not influence the subject's performances ($p>0.05$). Elevated heart rates were detected between 50-70% of the TT on the 3 mg·kg⁻¹ BW caffeine trial when compared to placebo ($p<0.05$). No RPE differences between treatments were noted throughout the TT. Post trial questionnaires revealed two subjects who were able to correctly identify the order of their caffeine doses, however only one of these subjects indicated a high degree of certainty over their predictions. Six subjects correctly identified their fastest TT.

Discussion

Paragraph Number 26 To our knowledge the present investigation is only the second to examine the effect of low dose ($\leq 3 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$) caffeine administration on endurance exercise lasting more than 60min. The findings of the present study indicate no significant ergogenic effect when endurance-trained athletes were provided with either a 1.5 or $3 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$ caffeine dose 1h prior to commencing an endurance cycling task in which CHO was readily available.

Paragraph Number 27 These results are in contrast to those of Cox and coworkers (10) who found significant positive effects from a low caffeine dose ($\sim 1.5 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$) and a larger dose ($6 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$) given to subjects completing precisely the same endurance cycling exercise protocol used in this study. In the present study, a trend for improvement in performance was shown at the higher caffeine dose only, whilst the lack of any ergogenic effect was more apparent following the $1.5 \text{ mg}\cdot\text{kg}^{-1} \text{ BW}$ dose. The modest effect size of the present data may provide some explanation for the inconsistent findings seen during endurance-exercise studies that have employed similar caffeine doses ingested by small numbers of research participants ($n=6-12$; 6, 8, 10, 21, 22, 23, 25, 38, 39).

Paragraph Number 28 The current lack of performance effect observed on these lower caffeine doses compared to the 3.1% improvement in performance observed by Cox et al (10) is more difficult to explain. Subjects recruited for both studies reported similar pre-trial training volumes but subtle differences in physical

characteristic such as body weight, VO_2 peak and average habitual caffeine intake. However, differences related to the method of low dose caffeine administration (i.e., Encapsulated pure caffeine [present study], Caffeine within cola beverage [10]) and the timing of the caffeine ingestion (i.e., 1h pre-exercise [present study], later stages of 2h SS exercise [10]) represent a feasible basis for the differing results. For example, it is possible that the effects of low doses of caffeine are more pronounced when they are consumed contemporaneously with the decline in exercise capacity or the onset of fatigue which was not the case in the present study. Whilst both Type I and II errors are a possibility, clearly, the effect of low caffeine doses on endurance performance requires further examination, especially given that these doses of caffeine are more likely to typically reflect amounts ingested by endurance athletes in competition (12).

Paragraph Number 29 It has been suggested that caffeine may augment endurance performance by increasing the gastrointestinal absorption of glucose (37). In support of this theory, Yeo and colleagues (40) reported that the consumption of a caffeinated beverage increased exogenous CHO oxidation by 26% during the 90 to 120min period of a 2h endurance cycling bout (Placebo = 0.57 ± 0.1 , Caffeine = 0.72 ± 0.1 $\text{g} \cdot \text{min}^{-1}$). In contrast, no increases in exogenous glucose oxidation were observed in the present study at either caffeine dose (Placebo = 0.95 ± 0.2 , $1.5 \text{ mg} \cdot \text{kg}^{-1}$ BW = 0.92 ± 0.2 , $3 \text{ mg} \cdot \text{kg}^{-1}$ BW = 0.96 ± 0.2 $\text{g} \cdot \text{min}^{-1}$). Participants in the current study received a diet in keeping with that recommended prior to endurance exercise (i.e. a high CHO diet 24h prior to all trials along with a high CHO breakfast 2h prior to the commencement of exercise), whereas VanNieuwenhoven et al (37) and Yeo et al

(40) both requested subjects to fast prior to trials. Additionally, the 6% CHO solution supplied during this study throughout exercise, resulted in peak exogenous CHO rates comparable with those seen in studies attempting to maximise exogenous CHO oxidation rates (27, 31). The combined results of VanNieuwenhoven et al (37) and Yeo et al (40) point to the potential for caffeine to improve the utilisation of exogenous glucose, however this has only been demonstrated when CHO intake throughout exercise was moderate (~30-35 [37], 48 g·h⁻¹ [40], respectively). Conversely, the present data demonstrates that when glucose is consumed in sufficient amounts to optimise its gastrointestinal transport (rates ~70 g·h⁻¹, 27) the addition of caffeine at either low or moderate doses does not further enhance the subsequent oxidation of this CHO.

Paragraph Number 30 Furthermore, the results of the current study and those of VanNieuwenhoven et al (37) and Yeo et al (40) suggests that caffeine's ergogenic potential may change based on exogenous CHO availability. That is, when exogenous CHO availability is moderate, caffeine has the potential to cause an increase in CHO absorption and subsequently may influence performance via an improvement in exogenous CHO oxidation. However, when exercising individuals are supplied with ample CHO either immediately prior to or throughout an endurance-exercise task this potential ergogenic mechanism may be beyond caffeine's influence.

Paragraph Number 31 Interestingly, the ergogenic properties of caffeine on exercise >1 h have often (10 of 19 studies) been examined using experimental protocols

devoid of CHO (i.e. using water or artificially sweetened beverages throughout exercise and in the absence of an immediate pre-event meal (6, 8, 9, 17, 18, 23, 34, 35, 36, 38). Eight of these ten studies report some ergogenic effect from caffeine administration. Whereas, the nine studies investigating caffeine's impact on performance over this duration where CHO has been provided either immediately prior to or throughout the trial (1, 10, 11, 16, 21, 22, 25, 37, 39) have produced more inconsistent findings (only 3 of 9 studies report ergogenic effects). Whilst the grouped interpretation of these studies must be made with caution due to differences in exercise protocols, caffeine doses, subject's training status and gender etc., and the direct influence of the CHO on performance, the interaction between CHO availability and caffeine warrants further clarification. Additionally, the ergogenic potential of caffeine on endurance-exercise should be assessed by emphasising those studies with the greatest "ecological" validity. That is, those providing CHO to subjects undertaking self-paced exercise protocols as CHO containing sports drinks are commonly consumed by athletes within competitive endurance events.

Paragraph Number 32 An alternative explanation for the lack of any metabolic change seen in the present study may relate to the differences observed in subjects' time-to-peak plasma caffeine rates. The concentration ranges of plasma caffeine 1h post ingestion of 1.5 and 3 mg·kg⁻¹ BW were 0-10.7 and 0-21.9 μmol·L⁻¹, respectively. Furthermore, we observed a consistent response from our subjects regarding their plasma caffeine appearance profiles. That is, similar time-to-peak rates were seen with our slowest (i.e., 180min post-ingestion) and fastest (i.e., 60min

post-ingestion) responders to caffeine irrespective of the dose, indicating some inter-individual differences in response to the caffeine ingestion. The differences in time-to-peak rates did not appear to correspond with the differences in habitual caffeine intake reported by our subjects. Caffeine pharmacokinetics both at rest and during exercise have been established for some years (2, 3, 7, 30, 32). These studies typically describe the rapid appearance of caffeine over the first hour following ingestion and a 4.5-6.5h average plasma half-life in men. However, many of these studies have employed relatively small numbers of subjects ([32] (n=6), [3] (n=4), [2] (n=10), [7] (n=12)) and several note considerable individual differences in time to reach peak caffeine values (2, 7, 32). With the observed differences in plasma caffeine concentrations throughout SS in the current study we cannot exclude the possibility of some subjects experiencing a greater exposure to caffeine-related effects compared to other subjects, or alternatively an over-exposure to these effects during the 2h SS period which may have potentially influenced subsequent performance. Furthermore, we can only speculate on an individual's sensitivity (or not) to the caffeine present within their circulation. Fortunately, the performance task in the current study was performed 3h post caffeine ingestion, when individual differences between plasma caffeine levels were smallest, suggesting that any direct impact of caffeine concentration on performance was well controlled in this study. The caffeine time-to-peak data does, however, demonstrate important differences between individual participants in the present study and the importance of measuring individual plasma caffeine values particularly when caffeine is provided ≤ 1 h prior to short duration performance tasks.

Paragraph Number 33 In summary, this study investigated the effect of two caffeine doses on endurance-exercise metabolism and performance in trained male cyclists. No significant effects were observed on substrate utilisation or performance following either the 1.5 or 3 mg·kg⁻¹ BW caffeine dose. Possible explanations for an absence of any ergogenic benefit may relate to the magnitude of the performance change being below that of our performance measures' sensitivity, a blunting of caffeine's ergogenic potential when exogenous CHO availability is high and/or possible differences in exposure to caffeine as a result of individual variability in caffeine appearance rates.

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Figure Titles

Figure 1 *Experimental Protocol.* † see METHOD for volume provided.

Figure 2 *Plasma caffeine concentration prior to and throughout SS exercise following ingestion of 0, 1.5 and 3 mg·kg⁻¹ BW of caffeine. Values are mean±SD (n=9).*

*significant difference between 0 and 1.5 and 3 mg·kg⁻¹ BW caffeine doses †significant difference between caffeine 1.5 and 3 mg·kg⁻¹ BW caffeine doses.

Figure 3 *Substrate utilisation throughout SS exercise following ingestion of 0, 1.5 and 3 mg·kg⁻¹ BW of caffeine. Values are mean±SD (n=9).*

No statistical differences observed.

Figure 4 *Exogenous glucose oxidation rate throughout SS exercise following ingestion of 0, 1.5 and 3 mg·kg⁻¹ BW of caffeine. Values are mean±SD (n=8).*

No statistical differences observed.

Figure 5 *Individual Subject TT data.*