EFFECT OF DIFFERENT PROTOCOLS OF CAFFEINE INTAKE ON
METABOLISM AND ENDURANCE PERFORMANCE

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ABSTRACT

We investigated the effect of different caffeine intake protocols on prolonged cycling in 2 separate studies using a similar experimental design, involving competitive athletes who undertook 4 x 2 h steady-state (SS) cycling bouts at 70% peak O₂ uptake followed by a 7 kJ/kg time-trial (TT) under conditions of ‘real-life’ practice: 2 h post-meal [2 g/kg carbohydrate (CHO)] and with intake of a 6% CHO drink during exercise. In Study A, 12 subjects received, in a Latin square design, either 6 mg/kg caffeine ingested 1 h pre-exercise (PRECAF), 6 x 1 mg/kg caffeine every 20 min throughout SS (DURCAF), 2 x 5 mL/kg Coca Cola™ consumed between 100-120 min SS and during TT (COKE), or placebo (PLACEBO). Compared to PLACEBO, improvements in TT were: PRECAF: 3.4% [0.2% to 6.5%, 95% C.I.], DURCAF: 3.1% [-0.1 to 6.5%] and COKE: 3.1% [-0.2 to 6.2%]. Post-exercise urinary [caffeine] were <12 ug/ml for all subjects. In Study B, 8 subjects received 3 x 5 mL/kg of 4 identically flavoured cola drinks during the last 40 min of SS and during TT: decaffeinated, 6% CHO (CONTROL); caffeinated, 6% CHO (CAFFEINE); decaffeinated, 11% CHO (extraCHO) and caffeinated, 11% CHO (COKE). Compared to CONTROL, COKE enhanced TT by 3.3% [0.8-5.9%], with all trials showing a 2.2% TT enhancement (0.5-3.8% ; P<0.05) due to the caffeine content and a non-significant 1% enhancement (-0.7-2.7%) due to the additional CHO intake. We conclude 1) intake of 6 mg/kg caffeine enhanced performance of a TT undertaken after 120 min of SS, independent of timing of intake and 2) replacing sports drink with Coca Cola™ during the latter stages of exercise was equally effective in enhancing endurance performance, primarily due to intake of small amounts of caffeine (~1.5 mg/kg).

Keywords: carbohydrate, ergogenic aids, cola drink, metabolism
Many studies have reported an enhancement of prolonged submaximal exercise following caffeine ingestion (2, 7, 9, 10, 14-16, 19, 22, 23, 27, 29). The mechanism(s) proposed to explain these benefits include an increased utilization of plasma free fatty acids (FFA) (27) and/or intramuscular triacylglycerol (IMTG)(10), which act to reduce the rate of muscle glycogenolysis (10, 27), as well as favourable changes in central nervous system cues (6) and/or neuromuscular function (21, 32).

In the majority of these studies (7, 9, 10, 14-16, 23, 27, 29), the ‘performance’ protocol used was a test of exercise ‘capacity’ (i.e. the time to exhaustion at a fixed submaximal power output). Furthermore in some of these studies (7, 9, 10, 19), subjects exercised after an overnight fast and usually with the intake of water (or no fluid) during the exercise task. Such conditions fail to simulate the nature of a sporting event and to represent the ideal nutritional preparation for endurance exercise (1). One recent study which simulated the conditions of real-life athletic competition reported enhancement of performance of a 1 h cycling time trial (TT) when caffeine was ingested in conjunction with a carbohydrate (CHO)-electrolyte drink (22). In that study caffeine was ingested prior to and during the TT, in contrast to the typical research protocols in which a single dose of caffeine is ingested 60 min prior to an exercise task (7, 9, 14-16, 23, 27, 29).

Caffeine intake during sporting events has become more popular and practical since the advent of specialized foods such as sports gels that contain both CHO and caffeine. We have also observed a widespread practice in endurance sports in which competitors drink de-fizzed Coca Cola™ during the latter stages of an event, in replacement of their earlier use of a CHO-electrolyte drink. Testimonials from athletes indicate that they believe that the intake of Coca Cola™ late in the event provides an ergogenic benefit due to the intake of caffeine (Martin
DT, unpublished observations), despite the caffeine concentration in cola drinks (~ 65 mg per typical 500 mL serve) being substantially less than the caffeine doses associated with ergogenic benefits in laboratory research protocols (4-9 mg/kg body mass [BM] ~280-630 mg).

We were interested to determine the effect of varying protocols of caffeine intake on metabolism and performance during prolonged cycling, undertaken in conjunction with the nutritional protocols recommended for endurance sport (viz. a pre-event CHO-rich breakfast, and the use of a sports drink to replace CHO and fluid throughout the event). We chose an exercise task involving a pre-load of steady-state cycling during which steady-state (SS) metabolic measurements could be made, followed by a TT of known and appropriate reliability to measure performance (20).

Our first intention was to determine the importance of the timing of intake of conventional doses of caffeine (6 mg/kg BM), comparing a protocol in which the caffeine was ingested 60 min prior to an endurance cycling task with a protocol in which this dose was consumed *throughout* the exercise task. We hypothesized that the different ingestion protocols would have diverse effects on substrate metabolism during submaximal exercise, but that both would enhance the performance of a subsequent TT. Furthermore we believed there would be minimal risk of producing a urinary caffeine concentration >12 ug/ml, which is the level at which the International Olympic Committee (IOC) deems a positive doping offence to have occurred (23). A secondary aim of this first study was to compare the effects of Coca Cola™ ingestion late in the exercise protocol against the intake of larger (conventional) caffeine doses. Originally we hypothesized that any benefits from Coca Cola™ intake would be largely mediated by a placebo effect, so that a Coca Cola™ treatment would not enhance
performance compared to the outcome of another placebo trial. However after data in our first study supported an ergogenic effect arising from the use of Coca Cola™, we undertook a second investigation to allow this treatment to be provided in a double-blind protocol, and to determine the separate effects of the caffeine content of Coca Cola™ and its higher CHO content (11% CHO compared to the 6% CHO content of the sports drink). We hypothesized that if we could replicate the ergogenic effects of Coca Cola™ treatment in a rigorous research design, either or both of these factors could be important in explaining any performance enhancement.

METHODS

Subjects and preliminary testing

Twelve highly-trained male cyclists or triathletes (age 27.1 ±1.3 y, mass 76.7 ±1.8 kg, peak $O_2$ uptake [VO$_{2\text{peak}}$] 66.4 ±1.3 ml/kg/min, values mean ±SEM) who were cycling >250 km/wk were recruited to participate in Study A. A separate group of 8 equally highly-trained male cyclists/triathletes with similar physiological characteristics and training history (age 27.8 ± 2.1 y, mass 69.4 ± 1.1 kg, VO$_{2\text{peak}}$ 71.2 ±2.2 ml/kg/min) were recruited for Study B. None of these subjects was a scholarship holder at the Australian Institute of Sport. Subjects’ background caffeine intake was investigated by questionnaire and found to vary between occasional intake during competitive events to habitual daily intake of ~150 mg/d. All subjects were fully informed of the nature and possible risks of the investigation before giving their written consent. The investigation was approved by the Human Research Ethics Committee of the Australian Institute of Sport.

On his first visit to the laboratory, each subject performed a maximal, incremental test to exhaustion on an electromagnetically braked cycle ergometer (Lode Instruments, Groningen,
The Netherlands). The maximal test protocol has been described in detail previously (18). During the maximal test, which typically lasted between 10-12 min, subjects inspired air through a two-way Hans Rudolf valve attached to a custom built automated Douglas bag gas analysis system (Australian Institute of Sport, ACT, Australia) for which calibration and operation details have been previously described (12). VO2peak was defined as the highest O2 uptake subjects attained during two consecutive 30 s sampling periods, while peak sustained power output (PPO) was calculated from the last completed work rate (W), plus the fraction of time spent in the final non-completed work rate (18). The results of this maximal test were used to establish a workrate that corresponded with ~70% of VO2peak (63% of PPO), which was to be used for all subsequent experimental trials.

**Overview of study designs**

In both Study A and Study B, each subject undertook 4 experimental trials with training and nutritional status being controlled prior to each trial. Subjects refrained from consuming caffeine-containing substances (coffee, chocolate and soft drinks) for 48 h prior to each experiment. Diet and exercise diaries were used to standardize food intake and training for each subject for the period lasting 24-48 h before each trial. During the 24 h period immediately prior to each trial subjects were instructed to refrain from all training, and were provided with a pre-packed standard diet with an energy content of 200 kJ/kg BM, composed of 63% carbohydrate (8 g/kg BM), 20% fat, and 17% protein. Food and exercise diaries were used to check compliance.

Each experimental trial consisted of 120 min of steady-state (SS) cycling at 70% immediately followed by a 7 kJ/kg time trial (TT). Each trial was undertaken under conditions designed to mimic recommended nutritional practices for endurance sport. Specifically, exercise
commenced 2 h after the intake of a standardized CHO-rich meal providing 2 g CHO/kg BM. In addition, subjects were provided with a commercial sports drink (6.3% CHO, 18 mmol/L sodium) to allow replacement of fluid (7 x 5 mL/kg over ~ 2.5 h) and CHO (total of 2.1 g/kg over ~ 2.5 h) during exercise.

The four experimental treatments undertaken in both Study A and Study B were provided according to a Latin Square design:

**Study A treatments**

1. 6 mg/kg caffeine intake, ingested 1 h before the cycling protocol (PRECAF);
2. 6 x 1 mg/kg caffeine intake, ingested every 20 min during the first 120 min of the cycling protocol (DURCAF);
3. Coca Cola™ ingested (2 x 5 mL/kg) during the latter stages of the cycling protocol to replace the standardized intake of sports drink (COKE)
4. Placebo capsule (PLACEBO).

In three of the trials (PRE-CAF, DUR-CAF and PLACEBO), subjects received a series of capsules in an identical procedure, with the knowledge that in two of the trials the capsules could contain caffeine. In the COKE trial, subjects received Coca Cola™ as a transparent treatment.

**Study B treatments**

The 3 x 5 ml/kg sports drinks provided at 80 and 100 min of SS and during the TT were replaced with a range of Coca-Cola™ beverages that were manipulated to provide:

1. Decaffeinated cola flavoured drink, 6% CHO (CONTROL)
2. Caffeinated (13 mg/100 mL) cola flavoured drink, 6% CHO (CAF)
3. Decaffeinated cola flavoured drink, 11% CHO (extraCHO)
4. Caffeinated (13 mg/100 mL) cola flavoured drink, 11% CHO (COKE)

At the end of each of the studies, subjects completed a questionnaire in which they were asked to identify the order of treatments received during the study and nominate which treatment and trial they perceived was associated with their best performance.

*Experimental protocol – Study A.*

On the morning of an experiment, subjects reported to the laboratory between 0700-0800 h after a 12-14 h overnight fast. After sitting quietly for 10-15 min, a Teflon Catheter (Terumo, 20G, Tokyo, Japan) was inserted into a vein in the antecubital fossa and a resting blood sample was taken. The catheter was subsequently flushed with 2-3 ml of 0.9% sterile saline to ensure patency of the vein. Subjects were then fed a standard breakfast (fruit juice, toasted bread and jam, and a Power Bar™), providing a CHO intake of 2 g/kg. This meal was consumed within 15 min and subjects then rested in the laboratory while post-prandial blood samples were taken at 60 and 90 min. In all trials except for COKE, subjects ingested opaque capsules containing either 6 mg/kg caffeine (PRECAF) or a placebo (Polycose™) [DURCAF and PLACEBO] immediately after the 60 min blood sample had been taken.

After resting for 115 min, subjects voided, were reweighed, then mounted the cycle ergometer. At 119 min, a blood sample was taken and in the 3 trials outlined above, subjects ingested another capsule containing either 1 mg/kg caffeine (DURCAF) or the placebo (PRECAF, PLACEBO). After 120 min, subjects began cycling at 100 W, increasing 50 W each min until a steady-state workload equal to 63% PPO (246 W) was achieved. A bottle containing 5 mL/kg of a 6.3% CHO- electrolyte (18 mmol/L sodium) drink was provided at the onset of exercise with instructions to be consumed within the next 20 min.
Respiratory gas was collected during 5-10 and 15-20 min of exercise, with a blood sample being collected at 20 min. Immediately after this sample had been taken, subjects were presented with a new bottle of CHO-electrolyte drink, and either a capsule containing 1 mg/kg caffeine (DURCAF), a placebo capsule (PRE-CAF, PLACEBO), or no capsule. This pattern of collection of respiratory gas and blood samples, and presentation of capsules and CHO-electrolyte drinks continued on a 20 min cycle until 100 min of SS. At this point in the COKE trial, the drink was changed to 5 mL/kg of defizzed Coca Cola™ (10.9 % CHO, 5 mmol/L sodium). A further cycle of data was collected from 115-120 min, and after exactly 120 min of SS cycling, subjects dismounted the ergometer to allow it to be adjusted into a pedaling rate-dependent (linear) mode. A linear factor was individualized so that at ~100 rev/min each subject would be cycling at a workload of 82.5% of PPO (~ 85% of VO2peak). This workload was chosen as it represents the maximum intensity that subjects can sustain for ~30 min after a 2 h pre-load (Hawley JA, unpublished observations).

After a 3 min rest subjects commenced a 7 kJ/kg TT that typically lasted ~30 min. During this ride they were provided with 5 mL/kg of CHO-electrolyte drink or defizzed Coca Cola™ depending on the nature of their trial. 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 standardized feedback to each subject. The only information available to subjects during a TT 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 respiratory or blood samples were collected during the TT. On completion of the TT subjects were towel-dried and weighed. A final blood
sample was taken 3 min after the completion of each TT. Subjects then provided a urine specimen of at least 80 mL, which was frozen for later determination of urinary [caffeine].

Throughout the SS ride subjective ratings of perceived exertion (RPE) were recorded following each gas collection using the modified Borg scale (3). Heart rate (HR) was recorded throughout all experimental trials using personal telemetry (Polar Accurex Plus, Polar Electro OY, Kempele, Finland).

**Blood sampling and analyses**

Twelve ml of blood were collected at each sampling time, of which 50 µl was immediately analyzed for blood glucose and lactate concentrations by a Blood and Oximetry Analyzer (ABL 725, Radiometer Medical A/S, Copenhagen, Denmark). A further 6 ml were placed in a tube containing fluoride heparin and centrifuged at 4,000 rpm for 5 min. The plasma was stored at –80°C and later analyzed for plasma caffeine and insulin concentration. Plasma insulin concentrations were determined by radioimmunoassay (Inestar, Stillwater, MN). A further 2 mL aliquot of blood was mixed in a tube containing lithium heparin and centrifuged at 4,000 rpm for 5 min. 500 µl of plasma were placed in a tube containing 500µl of ice-cold 3M perchloric acid, mixed vigorously on a vortex mixer and centrifuged for 5 min at 10,000 rpm. 800 µl of this supernatant were added to a tube containing 200 µl of 6 M potassium hydroxide (KOH), mixed and centrifuged. The resultant supernatant was analyzed for glycerol using an enzymatic spectrophotometric analysis (25). The remaining blood was added to an aliquot of preservative consisting of EGTA and reduced glutathione in normal saline, mixed gently and spun in a centrifuge. The plasma was later analyzed for free fatty acid concentration (FFA) using an enzymatic colorimetric method (Wako, NEFAC code 279-75409, Tokyo, Japan).
Plasma and Urinary caffeine analysis

The plasma and urinary caffeine analyses were undertaken using a HPLC technique according to the methods of Delbeke and De Backer et al. 1996 (8).

Reagents and Standards: Caffeine, theophylline and β-hydroxyethyltheophylline were purchased from Sigma (St Louis, MO). HPLC grade tetrahydrofuran and acetonitrile were from EM Science (Gibbstown, NJ) and aqueous HPLC solvent was prepared using water obtained from a Milli-Q water purification system from Millipore (Bedford, MA). Ammonia buffer (pH 9) was prepared by the addition of ammonia to a saturated ammonium chloride solution.

For the extraction of caffeine in urine, 1 mL of urine in a 10 mL screw capped plastic centrifuge tube was mixed with 100 mg of NaCl, 50 μL of the internal standard (β-hydroxyethyltheophylline 100 μg/mL), 100 μL of ammonium buffer and 5 mL of extraction solvent CHCl₃: MeOH (9:1, v/v). After vortexing for 2 min the samples were centrifuged at 4000 rpm for 5 min. The organic layer was isolated and passed through a Pasteur pipette containing anhydrous Na₂SO₄. The extract was evaporated to dryness under a stream of N₂ in a 40°C heating block. The residue was reconstituted in 150 μL HPLC eluant and 25 μL was injected onto the HPLC system. The concentration range for the standard curve was 2.5 to 20 μg/mL.

For the extraction of caffeine in plasma, 1 mL of plasma in a 10 mL screw capped plastic centrifuge tube was added 1 mL of 0.15 M Ba(OH)₂. The tube vortexed for 1 min, 1 mL of 5% zinc sulphate was added, and the tube was vortexed for a further 1 min. After protein
precipitation, the sample was centrifuged at 4000 rpm for 5 min. The upper layer was transferred to a Wassermann tube and 100 mg of NaCl, 50 μL of internal standard (β-hydroxyethyltheophylline, 100 μg/mL) and 100 μL of ammonia buffer was added. Extraction was performed with the addition of 5 mL CHCl₃: MeOH (9:1, v/v) by vortexing for 2 min followed by centrifugation at 4000 rpm for 5 min. The organic layer was isolated and passed through a Pasteur pipette containing anhydrous Na₂SO₄. The extract was evaporated to dryness under a stream of N₂ in a 40°C heating block. The residue was reconstituted in 150 μL HPLC eluant and 25 μL was injected onto the HPLC system. The concentration range for the standard curve was 2.5 to 20 μg/mL.

HPLC analysis of caffeine in urine and plasma samples was performed with a Waters Analytical HPLC system (Waters, Milford, MA). This consisted of a Waters Model 600E Powerline Quaternary Solvent Delivery System and a Waters WISP 717 Plus Autoinjector. The samples were separated at room temperature on an Adsorbosphere HS C₁₈ column, (5 μm, 4.6mm ID x 150mm, Alltech Associates, Deerfield, IL) with an Adsorbosphere C₁₈ guard column, (5 μm, 4.6mm ID x 7.5mm). The mobile phase for the separation was 10 mM KH₂PO₄: acetonitrile: tetrahydrofuran (94.5:3.5:1.25 v/v) and the flow rate was 1.0 mL/min. The peaks were determined using a Waters 484 Tunable Absorbance Detector at 273 nm. The data was processed with Waters Millenium 2010 Multisystem Software data analysis system. Peak heights were used for quantitation and the retention time for caffeine elution was 15.3 min and for the internal standard β-hydroxyethyltheophylline, 10.8 min.

Rates of fat and CHO oxidation

Instantaneous rates of whole-body carbohydrate and fat oxidation (g/min) were calculated from the respiratory gases collected during SS, using the non-protein RER values (24). Total
rates of substrate oxidation during the four 120 min of SS exercise bouts were estimated from the area under the CHO and fat vs. time curves for each subject.

**Experimental protocol – Study B**

Apart from the following minor differences, Study B was conducted using the same protocol as Study A:

1. Cola drinks were introduced at 80 min of SS instead of 100 min, following feedback from many of the subjects in Study A that they would not consume such large amounts of fluid in the last 20 min of a real race as asked to consume in our TT. Therefore, in Study B, they were offered 2 x 5 mL/kg of the cola-flavoured drinks at 80 min and 100 min of SS, and a further 5 mL/kg during TT with the understanding that they could chose to drink a comfortable or typical volume of this last drink. The volume that was freely consumed in the TT of the first trial was then provided in all subsequent trials. This design ensured that subjects would consume at least 10 mL/kg of cola-flavoured drink, as received in Study A, but allowed this dose to be consumed in a manner simulating the real life race behaviour of the subjects.

2. To suit subject availability, all trials were held in the evening. For the 48 h period to each trial subjects followed a standardised diet and exercise protocol, and caffeine withdrawal as in Study A.

3. Urinary and plasma caffeine measurements were not made.

**Statistical analyses**

The statistical analyses were undertaken using *Statistica* software for Windows (StatSoft, Inc. version 5.1, 1997, Tulsa, OK). Data from the four trials were compared using a two-factor (treatment and time) analysis of variance (ANOVA) with repeated measures. Newman-Keuls
post-hoc tests were conducted when ANOVA revealed a significant difference or interaction between treatments. Total carbohydrate and fat oxidation between trials were compared using a one-way ANOVA with Newman-Keuls post-hoc test. Analysis of TT performance data was by the mixed method methodology incorporating considerations of any effect due to the order of trials. This analysis was conducted using SAS versions 6.12 software package (SAS Institute, Carey, NC). Significant differences were accepted when $P<0.05$. All data are reported as mean ±SEM.

RESULTS

Study A

Pre-trial standardization

Examination of food and exercise diaries revealed that subjects complied with the pre-trial standardization. No exercise was undertaken during the 24 h period prior to each trial, and complete caffeine withdrawal was reported for 48 h before each trial. There were no differences between treatments for reported intakes of CHO and energy during the 24 h pre-trial: $8.0 \pm 0.1$ g/kg and $199 \pm 2$ kJ/kg (PRECAF), $7.9 \pm 0.1$ g/kg and $198 \pm 2$ kJ/kg (DURCAF), $8.0 \pm 0.1$ g/kg and $199 \pm 2$ kJ/kg (COKE) and $8.0 \pm 0.1$ g/kg and $199 \pm 2$ kJ/kg (PLACEBO), NS.

Drinks consumed during exercise

Subjects complied with the consumption of their drinks during each treatment. Mean total intake of CHO provided by drinks in PLACEBO, PRECAF and DURCAF was 151 g (~2.1 g/kg) with 187 g CHO (2.6 g/kg) being provided in the COKE treatment. Mean total intake of caffeine provided in the COKE treatment was 94 mg (1.3 mg/kg).
Plasma and urinary caffeine

Plasma caffeine profiles before and during exercise differed according to the treatment intervention (Fig. 1a). In both protocols involving caffeine intake of 6 mg/kg, plasma caffeine concentrations peaked ~2 h after the (first) dose. PRECAF trial resulted in higher plasma caffeine concentrations than DURCAF at all time points until 100 min of SS (P < 0.05). There were no differences in plasma caffeine concentrations after 120 min of SS or immediately after TT between these treatments. The COKE treatment produced a small rise in plasma caffeine concentrations, with peak values being observed immediately post-TT. The absence of caffeine in plasma samples at 60 min prior to exercise in all trials, in samples up to 100 min in the COKE trial, and in all samples in the PLACEBO trial confirms that the only caffeine consumed on the day of each trials was that provided to subjects according to the study protocol.

Urinary caffeine concentrations are shown in Fig. 1b. There was no difference between urinary caffeine concentrations in the PRECAF and DURCAF trials (NS), but urinary caffeine was significantly greater in these trials than in the COKE and PLACEBO treatments (P < 0.05). All subjects recorded urinary caffeine concentrations that were well below the IOC limit of 12 µg/mL.

Plasma metabolites

Figure 2 summarizes concentrations of blood glucose (2a), blood lactate (2b) and plasma insulin (2c) during the 2 h prior to, and throughout exercise for each trial. Consumption of the CHO-rich meal 2 h before exercise caused a rise in blood glucose concentrations (as evidenced by the increase in plasma insulin concentrations), but euglycemia was restored by the time of the next blood sampling after 60 min. There was a main effect of time (P < 0.05)
on blood glucose concentrations with values rising after the commencement of exercise and intake of the CHO-electrolyte drink and remaining elevated throughout exercise. Dietary treatment also produced a main effect on blood glucose, with the hierarchy being with PRECAF > DURCAF, COKE > PLACEBO (P < 0.05). The interaction of treatment and time produced several time points at which differences in blood glucose concentrations were observed (Fig. 2a). Intake of caffeine in the hour before exercise caused an increase in blood glucose at the onset of exercise in PRECAF compared to the other treatments (P < 0.05). At the end of the TT, blood glucose was highest in the COKE trial, and significantly lower in the PLACEBO trial (P < 0.05).

Blood lactate concentrations increased above fasting values in response to the pre-event CHO meal (P < 0.05), and remained constant throughout SS before rising significantly during the TT (P < 0.05) [Fig. 2b]. There was a main effect of treatment, with blood lactate concentrations being higher with the PRECAF and DURCAF trials than in the COKE and PLACEBO trials (P < 0.05). An interaction of time and treatment was also observed, with blood lactate concentrations at the completion of the TT being lower in the PLACEBO trial than in the other trials (P < 0.05). Plasma insulin concentrations increased in response to the pre-exercise meal, then gradually declined during exercise so that concentrations at 100 and 120 min were lower than pre-exercise values (P < 0.05 [Fig. 2c]. There were no differences in plasma insulin responses arising from the different treatments.

Plasma FFA and glycerol concentrations before and during exercise are summarized in Fig. 3. There was a treatment effect on plasma FFA responses with values being higher in the PRECAF trial compared to PLACEBO (P < 0.05) [Fig. 3a]. Plasma FFA concentrations increased gradually during exercise so that values at 100 and 120 min of SS were higher than
values at the onset of exercise (P < 0.05). Plasma glycerol concentrations also increased
during exercise and were higher at all time points after 100 min compared to pre-exercise (P
< 0.05) [Fig. 3b]. There was a time x treatment interaction for glycerol, with plasma glycerol
concentrations being higher at 120 min and post-TT in the PRECAF trial than other
treatments (P < 0.05).

*Ventilation (V<sub>E</sub>) and respiratory exchange data*

Ventilation (V<sub>E</sub>) increased over 120 min of SS (P < 0.05) and differed between treatments
with PRECAF, DUR CAF > COKE, PLACEBO (P < 0.05) [Fig.4]. There was a significant
interaction of time x treatment, with V<sub>E</sub> being greater at the onset of SS with the PRECAF
trial compared to the other treatments (P < 0.05). In the DURCAF trial, the commencement
of caffeine intake at the onset of exercise was associated with a larger increase in V<sub>E</sub>, so that
by 60 min of SS, V<sub>E</sub> was high in the two trials involving substantial caffeine intake than in
the COKE and PLACEBO trials (P < 0.05) [Fig 4].

There was a main effect of time for RER, with values falling from ~ 0.96 after 10 min of SS
to ~ 0.92 after 120 min (P < 0.05). However, changes in RER were not different between
treatments. Similarly, there were no differences in instantaneous rates of CHO and fat
oxidation at any time point, resulting from the different caffeine treatments (data not shown).
Total CHO oxidation over 120 min of SS did not differ according to treatment (448 ± 17 g,
442 ± 12 g, 438 ± 14 g and 442 ± 12 g for PRECAF, DURCAF, COKE and PLACEBO
respectively, NS). Total fat oxidation over this same period was also similar 42 ± 4 g, 42 ± 4
g, 44 ± 4 g and 42 ± 4 g (NS).
Perception of effort

The rating of perceived effort (RPE) increased over time during SS, from ~11 at 10 min to ~13 at 120 min (P < 0.05) [Fig. 5]. There was a main effect of treatment, with RPE being lower during the PRECAF trial than the PLACEBO trial (P = 0.05). The interaction of time x treatment showed differences between RPE with various treatments from 80 min onwards (see Fig. 5). At 120 min, subjects reported a lower RPE in the PRECAF and DURCAF treatments than with PLACEBO.

TT performance and success with blinding of treatments

The results of the TT are summarized in Table 1. All treatments produced a performance enhancement of ~ 3% compared with PLACEBO. There were no differences in performance between each of the treatment trials (e.g. COKE vs. PRECAF: P = 0.82; PRECAF vs. DURCAF: P = 0.92).

The post-trial questionnaires revealed that five of the 12 subjects were able to correctly identify the order of treatments in the PRE-CAF, DUR-CAF and PLACEBO trials. In addition, only four of the subjects were able to correctly identify which treatment was received during the trial in which they achieved their best TT performance, with 3 subjects incorrectly nominating the PLACEBO trial as the treatment in which they performed best.

Study B

Pre-trial standardization

Examination of food and exercise diaries revealed that subjects complied with the pre-trial standardization. No exercise was undertaken during the 24 h period prior to each trial, and complete caffeine withdrawal was reported for 48 h before each trial. Each subject consumed
the same pre-packaged diet during the 24 h prior to each pre-trial and consumed the same pre-event meal [2 g/kg CHO] 2 h prior to the trial. Total intake from these standardised meals was: 9.6 ± 0.1 g/kg CHO and 238 ± 2 kJ/kg.

Plasma metabolites, RER and perception of effort
Main effects of time (P <0.05) were observed for plasma concentrations of glucose, lactate, FFA and glycerol, but there was no effect of treatment. The typical patterns for each metabolite followed the responses seen in the COKE trial in Study A (data not shown). Similarly, RER and estimated rates of fat and carbohydrate oxidation changed with time (P <0.05) as would be predicted from Study A, but there were no differences between treatments (data not shown). RPE during exercise increased with time (P <0.05), but there were no differences between treatments at any time point.

Volume of fluid consumed during TT
The mean volume of cola drink consumed during the TT was 332 mL (4.8 mL/kg), providing a total intake of cola drinks of 14.8 mL/kg. The total CHO intake from drinks consumed during exercise on the COKE and extraCHO treatments was 284 g (compared with 210 g CHO on the CONTROL and CAF trial). The COKE and CAF trials provided a total intake of 133 mg of caffeine (~1.9 mg/kg).

TT performance and success with blinding of treatments
The results of the TT are summarised in Figure 6. There was a 3.3% improvement in TT performance following the COKE treatment compared with CONTROL (P<0.05). Comparison of the results of all trials show a ~2% enhancement of TT performance with caffeine compared with no caffeine (P<0.05), and a 1% (NS) improvement with 11% CHO intake compared with 6% CHO. Results of the post-study questionnaire showed that none of
the subjects in Study B was able to correctly identify the order of each treatment; in fact, 5 of
the 8 subjects were unable to correctly identify any of the treatments.

DISCUSSION
The first finding of this investigation was that caffeine intake of 6 mg/kg BM enhanced the
performance of a time-trial undertaken at the end of a prolonged cycling bout under
nutritional conditions recommended for endurance athletes; viz. after the intake of a CHO-
rich meal and with the intake of CHO throughout exercise. This performance enhancement
occurred whether the caffeine was consumed prior to the cycling bout or throughout exercise.
These results are consistent with the findings of other studies reporting enhanced capacity for
submaximal exercise with intakes of caffeine in the hour prior to exercise (7, 9, 10, 14-16, 19,
22, 23, 27, 29). They are also supported by the more recent work of Kovacs et al. (22) in
which a 1-h cycling time-trial was improved after caffeine consumption both prior to and
during exercise. The novelty of the present findings is the direct comparison of the timing of
the intake of caffeine and in the use of dietary conditions and an exercise protocol that
simulates the real life situation of competitive sport.

Another unique aspect of the present investigation was the study of the effect of consuming
Coca Cola™ as a replacement for sports drink during the latter stages of the endurance
cycling task. This treatment was investigated to replicate a practice of many endurance
athletes in competitive cycling, running, triathlon and skiing events, who believe that it
provides a useful “caffeine hit”. However, we found that the amounts of Coca Cola™
typically consumed by athletes caused only a minor increase in plasma caffeine concentration
(Fig. 1a) and minimal impact on urinary caffeine (Fig. 1b), compared to the doses of caffeine
typically associated with ergogenic benefits under laboratory conditions (4,22,23,30).
Nevertheless, in contrast to our hypothesis, intake of Coca Cola™ was associated with a performance enhancement similar to that attained by the larger caffeine doses, when compared directly using a placebo-controlled but unblinded presentation of the treatment design. The second part of the investigation (Study B), in which Coca Cola™ was provided in a rigorous double-blind placebo design, replicated the findings of Study A and found a performance enhancement of identical magnitude. The ergogenic effect was explained both by the intake of small amounts of caffeine and an increase in CHO intake.

Although the transparent allocation of the COKE treatment in the first study represents a weaker research design than a double-blind presentation, we considered it a necessary starting point for the investigation of any potential effects of the Coca Cola™ use by endurance athletes. Although COKE was provided as a transparent treatment to subjects, we took care in the organization of a placebo control so that subjects would receive psychological motivation in all trials. The researcher in charge of the TT measurements was kept blind to the treatment received by subjects to remove any bias in encouragement provided. Post-trial questionnaires revealed that most subjects were deceived by the blinding of treatments. Furthermore, 25% of the group nominated the PLACEBO trial as the treatment in which they felt they performed best, as would be expected if pure chance rather than knowledge of the treatments determined this outcome. The results of Study A provided justification for conducting a more elaborately controlled study on the effects of the COKE treatment on endurance performance, including investigation of the separate and additive effects of ingesting caffeine and higher concentrations of CHO towards the end of prolonged cycling.
The present investigation fails to provide clear evidence of the mechanism(s) underlying the performance enhancement seen with all of the active treatments. We originally hypothesized that the different caffeine intake protocols would result in different metabolic profiles; in particular the presence or absence of an early elevation in plasma FFA concentration with a concomitant CHO ‘sparing’ effect. Others have reported that intake of caffeine in the hour prior to exercise resulted in an increase in plasma FFA concentrations and increased fat oxidation both from blood-borne and intramuscular stores in the subsequent exercise task (7, 10, 19). Furthermore, a recent study has shown that this glycogen sparing effect is limited to the first 15-30 min of exercise (27), but is associated with enhancement of endurance performance (27). For this reason we hypothesized that pre-ingestion of caffeine would alter RER values early in exercise, whereas ingestion of caffeine throughout exercise would not further increase plasma fatty acid availability above those levels already associated with prolonged submaximal cycling. However, although the PRECAF trial was associated with higher overall concentrations of plasma FFA compared to the PLACEBO trial, we were unable to detect any differences in rates of substrate utilization between trials based on respiratory gas exchange data. There are several possibilities to explain our findings.

First, even if early intake of caffeine attenuates muscle glycogenolysis, it is possible that such changes in muscle metabolism are not detectable by conventional RER measures. For example, Spriet et al. (1992) reported that, compared to a placebo, pre-ingestion of a large dose of caffeine (9 mg.kg\(^{-1}\) BM) significantly reduced muscle glycogenolysis during the first 15 min of continuous cycling at 80% of VO\(_{2max}\) (27). This reduction in glycogen utilization occurred in the absence of any changes in whole body rates of CHO oxidation (estimated from RER values) during this period. Indeed, the RER values in the study of Spriet et al. (1992) are actually higher with caffeine ingestion than placebo (0.85 vs. 0.81), although it
should be noted that the exercise intensities of the two trials at this time point are different from each other and the (intended) exercise protocol of cycling at 80% \( \text{VO}_{2\text{max}} \) (viz. 82 vs. 74% of \( \text{VO}_{2\text{max}} \) for caffeine and placebo, respectively). Spriet and co-workers suggested their recreational cyclists might not have attained steady-state conditions at such high workrates (27). Furthermore, as in the present study, they found that ventilation was higher following caffeine intake. Therefore, it is possible that following moderate to high doses of caffeine (6-9 mg/kg) conventional pulmonary gas exchange data cannot detect differences in muscle metabolism, especially early in exercise.

Second, others have suggested that high CHO availability might inhibit the effect of caffeine on mobilization of FFA and fat oxidation (31). Indeed in our study, subjects were tested in conditions promoting optimal CHO status; 1-d of rest and high CHO intake, a pre-exercise CHO meal and regular intake of CHO throughout exercise. Other workers have also used conditions of pre-exercise CHO intake and feeding throughout exercise and found that caffeine ingestion had no effect on FFA concentrations (22).

Most importantly, however, there is considerable evidence that the ergogenic benefits of caffeine on exercise performance are not limited to, or always explained by, the so-called ‘metabolic theory’ (for review see (26). One early investigation in support of this view is of particular interest to the present study since caffeine was administered before and during prolonged exercise. Ivy et al. (1979) fed trained cyclists 250 mg of caffeine (~3.6 mg/kg) 60 min prior to a 2 h ride on an isokinetic ergometer followed by an additional 250 mg at 15 min intervals for the first 90 min of exercise. Caffeine ingestion increased the total work performed in the 2 h ride by 7.4% compared to control. However, plasma FFA concentrations
and estimated rates of CHO and fat oxidation were similar between trials, failing to explain the performance benefits (19).

More recent evidence against the metabolic theory is provided by Graham et al (13) who quantified muscle metabolism by a combination of direct arterio-venous balance methods and muscle biopsies after ingestion of 6 mg/kg caffeine during 1-h of submaximal exercise. They found that although caffeine ingestion stimulated the sympathetic nervous system, it did not alter leg FA uptake, net muscle glycogenolysis or rates of CHO and fat metabolism in the monitored leg (13). Another study from this group confirmed that intake of caffeine and another methyl-xanthine compound, theophylline, can prolong endurance during cycling at 80% VO_{2max} without affecting muscle glycogen utilization (17). However, others have found individual variability in the metabolic response to caffeine: half of a group of subjects was shown to “spare” glycogen during the first 15 minutes of exercise following caffeine intake (9 mg/kg) compared with a placebo treatment, while glycogen utilisation was unaffected in the other half of the group following caffeine treatment (5). Taken collectively, the results of these studies indicate that glycogen sparing following caffeine ingestion is a variable response, but seems most likely to occur with larger caffeine doses (> 6 mg/kg) and power outputs eliciting ≥ 70% VO_{2max}.

The mechanism for performance enhancement with caffeine ingestion in the present study could be due to an effect on the central nervous system (CNS) or a direct effect of caffeine on skeletal muscle (28). Caffeine ingestion has been shown effect the CNS (6) and to elicit greater motor unit recruitment and alter neurotransmitter function (32). Caffeine also affects the CNS in ways that cause it to override fatigue signals during exercise (6). Indeed, in the
present study, the subjective ratings of perception of effort at the end of the 120 min steady-state ride were lower with both caffeine ingestion protocols compared to placebo.

The design of Study B allows some insight into the mechanisms underlying the observed performance enhancement with Coca Cola™. The dose of caffeine associated with the late feeding of Coca-Cola™ (1.3 mg/kg and 1.9 mg/kg in Studies A and B respectively), although it did not cause substantial rises in plasma or urinary caffeine concentration in Study A, was found to provide a worthwhile performance enhancement. These caffeine doses are smaller than the lowest dose of caffeine that has been previously reported to enhance exercise performance (i.e. 2.1 mg/kg (22). These findings are in support of emerging evidence (15, 22) that caffeine achieves an ergogenic effect at intakes of 1-3 mg/kg); these doses are substantially lower than those used in earlier studies. Furthermore, there is no dose response to caffeine intake, since performance benefits are not increased when larger doses of caffeine are consumed (15, 22, 23).

The amount of CHO ingested late in exercise was greater with Coca-Cola™ than with the sports drink or 6% CHO cola drinks (74 g vs. 44 g in Study A and 114 g vs. 61 g in Study B), and caused subtle differences in the rate of change of blood glucose concentrations in the last 45 min of exercise (Fig. 2). As recently noted, the maintenance of high (i.e., 5-5 mM) plasma glucose concentrations typically associated with the ingestion of CHO throughout prolonged (2 h) moderate-intensity (~70% VO_{2max}) exercise can improve exercise performance by mechanisms other than alterations in substrate utilization (11). However, according to the results of Study B, the increase in CHO intake during the latter stages of prolonged cycling made only a small contribution to the overall performance enhancement.
An important practical finding of the study was that all protocols of caffeine intake produced urinary caffeine concentrations below 12 µg/ml, which is the level above which the anti-doping guidelines of the IOC deem a positive test for caffeine use to have occurred. Some individual variability in urinary caffeine levels following standard caffeine doses is noted (23), and there is one literature report of a subject returning a urinary caffeine level > 12 µg/mL following a caffeine intake of 6 mg/kg (4). However, our results support the consensus that caffeine intakes of up to 6 mg/kg pose a minor risk of causing an athlete to contravene the IOC urinary caffeine limits (26). Based on the results of the present study, the use of Coca Cola™ as a sports drink in the latter part of a race is likely to produce a minimal effect on urinary caffeine levels.

In summary, the results of the present investigation show that the intake of moderate doses of caffeine (6 mg/kg) enhanced the performance of a cycling time trial, undertaken with a sports-specific protocol and under the dietary conditions promoted for optimal sports performance. This ergogenic benefit to performance was observed whether caffeine was ingested one hour prior to exercise, or in a series of doses throughout the exercise bout, and was achieved without contravening the reporting limit for caffeine use by anti-doping codes of the IOC. We also found support for the observed practice of consuming Coca Cola™ as a replacement for sports drink during the last part of an endurance event. When provided in a double-blind design, the Coca Cola™ protocol also produced an enhancement of a TT performance at the end of the 2 h cycling task, with the benefits being largely due to the ingestion of the small amount of caffeine (~1.5 mg/kg). The direct comparison of the ingestion of larger amounts of caffeine with the Coca Cola™ protocol, albeit in a transparent but placebo-controlled design, suggests that all protocols of caffeine use are of equal and worthwhile benefit to the performance of a prolonged cycling task.
Acknowledgments.

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REFERENCES


Table 1. Results of 7 kJ/kg time trial (TT) following 120 min of steady-state cycling.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TT time (min: sec)</th>
<th>Enhancement compared to PLACEBO [confidence interval]</th>
<th>P value</th>
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<tbody>
<tr>
<td>PRECAF</td>
<td>28:18 ± 0:40</td>
<td>3.4% [0.2 to 6.5%]</td>
<td>0.04</td>
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<tr>
<td>DURCAF</td>
<td>28:24 ± 0:57</td>
<td>3.1% [-0.1 to 6.5%]</td>
<td>0.06</td>
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<tr>
<td>COKE</td>
<td>28:24 ± 0:30</td>
<td>3.1% [-0.2 to 6.2%]</td>
<td>0.06</td>
</tr>
<tr>
<td>PLACEBO</td>
<td>29:18 ± 0:44</td>
<td></td>
<td></td>
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Figures

**Figure 1.** Effect of 6 mg/kg BM caffeine ingested 1 h pre-exercise (PRECAF), 6 x 1 mg/kg BM caffeine ingested during exercise (DURCAF), or 2 x 5 mL/kg Coca Cola™ ingested late in exercise (COKE) on plasma caffeine concentration before and during exercise (1a) and post-exercise urinary caffeine concentration (1b) associated with a cycling task (2 h steady-state @ 70% of VO$_{2\text{peak}}$ + time-trial). Values are mean ± SEM for 12 subjects. * = different from – 60 min (P < 0.05); a = different from DURCAF, COKE, PLACEBO (P < 0.05); b = different from COKE, PLACEBO (P < 0.05).

**Figure 2.** Effect of 6 mg/kg BM caffeine 1 h pre-exercise (PRECAF), 6 x 1 mg/kg BM caffeine during exercise (DURCAF), or 2 x 5 mL/kg Coca Cola™ late in exercise (COKE) on blood glucose concentrations (Fig. 2a), blood lactate concentrations (Fig. 2b) and plasma insulin concentrations (Fig. 2c) for 2 h after a CHO-rich meal and during a subsequent cycling task (2 h steady-state @ 70% of VO$_{2\text{peak}}$ + TT). Values are mean ± SEM for 12 subjects. a = PRECAF > DURCAF, COKE, PLACEBO; b = PLACEBO < PRECAF, DURCAF < COKE; c = PLACEBO < PRECAF, DURCAF, COKE; d = main effect treatment: PRECAF > DURCAF, COKE > PLACEBO; e = main effect treatment: PRECAF, DURCAF > COKE, PLACEBO; * = main effect time: different from t = 0 min; # = main effect time; different from t = -120 min. All differences are significant at P < 0.05.
**Figure 3.** Effect of 6 mg/kg BM caffeine 1 h pre-exercise (PRECAF), 6 x 1 mg/kg BM caffeine during exercise (DURCAF), or 2 x 5 mL/kg Coca Cola™ late in exercise (COKE) on plasma free fatty acid [FFA] concentrations (Fig. 3a) and plasma glycerol concentrations (Fig. 3b), for 2 h after a CHO-rich meal and during a subsequent cycling task (2 h steady-state @ 70% of VO₂peak + TT). Values are mean ± SEM for 12 subjects. * = main effect time: different from t = 0 (P < 0.05); a = PRECAF > DURCAF, COKE, PLACEBO; b = PRECAF > COKE, PLACEBO; c = main effect treatment: PRECAF > PLACEBO (P < 0.05).

**Figure 4.** Effect of 6 mg/kg BM caffeine 1 h pre-exercise (PRECAF), 6 x 1 mg/kg BM caffeine during exercise (DURCAF), or 2 x 5 mL/kg Coca Cola™ late in exercise (COKE) on ventilation (Vₑ) during 2 h steady-state cycling @ 70% of VO₂peak. Values are mean ± SEM for 12 subjects. main effect time: increase from t = 10 min (P < 0.05). main effect treatment: PRECAF > DURCAF > COKE, PLACEBO. Interactions of treatment and diet: a = PRECAF > DURCAF, COKE, PLACEBO; b = PRECAF, DURCAF > COKE, PLACEBO. All differences are significant at P < 0.05.
**Figure 5.** Effect of 6 mg/kg BM caffeine 1 h pre-exercise (PRECAF), 6 x 1 mg/kg BM caffeine during exercise (DURCAF), or 2 x 5 mL/kg Coca Cola™ late in exercise (COKE) on ratings of perceived exertion (RPE) during 2 h steady-state cycling @ 70% of $\text{VO}_2\text{peak}$. Values are mean ± SEM for 12 subjects. * = main effect time: all time points different from t = 10 min; a = main effect treatment: PRECAF < PLACEBO; b = DURCAF > PRECAF, PLACEBO; c = PRECAF, DURCAF < PLACEBO; d = DURCAF < PLACEBO, COKE. All differences are significant at P ≤ 0.05.

**Figure 6.** Effect of intake of 3 x 5 mL/kg of various Cola-flavoured drinks (CONTROL, CAFFEINE, ExtraCHO, COKE) late in exercise on the performance of a 7 kJ/kg time trial at the end of 2 h steady-state cycling @ 70% of $\text{VO}_2\text{peak}$. Values are mean ± SEM for 8 subjects, with % improvement [±95% Confidence Intervals] compared with CONTROL treatment. * = different to CONTROL, P < 0.05.
<table>
<thead>
<tr>
<th>Control</th>
<th>Caffeine</th>
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</thead>
<tbody>
<tr>
<td>27:05 ± 0:42min</td>
<td>26:36 ± 0:42 min</td>
</tr>
<tr>
<td>1.9% [-0.6 – 4.41%]</td>
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</table>

<table>
<thead>
<tr>
<th>ExtraCHO</th>
<th>Coke</th>
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<tbody>
<tr>
<td>26:55 ± 0:43 min</td>
<td>26:15* ± 0:43 min</td>
</tr>
<tr>
<td>0.6% [-1.8 – 3.1%]</td>
<td>3.3% [0.8 – 5.9%]</td>
</tr>
</tbody>
</table>

Effect of caffeine:
2.2%* [0.5 – 3.8%]

Effect of additional CHO:
1.0% [-0.7 – 2.7%]