

Caffeine increases exogenous carbohydrate oxidation during exercise

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Yeo, Sophie E., Roy L. P. G. Jentjens, Gareth A. Wallis, and Asker E. Jeukendrup. Caffeine increases exogenous carbohydrate oxidation during exercise. *J Appl Physiol* 99: 844–850, 2005. First published April 14, 2005; doi:10.1152/jappphysiol.00170.2005.—Both carbohydrate (CHO) and caffeine have been used as ergogenic aids during exercise. It has been suggested that caffeine increases intestinal glucose absorption, but there are also suggestions that it may decrease muscle glucose uptake. The purpose of the study was to investigate the effect of caffeine on exogenous CHO oxidation. In a randomized crossover design, eight male cyclists (age 27 ± 2 yr, body mass 71.2 ± 2.3 kg, maximal oxygen uptake 65.7 ± 2.2 ml·kg⁻¹·min⁻¹) exercised at $64 \pm 3\%$ of maximal oxygen uptake for 120 min on three occasions. During exercise subjects ingested either a 5.8% glucose solution (Glu; 48 g/h), glucose with caffeine (Glu+Caf, 48 g/h + 5 mg·kg⁻¹·h⁻¹), or plain water (Wat). The glucose solution contained trace amounts of [U-¹³C]glucose so that exogenous CHO oxidation could be calculated. CHO and fat oxidation were measured by indirect calorimetry, and ¹³C appearance in the expired gases was measured by continuous-flow IRMS. Average exogenous CHO oxidation over the 90- to 120-min period was 26% higher ($P < 0.05$) in Glu+Caf (0.72 ± 0.04 g/min) compared with Glu (0.57 ± 0.04 g/min). Total CHO oxidation rates were higher ($P < 0.05$) in the CHO ingestion trials compared with Wat, but they were highest when Glu+Caf was ingested (1.21 ± 0.37 , 1.84 ± 0.14 , and 2.47 ± 0.23 g/min for Wat, Glu, and Glu+Caf, respectively; $P < 0.05$). There was also a trend ($P = 0.082$) toward an increased endogenous CHO oxidation with Glu+Caf (1.81 ± 0.22 g/min vs. 1.27 ± 0.13 g/min for Glu and 1.12 ± 0.37 g/min for Wat). In conclusion, compared with glucose alone, 5 mg·kg⁻¹·h⁻¹ of caffeine coingested with glucose increases exogenous CHO oxidation, possibly as a result of an enhanced intestinal absorption.

ergogenic aid; exogenous carbohydrate oxidation; endurance

IT IS WELL ESTABLISHED THAT carbohydrate (CHO) feeding during prolonged exercise can postpone fatigue and enhance endurance capacity (4, 5, 12, 28). The benefits of ingesting CHO during exercise have been attributed to the maintenance of plasma glucose concentrations and high rates of CHO oxidation late in exercise when muscle and liver glycogen levels are low (2, 5, 6, 13, 43). Ingested CHO can be oxidized at rates of up to 1.0 g/min during prolonged exercise (20, 30). It has been suggested that absorption of glucose in the intestine (21, 26, 46), or output by the liver, regulates glucose appearance into the circulation (31, 32), and they are therefore rate-limiting factors for exogenous CHO oxidation. There is emerging indirect evidence that the sodium-dependent glucose transporter (SGLT1), which is responsible for the intestinal transport of glucose, becomes saturated at high glucose ingestion rates (>1.2 g/min) (26, 27, 30). This would prevent further

absorption and thus limit CHO availability to the circulation (25).

Caffeine doses of 3–9 mg/kg have been shown to improve exercise time to exhaustion (9, 10, 17, 40, 51), although there is still considerable debate about the exact mechanism by which caffeine exerts its effect. The performance effects are typically observed when caffeine is ingested 1 h preexercise, but they are also evident when it is ingested during prolonged exercise. Kovacs et al. (37) identified improvements in time trial cycling performance when moderate amounts of caffeine (2.1 and 4.5 mg/kg) were ingested in combination with a 7% CHO solution during exercise. Both 2.1 and 4.5 mg/kg of caffeine added to a CHO solution resulted in faster performance times than water placebo or CHO alone.

Interestingly, a recent study in which liquid chromatography with fluorescence labeling was applied to determine intestinal permeability and glucose absorption, caffeine significantly increased intestinal glucose absorption (55). In that study, consumption of small amounts of caffeine and glucose (1.4 mg/kg and 0.5 g/min, respectively) during 90 min cycling at 70% of maximum power output (\dot{W}_{\max}) produced 23% greater intestinal glucose absorption compared with glucose or water consumption. Because it has previously been suggested that intestinal absorption is one of the main limiting factor for exogenous CHO oxidation (26, 27), we speculated that the ingestion of caffeine could increase the availability of ingested CHO and increase exogenous CHO oxidation during prolonged exercise.

Oral and intravenous caffeine administration with glucose ingestion has been shown to induce a rise in blood glucose concentrations (3, 44) and reduce glucose uptake into tissues (36, 54). Although muscle glucose uptake is not thought to be an important rate-limiting factor for exogenous CHO oxidation (19, 30), these effects of caffeine on glucose disposal have the potential to reduce exogenous CHO oxidation.

Therefore, the purpose of the present study was to investigate the effect of combined ingestion of glucose and caffeine during 2 h of cycling exercise on exogenous CHO oxidation rates. We hypothesized that caffeine, when coingested with glucose, will result in higher exogenous CHO oxidation rates compared with the ingestion of similar amounts of glucose only.

METHODS

Subjects. Eight trained male cyclists, aged 27 ± 2 yr with a body mass of 71.2 ± 2.3 kg, a maximal oxygen consumption ($\dot{V}O_{2\max}$) of 65.7 ± 2.2 ml·kg⁻¹·min⁻¹, and a maximal power output (\dot{W}_{\max}) of 356 ± 18 W took part in this study. Inclusion criteria required that subjects trained at least three times a week for >2 h/day and had been

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involved in endurance-based training for at least 2–3 yr. The experimental procedures and possible risks associated with the study were fully explained to each individual, and to support this they were required to sign an informed consent form before participation and complete and sign a general health questionnaire. The study was approved by the Ethics Committee of the School of Sport and Exercise Sciences of the University of Birmingham, United Kingdom.

Preliminary testing. To establish each subject's $\dot{V}_{\text{O}_{2\text{max}}}$ and \dot{W}_{max} , an incremental cycle exercise test to volitional exhaustion was performed at least 1 wk before the start of the first experimental trial. This test was performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), modified to the configuration of a racing bicycle with adjustable saddle height and handlebar position. After the subjects reported to the laboratory, body mass (Seca Alpha, Hamburg, Germany) and height were recorded. Subjects then began cycling at 95 W for 3 min, followed by incremental steps of 35 W every 3 min until volitional exhaustion. Heart rate (HR) was recorded continuously by a radiotelemetry HR monitor (Polar Vantage, Kempele, Finland). \dot{W}_{max} was calculated from the last completed work rate, plus the fraction of time spent in the final noncompleted work rate, multiplied by the work rate increment. The results were used to determine the work rate corresponding to 55% \dot{W}_{max} , which was later employed in the experimental exercise trials. Breath-by-breath measurements were performed throughout exercise by using an online automated gas-analysis system (Oxycon Pro, Jaeger, Wuerzburg, Germany). The volume sensor was calibrated by using a 3-liter calibration syringe, and the gas analyzers were calibrated by using a 5.03% CO_2 -94.97% N_2 gas mixture. Oxygen consumption (\dot{V}_{O_2}) was considered to be maximal ($\dot{V}_{\text{O}_{2\text{max}}}$) when at least two of the three following criteria were met: 1) a leveling off of \dot{V}_{O_2} with increasing workload (increase of no more than 2 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), 2) a HR within 10 beats/min of predicted maximum (HR 220 minus age), and 3) a respiratory exchange ratio (RER) >1.05 . $\dot{V}_{\text{O}_{2\text{max}}}$ was calculated as the average \dot{V}_{O_2} over the last 60 s of the test.

Experimental design. Each subject completed three exercise trials, each consisting of 120 min of cycling at 55% \dot{W}_{max} . Throughout the trials, subjects received either a glucose drink (Glu), a glucose and caffeine drink (Glu+Caf), or plain water (Wat). The glucose drink had a natural high ^{13}C abundance [-10.8 , ‰ vs. Pee Dee Belemnite (PDB)] and to increase the ^{13}C enrichment of the glucose solutions even further, a trace amount of uniformly labeled [^{13}C]glucose was added (~ 0.04 g [^{13}C]glucose/l) (Cambridge Isotope Laboratories, Cambridge, MA). We had to make up a new batch of [^{13}C]glucose solution for the last few trials. The ^{13}C enrichment of the first batch was 24.67 ‰ vs. PDB, but for five trials we used a solution with a ^{13}C enrichment of 36.61 ‰ vs. PDB. As a result, there was a small difference in the enrichments of the drinks between the Glu and Glu+Caf trials (29.15 ± 2.18 and 26.17 ± 1.49 , ‰ vs. PDB respectively). The ^{13}C enrichment was determined by elemental analyzer-isotope ratio mass spectrometry (model GEO 20-20, Europa Scientific, Crewe, UK). The order of the experimental drinks was randomly assigned in a crossover design. Experimental trials were separated by 1 wk.

Diet and activity before testing. Subjects were asked to keep a record of their food intake and activity patterns on the day before the first trial and to replicate the same diet and exercise activities 24 h before trials 2 and 3. They were also instructed to maintain their usual caffeine intake, refrain from any strenuous exercise, and avoid alcohol intake 24 h before the start of each experimental procedure. In addition, 5–7 days before each experimental trial subjects were asked to perform “an exhaustive exercise bout” to reduce ^{13}C -enriched glycogen stores to a minimum. They were further instructed not to consume any food products with a high natural abundance of ^{13}C (CHO derived from C4 plants such as maize and sugar cane) during the week preceding each exercise trial. This ensured minimal back-

ground shift (change in $^{13}\text{CO}_2$ enrichment) from endogenous substrate stores.

Protocol. Subjects arrived at the Human Performance Laboratory in the morning (7:00–9:00 AM) after an overnight fast (10–12 h). The start time allocated to each subject remained consistent for all trials to avoid any influence of circadian variance. On arrival in the laboratory, subjects were asked to empty their bladder before body mass was recorded. After this, a flexible 20-gauge Teflon catheter (Venflon, BD, Plymouth, UK) was inserted into an antecubital vein and attached to a three-way stopcock (Sims Portex, Kingsmead, UK) to allow for repeated blood sampling during exercise. The catheter was kept patent by flushing with 1.0–1.5 ml of isotonic saline (0.9%, Baxter Healthcare, Norfolk, UK) after each sample collection. The cycle ergometer was adjusted to a comfortable position as specified by the subject, and once mounted, participants were asked to remain stationary on the ergometer for 2–3 min. Resting breath samples were collected in Extainer tubes (Labco Brow Works, High Wycombe, UK), which were filled directly from a mixing chamber in duplicate to determine the $^{13}\text{CO}_2$ -to- $^{12}\text{CO}_2$ ratio ($^{13}\text{CO}_2/^{12}\text{CO}_2$) in the expired gases. In addition, a resting blood sample (10 ml) was taken and stored on ice until centrifugation.

Subjects then began cycling and maintained a work rate equivalent to 55% \dot{W}_{max} (199.5 ± 8.7 W) for 120 min. Expiratory breath samples were collected and blood samples drawn at 15-min intervals during the trial until termination of exercise. \dot{V}_{O_2} , carbon dioxide production (\dot{V}_{CO_2}), and RER were measured every 15 min for periods of 4 min.

Before the start of exercise, but after baseline gas and blood sample collections, subjects drank an initial bolus of one of three experimental drinks: Glu, Glu+Caf, or Wat. After the subject exercised for 15 min, and at 15-min intervals thereafter, a beverage volume of 150 ml was provided. The amount of glucose and fluid provided during the 120 min exercise bout was 96 g (equal to an average intake of 0.8 g of glucose/min) and 1.65 liters, respectively. The total amount of caffeine in the Glu+Caf trial was 5 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (~ 353 mg/h). All exercise trials were performed under normal and standard environmental conditions (19–22°C dry-bulb temperature and 50–60% relative humidity). Standing floor fans were available to the subjects to minimize thermal stress.

Additional measures. Subjects were asked to rate their perceived exertion (RPE) for whole body and legs every 30 min on a scale from 6 to 20 using the Borg category scale (2). In addition, subjects were asked to complete a questionnaire (17 items) containing questions regarding the occurrence of gastrointestinal (GI) symptoms during the trial (15). While continuing to exercise, subjects were asked to read through a list of questions and point to or verbally indicate a number within a numerical rating scale from 1 to 10 (1 = not at all; 10 = very, very much), corresponding to severity of the GI complaint. Symptoms addressed included stomach problems, GI cramping, bloated feeling, diarrhea, nausea, dizziness, headache, belching, vomiting, and urge to urinate and defecate. The severities of the GI symptoms were divided into two categories: severe and nonsevere symptoms (34). Severe complaints included nausea, stomach problems, bloated feeling, diarrhea, urge to vomit, and stomach and intestinal cramps because these are symptoms that might impair performance and may bring their health risks. The above symptoms were only registered as severe when a score ≥ 5 out of 10 was reported. When a score < 5 was indicated, they were registered as nonsevere. All other symptoms were reported as nonsevere regardless of score reported.

Analysis. Blood samples were collected into EDTA-containing tubes, stored on ice, and centrifuged at 2,300 g and 4°C for 10 min. Aliquots of plasma were immediately frozen in liquid nitrogen and stored at -25°C until analyses for selected metabolites. Glucose (Glucose HK, ABX Diagnostics, Montpellier, France), lactate (Lactic Acid, ABX Diagnostics), free fatty acids (FFA) (NEFA-C Kit, Wako, Neuss, Germany), and glycerol (Scil Diagnostics) concentrations were analyzed enzymatically on a semiautomatic analyzer (Cobas Mira S-Plus, ABX). Breath samples were analyzed for $^{13}\text{CO}_2/^{12}\text{CO}_2$ by gas

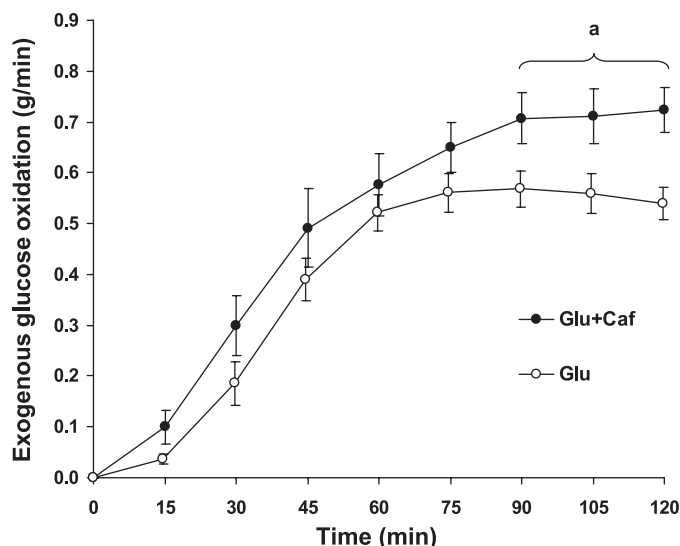


Fig. 1. Exogenous carbohydrate oxidation during exercise with ingestion of glucose (Glu) or glucose+caffeine (Glu+Caf). Values are means \pm SE; $n = 8$ men. ^a Significant difference between Glu and Glu+Caf, $P < 0.05$.

chromatography continuous-flow isotope ratio mass spectrometry (Europa Scientific). From indirect calorimetry ($\dot{V}O_2$ and $\dot{V}CO_2$) and stable isotope measurements (breath $^{13}CO_2/^{12}CO_2$), oxidation rates of total fat, total CHO, and exogenous glucose and endogenous CHO oxidation were calculated.

Calculations. From $\dot{V}CO_2$ and $\dot{V}O_2$ (l/min), total CHO and fat oxidation rates (g/min) were calculated by using the stoichiometric equations of Jeukendrup and Wallis (29), with the assumption that protein oxidation during exercise was negligible

$$\text{CHO oxidation} = 4.210\dot{V}CO_2 - 2.962\dot{V}O_2 \quad (1)$$

$$\text{Fat oxidation} = 1.695\dot{V}O_2 - 1.701\dot{V}CO_2 \quad (2)$$

The isotopic enrichment was expressed as $\delta\text{‰}$ difference between the $^{13}C/^{12}C$ of sample and a known laboratory reference standard, according to the formula of Craig (14)

$$\delta^{13}C = \left[\left(\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard}} \right) - 1 \right] \cdot 10^3 \quad (3)$$

The $\delta^{13}C$ was then related to an international standard (PDB). In the CHO trials, the rate of endogenous CHO oxidation was calculated by using the following formula:

$$\text{Exogenous CHO oxidation} = \dot{V}CO_2 \cdot \left(\frac{\delta \text{Exp} - \delta \text{Exp}_{\text{bkg}}}{\delta \text{Ing} - \delta \text{Exp}_{\text{bkg}}} \right) \left(\frac{1}{k} \right) \quad (4)$$

where δExp is the ^{13}C enrichment of expired air during exercise at different time points, $\delta \text{Ing}_{\text{bkg}}$ is the ^{13}C enrichment of the ingested CHO solution, $\delta \text{Exp}_{\text{bkg}}$ is the ^{13}C enrichment of expired air in the

Wat trial (background) at different time points, and k is the amount of CO_2 (in liters) produced by the oxidation of 1 g glucose ($k = 0.7467$ liter of CO_2 derived from the oxidation of 1 g of glucose). Endogenous CHO oxidation was calculated by subtracting exogenous CHO oxidation from total CHO oxidation.

A methodological consideration when using $^{13}CO_2$ in expired air to calculate exogenous substrate oxidation is the temporary trapping of $^{13}CO_2$ in the bicarbonate pool, in which an amount of CO_2 arising from CHO and fat oxidation is retained. However, during exercise, the turnover of this pool increases severalfold, so a physiological steady-state condition will occur relatively quickly and $^{13}CO_2$ in the expired air will be equilibrated with the $H^{13}CO_3$ pool. Recovery of $^{13}CO_2$ from oxidative processes will approach 100% after 60 min of exercise when dilution in the bicarbonate pool becomes negligible (39, 49). All calculations on substrate oxidation were performed over the last 30 min of exercise (90–120 min).

Statistical analysis. Differences in substrate utilization and blood-related parameters between trials and over time were compared using two-way repeated-measures ANOVA. A Tukey post hoc test was applied in the event of a significant F ratio to locate the differences. Data evaluation was performed by using the SPSS for Windows Version 10.0 software package (Chicago, IL). All data are reported as means \pm SE. Statistical significance was set at $P < 0.05$.

RESULTS

Stable-isotope measurements. The mean $^{13}CO_2$ enrichment of the resting breath samples was $-26.86 \pm 0.4 \text{‰}$ vs. PDB. In the Glu and Glu+Caf trials, there was a significant increase ($P < 0.01$) in the $^{13}CO_2$ enrichment of expired breath, reaching an enrichment difference of 8‰ vs. PDB toward the end of the 120-min exercise period, compared with the corresponding water trial. $^{13}CO_2$ enrichment in Glu was not significantly different from that in Glu+Caf. During the Wat trial, there was a small but significant increase in $^{13}CO_2$ enrichment of the expired gases ($P < 0.01$). A background correction was made for the calculation of exogenous CHO oxidation in Glu and Glu+Caf using data from Wat.

Exogenous and endogenous CHO oxidation. In the Glu trial, exogenous CHO oxidation rates gradually increased during the first 75 min of exercise and then remained relatively constant until termination of the exercise bout (120 min; Fig. 1). In the Glu+Caf trial, exogenous CHO oxidation rates increased steadily during the first 90 min of exercise and leveled off during the final 30 min of exercise (Fig. 1). During the final 30 min of exercise, exogenous CHO oxidation rates were $\sim 26\%$ higher ($P < 0.05$) in Glu+Caf (0.72 ± 0.04 g/min) compared with Glu (0.57 ± 0.04 g/min; Table 1, Fig. 1). In Glu+Caf, peak exogenous CHO oxidation rates (0.72 ± 0.05 g/min) were reached at the end of exercise (120 min) and were significantly higher ($P < 0.05$) than peak exogenous CHO rates in Glu (0.57 ± 0.04 g/min). There were no significant differences in

Table 1. Gas-exchange data and substrate oxidation during exercise (90–120 min)

	$\dot{V}O_2$, l/min	$\dot{V}CO_2$, l/min	RER	Total CHO, g/min	Total Fat, g/min	Endogenous CHO, g/min	Exogenous CHO, g/min
Wat	3.32 ± 0.10	2.63 ± 0.13	0.79 ± 0.03	1.12 ± 0.37	1.13 ± 0.17	1.12 ± 0.37	
Glu	3.34 ± 0.13	2.79 ± 0.10	$0.84 \pm 0.01^*$	$1.84 \pm 0.14^*$	$0.93 \pm 0.11^*$	1.27 ± 0.13	0.57 ± 0.04
Glu + Caf	3.29 ± 0.13	2.90 ± 0.11	$0.88 \pm 0.02^{*\dagger}$	$2.47 \pm 0.23^{*\dagger}$	$0.65 \pm 0.11^{*\dagger}$	1.81 ± 0.22	$0.72 \pm 0.04^\dagger$

Values are means \pm SE; $n = 8$ men. Data presented are total carbohydrate (CHO), total fat, endogenous carbohydrate, and exogenous CHO oxidation during exercise with ingestion of water (Wat), glucose (Glu), or glucose plus caffeine (Glu + Caf). $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production. *Significant difference between Wat and CHO trials, $P < 0.05$. † Significant difference between Glu and Glu+Caf, $P < 0.05$.

endogenous CHO oxidation rates among trials. However, over the final 30 min of exercise, there was a trend ($P = 0.082$) for increased endogenous CHO oxidation with Glu+Caf (1.81 ± 0.22 g/min) compared with Glu and Wat, 1.27 ± 0.13 g/min and 1.12 ± 0.37 g/min respectively (Table 1).

$\dot{V}O_2$, RER, and total CHO and fat oxidation. Data for $\dot{V}O_2$, RER, and total CHO and fat oxidation over the 90- to 120-min exercise period are shown in Table 1. No significant differences were observed in $\dot{V}O_2$ among the three experimental trials. RER was significantly lower in Wat compared with the two CHO trials ($P < 0.01$), and RER was highest in Glu+Caf ($P < 0.01$). Total CHO oxidation was significantly higher after CHO ingestion compared with Wat ingestion ($P < 0.01$). Furthermore, over the final 30 min of exercise, total CHO oxidation was significantly higher with Glu+Caf ingestion compared with Glu alone (2.47 ± 0.23 and 1.84 ± 0.14 g/min, respectively; $P < 0.05$). Fat oxidation was significantly different between the three exercise trials during the final 30 min of exercise ($P < 0.05$). Fat oxidation was markedly suppressed in Glu+Caf (0.65 ± 0.11 g/min) compared with Glu (0.93 ± 0.11 g/min) and Wat (1.13 ± 0.17 g/min). The contribution of substrates to total energy expenditure during the 90- to 120-min period is depicted in Fig. 2. The relative contribution of fat oxidation decreased from 68% in Wat to 53% in Glu and 38% in Glu+Caf ($P < 0.05$). The relative contribution of endogenous CHO oxidation to total substrate utilization increased from 32% in Wat to 34% and 45% in Glu and Glu+Caf, respectively, although this did not reach statistical significance. The contribution of exogenous CHO oxidation to total substrate utilization increased from 14% in Glu to 16% in Glu+Caf ($P < 0.05$).

Plasma metabolites. Plasma glucose, lactate, glycerol, and FFA concentrations at rest and during exercise are shown in Fig. 3. Resting concentrations of plasma glucose were not different among trials (on average, 4.3 ± 0.6 mmol/l; Fig. 3A). There was a significant effect of time in concentration of

plasma glucose in all three trials ($P < 0.01$). Plasma glucose concentrations during Wat gradually decreased from 4.2 ± 0.6 mmol/l at the start of exercise to 3.4 ± 0.8 mmol/l at cessation of exercise. In the CHO trials, plasma glucose concentrations increased to peak values around 5.6 ± 1.4 mmol/l during the first 30–60 min of exercise, and then they gradually dropped to reach values of 4.4 ± 0.7 mmol/l after 120 min of exercise. Throughout exercise, plasma glucose concentrations were highest in the CHO trials (average 4.83 ± 0.84 mmol/l) but not significantly different from the Wat trial (4.0 ± 0.6 mmol/l). There was no significant difference in glucose concentrations between Glu and Glu+Caf (4.18 ± 0.27 and 4.78 ± 0.25 mmol/l respectively).

Fasting plasma lactate concentrations were similar in all three trials (on average 1.4 ± 0.5 mmol/l; Fig. 3B). At all time points during exercise, plasma lactate concentrations were significantly higher ($P < 0.01$) compared with lactate concentrations at rest. Throughout exercise, plasma lactate concentrations of Glu were not significantly different from those of Wat (average 2.0 ± 0.9 mmol/l). In these trials, plasma lactate concentrations increased during the first 30 min of exercise and thereafter remained at similar concentrations for the duration of exercise. Plasma lactate concentrations in Glu+Caf gradually increased from resting concentrations to reach values twofold higher at the cessation of exercise (2.8 ± 1.5 mmol/l). During the last 45 min of exercise, plasma lactate concentrations were significantly higher ($P < 0.05$) in Glu+Caf compared with Glu and Wat.

Fasting plasma glycerol concentrations were not different among trials (on average, 84 ± 16 μ mol/l; Fig. 3C). Plasma glycerol concentrations gradually increased from resting concentrations until the end of exercise in all three trials ($P < 0.01$). During the last 60 min of exercise, plasma glycerol concentrations were significantly lower in the CHO trials than in the Wat trial ($P < 0.05$). No significant differences were observed in plasma glycerol concentrations between Glu and Glu+Caf.

Fasting plasma FFA concentrations (Fig. 3D) were not significantly different in the three trials (on average, 286 ± 41 μ mol/l). In all exercise trials, plasma FFA concentrations decreased within the first 30 min of exercise ($P < 0.01$) before gradually increasing to reach peak values at the end of the 120-min exercise bout. Plasma FFA concentrations were significantly higher in Wat compared with the CHO trials at time points 60, 75, 90, and 105 min ($P < 0.01$). There was no difference in plasma FFA concentrations between Glu and Glu+Caf.

GI discomfort and RPE. The most frequently reported complaints were bloated feeling, belching, and urge to urinate. There were no reports of severe GI discomfort during any of the trials. No differences in GI discomfort were observed between trials. No significant differences in RPE overall or RPE in legs were observed among the four experimental trials. The mean values for RPE "overall" and RPE "legs" during the 120-min exercise protocol were 12.8 ± 0.6 and 13.1 ± 0.6 , respectively.

DISCUSSION

Both CHO (4, 5, 11, 12, 28) and caffeine (9, 10, 17, 40, 51) ingested before and during exercise have been shown to delay the onset of fatigue and increase exercise capacity. It is gen-

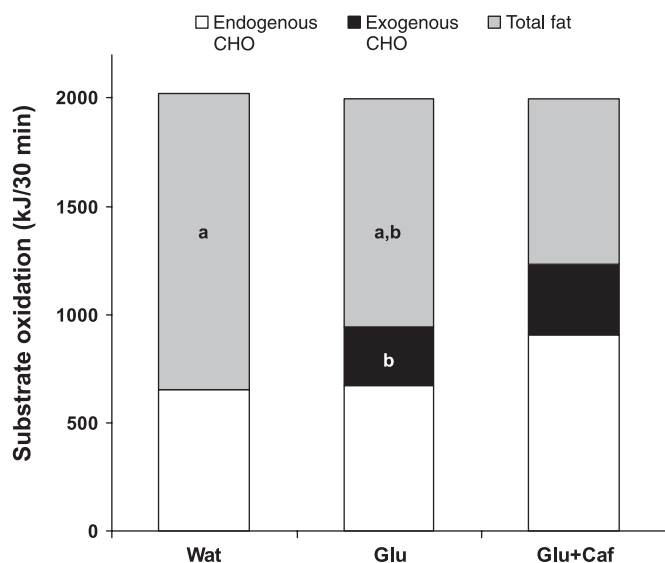


Fig. 2. Absolute contributions of substrates to total energy expenditure calculated for the 90- to 120-min period of exercise with ingestion of Wat, Glu, or Glu+Caf. Values are means \pm SE; $n = 8$ men. ^a Significant difference between Wat and CHO trials, $P < 0.05$. ^b Significant difference between Glu and Glu+Caf, $P < 0.05$.

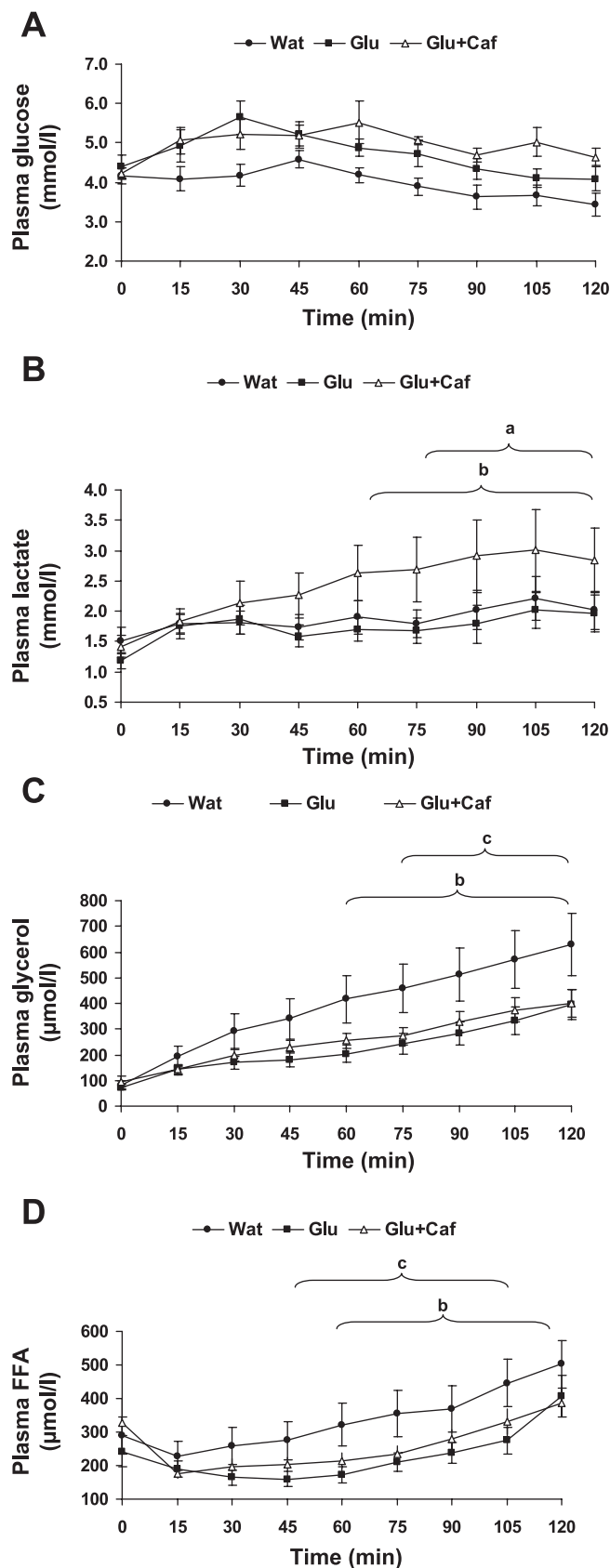


Fig. 3. Plasma glucose (A), lactate (B), glycerol (C), and free fatty acids (FFA; D) during exercise with ingestion of Wat, Glu, or Glu+Caf. Values are means \pm SE; $n = 8$ men. ^a Significantly different from Wat, $P < 0.05$. ^b Significantly different from Glu, $P < 0.05$. ^c Significantly different from Glu+Caf, $P < 0.05$.

erally believed that an increased contribution of exogenous CHO oxidation is beneficial because it reduces the reliance on endogenous CHO sources (30). It is thought that exogenous CHO oxidation rates are mainly limited by intestinal glucose absorption (21, 30). When ingested during exercise, moderate doses of caffeine (2.1 and 4.5 mg/kg) in combination with a 7% CHO solution significantly improved 1-h cycling time trial performance (60.4 ± 1.0 and 58.9 ± 1.0 min for 2.1 and 4.5 mg/kg caffeine, respectively) compared with the ingestion of water placebo (62.5 ± 1.3 min) or CHO alone (61.5 ± 1.1 min) (37). The exact mechanisms responsible for these ergogenic effects are unclear but may partly be explained by central effects. Interestingly, a recent study suggested that caffeine may also increase intestinal glucose absorption during exercise (55). We hypothesized that caffeine may contribute to a faster intestinal glucose absorption rate, thereby allowing higher rates of exogenous CHO oxidation during exercise. The main finding of the present study was that ingestion of caffeine ($5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) in combination with glucose (0.8 g/min) resulted in $\sim 26\%$ higher exogenous CHO oxidation rates during the final 30 min of exercise compared with the ingestion of glucose alone (Table 1, Fig. 1).

Exogenous CHO oxidation during exercise is potentially limited by gastric emptying, digestion and intestinal absorption, hepatic extraction and storage of glucose, muscle glucose uptake or a combination of these factors (30). Caffeine, at least in theory, could affect any of these steps. Van Nieuwenhoven et al. (55) measured the effects of caffeine on gastric emptying using the [^{13}C]acetate breath test. Caffeine coingested with glucose during exercise did not affect gastric emptying or orocecal transit time compared with CHO ingestion alone. In addition, it has been shown that when CHO is ingested, most is emptied from the stomach, and therefore gastric emptying does not appear to be a rate-limiting step for exogenous CHO oxidation (47, 50). In the same study, Van Nieuwenhoven et al. (55) demonstrated that consumption of small amounts of caffeine (1.4 mg/kg) and glucose (45 g) during 90 min of exercise at $70\% \dot{W}_{\text{max}}$ resulted in significantly higher intestinal glucose absorption rates compared with glucose alone (23% higher). The authors attributed their findings to a caffeine-induced increase in the glucose flux through SGLT1 into the enterocyte or GLUT2 transport from the enterocyte into the capillary. Direct evidence for a mechanism by which caffeine can increase intestinal glucose absorption is lacking. Caffeine inhibits the enzyme phosphodiesterase and therefore enables cAMP to remain active at the cell membrane for longer. cAMP has been shown to acutely increase glucose absorption via SGLT1 in the small intestine (52, 53), which could potentially lead to higher availability of CHO for oxidation. Therefore, it is possible that caffeine exerts its effects on exogenous CHO oxidation through modifying SGLT1 function and thus enhancing intestinal glucose absorption and delivery to the circulation. However, further investigation is required to fully understand how caffeine increases exogenous CHO oxidation. It is interesting to note that the magnitude of increase in exogenous CHO oxidation with caffeine in the present study (26%) is similar to the 23% increase in absorption reported by van Nieuwenhoven et al. (55).

Evidence from previous studies suggests that the mechanism by which caffeine increases exogenous CHO oxidation is unlikely to be located at the level of the liver. For example,

combined ingestion of caffeine and glucose has been shown to increase plasma insulin concentrations compared with glucose alone (16). This may result in increased glucose retention in the liver and therefore a decrease in the availability of blood glucose for exogenous CHO oxidation. Furthermore, it seems unlikely that the observed effects of caffeine on exogenous CHO oxidation are a result of increased muscle glucose uptake. In fact, caffeine has been shown to reduce muscle glucose uptake at rest (18, 36) and during muscle contraction (56). Even in the presence of elevated insulin concentrations, as often observed with caffeine ingestion, glucose uptake may be reduced (36, 42). The increase in exogenous CHO oxidation in the present study occurred despite suggestions that caffeine could reduce glucose uptake by the muscle. Although plasma glucose kinetics were not determined in the present study, from the evidence presented above it seems unlikely that caffeine increases the oxidation of ingested CHO by increase hepatic glucose output or muscle glucose uptake.

During the final 30 min of exercise, there was a ~35% increase in total CHO oxidation in Glu+Caf compared with Glu and this was partly an effect of increased exogenous CHO oxidation. There was also a trend for endogenous CHO oxidation to be higher with caffeine. Although endogenous CHO oxidation was not statistically significant, the higher plasma lactate concentrations found in the present study could reflect higher rates of muscle glycogen breakdown. The finding of increased plasma lactate concentrations with Glu+Caf compared with Glu and Wat is consistent with findings by others (17, 18, 23, 51). An increased rate of muscle glycogenolysis could be explained by the increase in circulating catecholamine concentrations with caffeine. Although epinephrine was not measured in the present study, a large number of studies have reported greater increases in plasma epinephrine concentrations during prolonged exercise with caffeine ingestion (7, 8, 17, 18, 51). Infusion of epinephrine has been shown to increase muscle glycogenolysis and lactate formation during exercise (15, 24, 48, 57), although the concentrations in those studies were much higher than the concentrations observed after caffeine ingestion.

In summary, our findings show that glucose, when co-ingested with caffeine during exercise, resulted in higher exogenous CHO oxidation than glucose ingestion alone. This effect is probably mediated by an increase in intestinal glucose absorption. It is unlikely that the observed increase in exogenous CHO oxidation is caused by an effect of caffeine on gastric emptying, liver glucose disposal, or muscle glucose uptake. Although further investigation is warranted, the findings of the present study suggest a novel mechanism by which caffeine may exert ergogenic properties during exercise.

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