

# Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance

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**Kovacs, Eva M. R., Jos H. C. H. Stegen, and Fred Brouns.** Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. *J. Appl. Physiol.* 85(2): 709–715, 1998.—The effect of addition of different dosages of caffeine (Caf) to a carbohydrate-electrolyte solution (CES) on metabolism, Caf excretion, and performance was examined. Subjects ( $n = 15$ ) ingested 8 ml/kg of water placebo (Pla-W), 7% CES (Pla-CES), or 7% CES with 150, 225, and 320 mg/l Caf (CES-150, CES-225, and CES-320, respectively) during a warm-up protocol (20 min) and 3 ml/kg at one-third and two-thirds of a 1-h time trial. Performance was improved with Caf supplementation:  $62.5 \pm 1.3$ ,  $61.5 \pm 1.1$ ,  $60.4 \pm 1.0$ ,  $58.9 \pm 1.0$ , and  $58.9 \pm 1.2$  min for Pla-W, Pla-CES, CES-150, CES-225, and CES-320, respectively. The postexercise urinary Caf concentration (range 1.3–2.5  $\mu\text{g/ml}$ ) was dose dependent and always far below the doping level of the International Olympic Committee (12  $\mu\text{g/ml}$ ) in all subjects. Sweat Caf excretion during exercise exceeded postexercise early-void urinary Caf excretion. Caffeinated CES did not enhance free fatty acid availability, ruling out the fact that performance improvement resulted from enhanced fat oxidation. It is concluded that addition of relatively low amounts of Caf to CES improves performance and that postexercise urinary Caf concentration remained low.

caffeine; exercise; dose-response; doping

SEVERAL STUDIES OBSERVED that caffeine (Caf) ingestion improves endurance performance (9, 19, 20, 28, 32, 33). Explanations are increased lipolysis and fat oxidation and decreased muscle glycogen breakdown (9, 14, 21) in combination with a lessening of the subjective perception of effort (8). Most of these studies examined the ergogenic effects of Caf by measuring performance as the time taken to reach exhaustion at a given power output. This is, however, more a measure of endurance capacity rather than of absolute performance, like the time needed to cover a certain distance. Only a few studies have examined the effect of Caf on exercise time over a specified distance or on completion of a given amount of work (3, 7, 26). Little is known about the effect on physical performance of Caf added to a solution containing carbohydrates (CHO) (17, 30). Therefore, the first purpose of this study was to determine the effect of adding Caf to a CHO-electrolyte solution (CES) on time trial cycling performance of  $\sim 1$  h. Four levels of Caf (0, 150, 225, and 320 mg/l) in combination with CES were examined, compared with a water placebo (Pla-W). In addition, Caf concentration in postexercise urine was measured in relation to the doping limit of the International Olympic Committee (IOC) of 12  $\mu\text{g/ml}$ . It was hypothesized that Caf enhances performance metabolism and urinary Caf levels in a dose-dependent way.

A substantial number of studies have been published on the composition of sweat produced during exercise (4). To our knowledge, however, the excretion of Caf with sweat during exercise has never been investigated. Therefore, a second purpose of this study was to examine Caf excretion with sweat and its relationship to Caf intake and urinary Caf excretion.

## METHODS

### Subjects

Fifteen healthy and well-trained ( $\geq 2$  h/day and  $\geq 4$  times/wk) male subjects (triathletes and cyclists) participated in this study. All subjects were Caf users in varying gradations (20–410 mg/day), and no hypersensitivity to Caf was known. Their physical characteristics (mean  $\pm$  SE) were age,  $23.3 \pm 0.9$  yr; height,  $185 \pm 2$  cm; body weight (BW),  $72.6 \pm 1.9$  kg; maximal work capacity ( $\dot{W}_{\text{max}}$ ),  $401 \pm 10$  W; and maximal heart rate,  $188 \pm 2$  beats/min. All subjects were familiar with the time trial protocol and had previously been subjects in laboratory exercise protocols. They were fully informed about all procedures and risks of the investigation before their written informed consent was obtained. The study was approved by the local medical ethical committee.

### Experimental Procedures

The study had a randomized double-blind, placebo-controlled, crossover design. Each subject followed the protocol with an interval of 7 days between all five tests.

**Treatment.** The treatment consisted of drinking 14 ml/kg BW of a Pla-W and four CES containing 68.8 g/l CHO (Pla-CES) or 68.8 g/l CHO combined with 150 mg/l Caf (CES-150), 225 mg/l Caf (CES-225), and 320 mg/l Caf (CES-320). All CES further contained (mg/l) 100 vitamin C, 100 vitamin E, 690  $\text{Na}^+$ , 180  $\text{K}^+$ , 300  $\text{Cl}^-$ , 10  $\text{Mg}^{2+}$ , 10  $\text{Ca}^{2+}$ , 70 phenylalanine, 70 tyrosine, 70 taurine, 51 myoinositol, and 100 choline. The drinks were similar in color and taste. The temperature of the drinks was kept at 8–10°C. In general, the drinks were well tolerated. None of the subjects complained of adverse effects. The subjects ingested a mean volume of 1,026 ml of a drink. Mean Caf intake, therefore, was 154 mg (2.1 mg/kg BW) in the CES-150 trial, 230 mg (3.2 mg/kg BW) in the CES-225 trial, and 328 mg (4.5 mg/kg BW) in the CES-320 trial.

**Instructions to subjects.** The subjects were instructed to schedule the experimental test into their training program as a competition. They were asked to record food consumption and activities during the 2 days before the first test. To minimize variation in preexercise glycogen stores, they were asked to repeat the same food consumption and activities protocol before all remaining tests. From 48 h before the test, training was not allowed to be exhaustive, and subjects were asked to refrain from Caf consumption (coffee, tea, chocolate, cola).

**Initial procedure.** In a pretest, each subject performed an incremental exercise test on an electromagnetically braked

cycle ergometer (Lode Excalibur, Groningen, The Netherlands) to determine the actual  $\dot{W}_{\max}$  (24).

**Test design.** The subjects arrived at the laboratory in a fasted state, always at the same time (7:30 or 8:15 AM) and then consumed a standardized breakfast, consisting of 1.5 g/kg BW of bread, 0.5 g/kg BW of cheese, 10 g of butter, and 200 ml of mineral water.

Thirty minutes after breakfast, nude BW was measured on a precision scale (Chyo-MW-150K, Japan; weighing accuracy of  $\pm 20$  g), and a urine sample was collected. A catheter was inserted in an antecubital vein, and a resting blood sample ( $t = -75$  min) was taken. When starting the warm-up protocol, the subjects ingested 8 ml/kg BW of one of the solutions. The warm-up protocol consisted of 5-min exercise at 1.5 W/kg BW followed by 15 min at 2.5 W/kg BW, after which a blood sample was taken ( $t = -45$  min). The subjects then moved to another room to perform a number of psychological tests, lasting about 35 min. These tests are described in a separate paper (E. Hogervorst, W. Riedel, E. Kovacs, F. Brouns, and J. Jolles, unpublished observations). When the subjects returned to the exercise laboratory, nude BW was remeasured and a blood sample was taken ( $t = 0$  min). The skin of the upper back was cleaned with distilled water and dried. Two airtight capsules with cotton were placed in the infrascapular fossa of the scapula and fixed by an elastic-mesh vest (Elastofix, Beiersdorf, Hamburg, Germany) for local sweat collection (25). After a short warm-up (5 min, 100 W), the subjects started the time trial protocol. The ergometer was adjusted into a pedaling rate-dependent (linear) mode according to the formula

$$\dot{W} = L \cdot (\text{rpm})^2$$

where work is  $\dot{W}$ , rpm is the pedaling rate, and  $L$  the linear factor.  $L$  was chosen in a way that would result in a pedaling rate of 90 rpm at 70% of  $\dot{W}_{\max}$ . The subjects were asked to perform a set amount of work (equal to  $\sim 1$  h cycling) as quickly as possible. They were encouraged by the same person in every test. The target amount of work ( $T$ ) was calculated according to the formula

$$T(J) = 0.75 \cdot \dot{W}_{\max} \cdot 3,600$$

The time needed to complete the target amount of work and the work output at which the subjects cycled were representative for performance. To avoid test-retest influence, the subjects received no information on performance time, workload, pedaling rate, and heart rate. This validated time trial protocol has been shown to be highly reproducible in trained athletes (23) (coefficient of variation 3.4%). During the time trial, the subjects ingested 3 ml/kg BW of the test solution twice, at one-third ( $t = +20$  min) and two-thirds ( $t = +40$  min) the target amount of work. A blood sample was taken at the same time points and at the end of the time trial ( $t = +60$  min). After a short cooldown (5 min, 100 W), the sweat-sampling capsules with cotton were removed, a urine sample was collected, and nude BW was measured. Heart rate was continuously recorded by using a Sporttester (Polar, Finland) during the whole investigation period.

Environmental conditions were standardized during all tests; temperature and relative humidity were kept at 21°C and 50–60%, respectively.

#### Analysis

**Blood samples.** Blood samples (5 ml) were drawn into 10-ml EDTA tubes. Hemoglobin and hematocrit were determined

immediately by using a hematology system (Coulter Micro-Diff 18, Coulter Electronics, Mijdrecht, The Netherlands) to calculate changes in plasma volume (11). Blood samples were immediately centrifuged for 10 min at 3000 rpm (4°C), and plasma was immediately frozen in liquid nitrogen and then stored at  $-20^\circ\text{C}$  until chemical analysis. Glucose (hexokinase method, Roche), free fatty acids (FFA; acyl-CoA-synthetase/acyl-CoA oxidase method, Wako), glycerol (pyruvic kinase/lactate dehydrogenase method), and lactate (lactate dehydrogenase method) were analyzed by a semiautomated centrifugal spectrophotometer (Cobas-Fara). Caf was determined by using HPLC (Clin Rep Komplettkit für Theophyllin, Theobromin und Coffein, Recipe Chemical+Instruments Labortechnik, Munich, Germany).

**Urine samples.** Immediately after collection, urine samples were weighed and stored at  $-20^\circ\text{C}$  for later analysis of Caf by using HPLC (Clin Rep Komplettkit für Theophyllin, Theobromin und Coffein, Recipe Chemical+Instruments Labortechnik).

**Sweat samples.** The wet cotton was eliminated from the sweat capsule and put in a syringe, and sweat was pressed out. Sweat samples were stored at  $-20^\circ\text{C}$  until analysis for Caf by using HPLC (Clin Rep Komplettkit für Theophyllin, Theobromin und Coffein, Recipe Chemical+Instruments Labortechnik).

Sweat loss was calculated as the difference among BW change, fluid intake, and urine output, corrected for metabolic and respiratory losses according to the following formulas (12, 27)

$$\text{Sweat loss } (L_S) = \Delta BW - L_M - L_R \text{ (g)}$$

$$\text{Metabolic loss } (L_M) = \frac{\dot{V}_{\text{CO}_2} \times 12}{22.4} = \dot{V}_{\text{CO}_2} \times 0.53 \text{ (g/min)}$$

where  $\dot{V}_{\text{CO}_2}$  is the volume of  $\text{CO}_2$  produced (l/min), 22.4 is the volume of 1 mol of gas (l/mol), and 12 is the mole mass of carbon (g/mol)

$$\text{Respiratory loss } (L_R) = 0.019 \cdot \dot{V}_{\text{O}_2} \cdot (44 - P_a) \text{ (g/min)}$$

where  $\dot{V}_{\text{O}_2}$  is the volume of assimilated  $\text{O}_2$  (l/min) and  $P_a$  is water vapor pressure (mmHg) and

$$P_a = \varphi \cdot P_D \text{ (mmHg)}$$

where  $\varphi$  is the relative humidity (%) and  $P_D$  is the saturation water vapor pressure dependent on temperature (mmHg) (41).  $\dot{V}_{\text{CO}_2}$  and  $\dot{V}_{\text{O}_2}$  were estimated from values obtained previously during time trial tests at similar intensities. Metabolic and respiratory losses during rest were considered to be negligible.

#### Statistical Analysis

All results are expressed as means  $\pm$  SE. Statistical significance between treatments was assessed by using repeated-measures analysis of variance, and differences were located by using the Fisher protected least significant differences post hoc test. The level of significance was set at  $P < 0.05$ . Calculations of correlation coefficients,  $r$ , between variables were carried by the using the Pearson product-moment method.

#### RESULTS

All results refer to 15 subjects, unless otherwise indicated. Statistical analysis pertains to results obtained in 14 subjects; one subject was excluded from

analysis because Caf had been detected in plasma during the Pla-W trial, most probably due to earlier Caf consumption.

### Performance Parameters

Subjects completed the time trial significantly faster and with a significantly higher mean work output after ingestion of CES-225 ( $58.9 \pm 1.0$  min,  $308 \pm 9$  W) and CES-320 ( $58.9 \pm 1.2$  min,  $309 \pm 10$  W) compared with Pla-W ( $62.5 \pm 1.3$  min,  $292 \pm 10$  W), Pla-CES ( $61.5 \pm 1.1$  min,  $295 \pm 9$  W), and CES-150 ( $60.4 \pm 1.0$  min,  $299 \pm 10$  W) ( $P < 0.001$ ). Additionally, the fixed amount of work was also achieved significantly faster and with a higher mean work output after ingestion of CES-150 compared with Pla-W ( $P < 0.001$ ). Performance time and work output with Pla-CES were not different compared with Pla-W. No significant difference in heart rate was observed between treatments during the 20-min warm-up protocol. During the time trial, a higher heart rate was observed after ingestion of CES-150 ( $174 \pm 2$  beats/min), CES-225 ( $177 \pm 1$  beats/min), and CES-320 ( $176 \pm 2$  beats/min) compared with Pla-W ( $171 \pm 2$  beats/min) and Pla-CES ( $172 \pm 2$  beats/min) ( $P < 0.001$ ).

### Blood Parameters

The plasma volume response during exercise was similar for all trials, showing a mean reduction of  $3.5 \pm 1.2\%$  during the warm-up protocol and  $8.6 \pm 1.1\%$  during the time trial ( $P < 0.001$ ,  $n = 6$ ). There was no difference in plasma volume between the trials at any time point.

During the experiment, the plasma Caf levels increased after consumption of the Caf-containing drinks in a dose-related manner (Fig. 1).

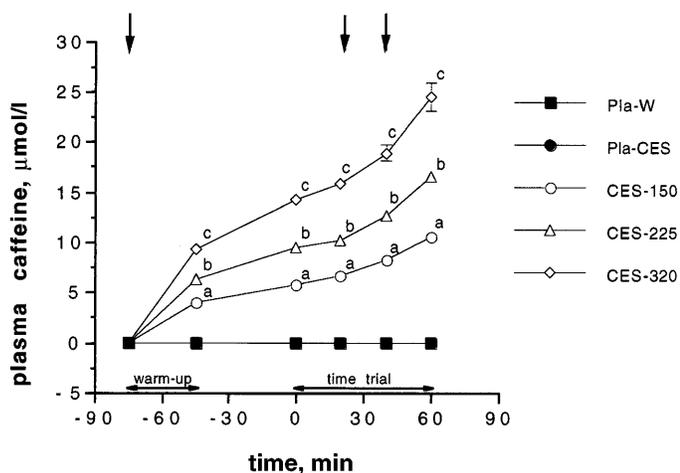


Fig. 1. Plasma caffeine ( $\mu\text{mol/l}$ ). Values are means  $\pm$  SE. Pla-W and Pla-CES, water and carbohydrate-electrolyte solution (CES; 7% CHO) placebo, respectively; CES-150, CES-225, and CES-320: CES with addition of 150, 225, and 320 mg/l caffeine, respectively. Values for Pla-W and Pla-CES are on same line. Arrows indicate time of drink ingestion. <sup>a</sup>Significantly higher compared with Pla-W and Pla-CES,  $P < 0.001$ . <sup>b</sup>Significantly higher compared with Pla-W, Pla-CES, and CES-150,  $P < 0.001$ . <sup>c</sup>Significantly higher compared with Pla-W, Pla-CES, CES-150, and CES-225,  $P < 0.001$ .

Consumption of all CES slightly increased plasma glucose during the warm-up protocol, whereas Pla-W resulted in a significantly lower glucose level at  $t = -45$  min ( $P < 0.001$ ). Plasma glucose levels in all trials decreased below the preexperiment levels during the subsequent 35-min nonexercise period (psychological tests). The subsequent time trial resulted in an increased plasma glucose concentration in all trials. At  $t = +20$  min, plasma glucose concentration was significantly lower with Pla-CES compared with CES-150, CES-225, and CES-320 ( $P < 0.001$ ). At  $t = +40$  min and at the end of the time trial, plasma glucose concentration was lower with Pla-W compared with CES-150, CES-225, and CES-320 and with Pla-CES compared with CES-150 and CES-320 ( $P < 0.001$ ). At the end of the time trial, plasma glucose concentration with Pla-W was also lower than with Pla-CES ( $P < 0.001$ ).

During the warm-up procedure plasma FFA decreased similarly in all treatments (Fig. 2). Before the time trial protocol began, FFA with ingestion of CES-320 was elevated compared with Pla-CES, CES-150, and CES-225 ( $P < 0.001$ ). During the time trial, plasma FFA levels increased in all trials and were higher with Pla-W compared with Pla-CES, CES-150, and CES-225 at  $t = +20$  min,  $t = +40$  min, and at the end of the time trial ( $P < 0.001$ ). Also, at the end of the time trial plasma FFA concentration with CES-320 was higher compared with CES-225 ( $P < 0.001$ ). Plasma glycerol increased during the warm-up protocol and returned to the preexperiment level during the rest period. Before the time trial protocol began, plasma glycerol was higher with CES-320 compared with Pla-CES, CES-150, and CES-225 ( $P < 0.001$ ). A rapid increase in glycerol levels was observed during the time trial. At the end of the time trial plasma glycerol concentration was higher with Pla-W compared with Pla-CES and CES-150 and with CES-320 compared with CES-150 ( $P < 0.001$ ).

Before the start of the time trial, lactate levels were lower with Pla-W compared with all other trials and with Pla-CES compared with CES-150, CES-225, and CES-320 ( $P < 0.001$ ). During the time trial, plasma lactate increased rapidly in all trials ( $P < 0.001$ ). Significantly lower lactate levels were observed after ingestion of Pla-W and Pla-CES compared with CES-150, CES-225, and CES-320 at  $t = +20$  min,  $t = +40$  min, and at the end of the time trial ( $P < 0.001$ ).

### Urine Parameters

There was no difference in urine output between trials at the start (Pla-W,  $85 \pm 21$  ml; Pla-CES,  $85 \pm 20$  ml; CES-150,  $87 \pm 24$  ml; CES-225,  $121 \pm 35$  ml; CES-320,  $94 \pm 16$  ml) or at the end of the experiment (Pla-W,  $322 \pm 65$  ml; Pla-CES,  $199 \pm 57$  ml; CES-150,  $216 \pm 49$  ml; CES-225,  $290 \pm 58$  ml; CES-320,  $215 \pm 48$  ml). After the time trial, urinary Caf was positive in the Caf trials and negative in the placebo trials. The Caf concentration in urine was well below the doping limit of the IOC, which amounts to  $12 \mu\text{g/ml}$ :  $1.3 \pm 0.2 \mu\text{g/ml}$

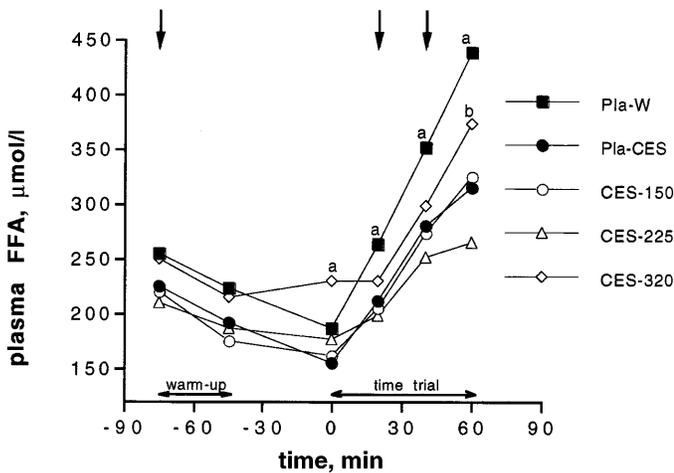


Fig. 2. Plasma free fatty acids (FFA;  $\mu\text{mol/l}$ ). Values are means  $\pm$  SE. <sup>a</sup>Significantly higher compared with Pla-CES, CES-150, and CES-225,  $P < 0.001$ . <sup>b</sup>Significantly higher compared with CES-225,  $P < 0.001$ . Error bars are not shown. Arrows indicate time of drink ingestion.

with CES-150,  $1.9 \pm 0.2 \mu\text{g/ml}$  with CES-225, and  $2.5 \pm 0.2 \mu\text{g/ml}$  with CES-320 (Fig. 3). Urinary Caf concentration was related to Caf intake and plasma Caf level in a dose-response manner ( $r = 0.87$  and  $r = 0.92$ , respectively;  $P < 0.001$ ). There was no correlation between urinary Caf concentration and urine output ( $r = 0.13$ ;  $P > 0.1$ ). Urinary Caf excretion after the time trial was  $243 \pm 45$ ,  $504 \pm 96$ , and  $540 \pm 138 \mu\text{g}$  with CES-150, CES-225, and CES-320, respectively.

**Sweat Parameters**

BW loss during the entire experiment, corrected for urine output, was  $\sim 1.8 \text{ kg}$  in all trials. This large quantity of fluid lost was primarily due to sweat loss (82%) and in minor part to metabolic and respiratory losses (8 and 10%, respectively). No differences in

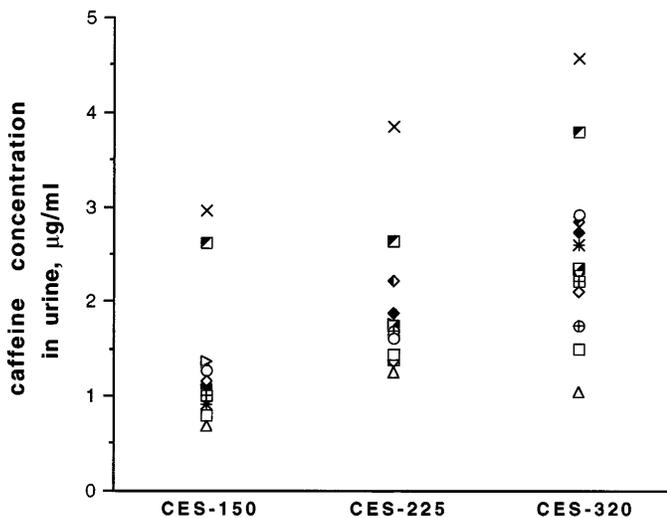


Fig. 3. Individual caffeine concentrations in urine obtained after the time trial after ingestion of caffeine-containing solutions ( $\mu\text{g/ml}$ ). Mean (range) urinary caffeine concentrations after the time trial were  $1.3 (0.7\text{--}3.0)$ ,  $1.9 (1.3\text{--}3.8)$ , and  $2.5 (1.0\text{--}4.6) \mu\text{g/ml}$  for CES-150, CES-225, and CES-320, respectively.

metabolic, respiratory, or sweat losses were found among trials. Sweat Caf concentration amounted to  $1.7 \pm 0.1 \mu\text{g/ml}$  with CES-150,  $2.7 \pm 0.1 \mu\text{g/ml}$  with CES-225, and  $3.7 \pm 0.2 \mu\text{g/ml}$  with CES-320. Sweat Caf concentration correlated in a dose-response manner with Caf intake ( $r = 0.96$ ;  $P < 0.001$ ) and also correlated with plasma and urinary Caf concentration ( $r = 0.98$  and  $r = 0.92$ , respectively;  $P < 0.001$ ) (Fig. 4). The calculated Caf excretion with sweat during exercise amounted to  $2,087 \pm 137$ ,  $3,223 \pm 162$ , and  $4,446 \pm 297 \mu\text{g}$  with CES-150, CES-225 and CES-320, respectively.

**DISCUSSION**

The results show that the addition of Caf to CES improves 1-h time trial cycling performance in well-trained athletes. An ergogenic effect of Caf was observed even at the lowest dosage of  $150 \text{ mg/l}$  and was greater with the medium dosage of  $225 \text{ mg/l}$ . However, the highest dosage of  $320 \text{ mg/l}$  Caf did not result in a further improvement compared with the  $225 \text{ mg/l}$  dosage. The ergogenic effect of Caf observed in the present study is in agreement with the results of several other investigations (3, 9, 16, 19, 20, 21, 26, 28, 30, 32, 33). In the majority of these studies, the ingested Caf dosages were  $\geq 5 \text{ mg/kg BW}$ . Only Ivy et al. (21) and Graham and Spriet (20) used a lower Caf dosage,  $250 \text{ mg}$  and  $3 \text{ mg/kg BW}$ , respectively. However, few studies did not find any performance-enhancing effect (1, 7, 31). This variety in results may be explained by differences in the dosage of Caf, intensity and duration of exercise, type and reliability of the exercise protocol, Caf use and training status of the subjects, or variability in individual and environmental conditions.

Most of the studies investigated the effects of Caf on endurance capacity during a cycling or running protocol at a given intensity to reach exhaustion. Few studies investigated the ergogenic effects of Caf over a specified distance. Berglund and Hemmingsson (3) observed that Caf may improve performance during 21-km cross-country skiing at low and high altitudes.

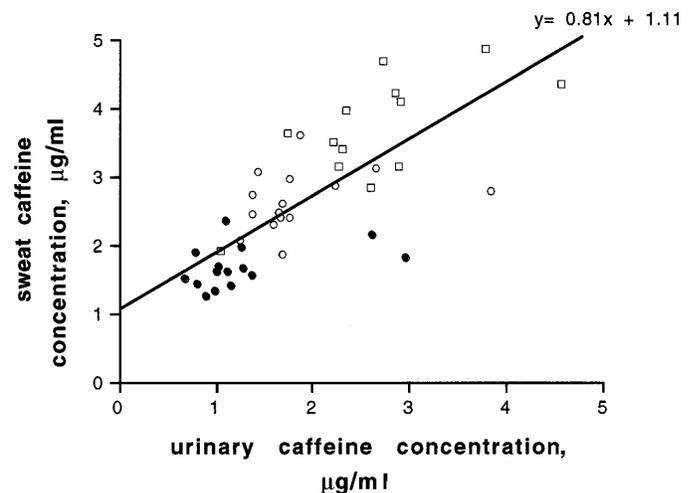


Fig. 4. Correlation between urinary and sweat caffeine concentration ( $r = 0.92$ ;  $P < 0.001$ ). ●, ○, and □: Data from CES-150, CES 225, and CES-320, respectively.

However, Cohen et al. (7) did not find a positive effect of Caf on performance during a 21-km outdoor footrace in hot and humid conditions. Different findings may have resulted from differences in environmental conditions. MacIntosh and Wright (26) observed improved performance during 1,500-m swimming after ingestion of Caf. In the present study, a time trial protocol was used in which the subjects had to complete a given amount of work. By maintaining standardized conditions, this protocol represents, in a reliable way, a competitive race in which athletes have to cover a certain distance. The results of the present study are therefore well applicable to the practice.

Only a few studies have investigated the effect of Caf addition to a solution that contains CHO (17, 30). These studies found that Caf added to CHO did not further improve performance. In the present study, Caf addition to CES improved performance.

Different explanations for the ergogenic effects of Caf have been put forward. Caf may stimulate lipolysis (28, 33) and may enhance central nervous system activity as well as the release of epinephrine (19, 20). However, no differences in FFA were observed in the present study when Caf was added to CES. This is in line with observations that epinephrine may not play the major role in the metabolic changes that result from Caf ingestion. Chesley et al. (5) did not find any effect on FFA or glycogenolysis during exercise after infusing epinephrine in an amount such that the concentration of plasma epinephrine was similar to that induced by Caf. Also, Graham and Spriet (20) observed no increase in epinephrine and FFA concentration during exercise after ingestion of 3 mg/kg BW Caf, although this dosage improved endurance capacity. In contrast, they found that a dosage of 9 mg/kg BW Caf increased FFA and epinephrine but had no effect on endurance performance. Van Soeren et al. (36) suggest that Caf directly stimulates specific tissues, i.e., adipose and peripheral vascular tissue, and that these effects are not secondary to increases in epinephrine. They observed an increase in FFA and glycerol after Caf ingestion in tetraplegic subjects with impaired epinephrine response. Alternatively, a stimulating effect of Caf on lipolysis may have been counteracted by an inhibiting effect due to the supply of CHO and the resulting increased blood glucose and insulin values.

Interestingly, Vergauwen et al. (38) observed that Caf acts as an adenosine-receptor antagonist, thereby reducing glucose uptake by the muscle during contractions. In the present conditions, this should favor the use of FFA as substrate, which is not observed.

The observation that plasma FFA and glycerol were significantly more elevated with Pla-W compared with CES-Caf drinks but that this resulted in the worst performance overrules the explanation that performance improvements due to Caf are caused by improved fat metabolism. It is therefore supposed that the ergogenic effect of Caf may not be explained by effects on muscle substrate metabolism but is more likely due to Caf stimulation of the central nervous system, through alterations in neurotransmitter function or

through a greater recruitment of motor units because of a decreased neuron activation threshold (35, 39). The latter is suggested to reduce the perception of work intensity (8, 9, 15, 26), which may result in performance improvement, by allowing athletes to work at a higher intensity at the same perceived level of effort (8).

During the present experiment, subjects were unaware of the composition of the drinks. After each test, they were asked to guess which test drink they ingested and to tell the reason for their choice (taste, cycling performance, urine volume, or subjective feeling, e.g., nervousness, concentration). Percentages of correct guesses were 50% for Pla-W, 63% for Pla-CES, 22% for CES-150, 38% for CES-225, and 41% for CES-320 (E. Hogervorst, W. Riedel, E. Kovacs, F. Brouns, and J. Jolles, unpublished observations). Although two studies observed that heart rate and/or plasma lactate was higher after Caf ingestion (30, 31), other studies did not find any difference (9, 14, 29, 33, 41). The higher heart rate and plasma lactate concentrations found in the present study when Caf was ingested were probably not a direct effect of Caf but rather a result of the higher work output at which subjects were cycling during exercise.

The ingestion of CES reduced mean performance time by 1 min compared with the Pla-W, but this improvement was not significantly different. This is in contrast to data from a study of Jeukendrup et al. (22), who observed a performance-enhancing effect of CES during a 1-h time trial compared with a Pla-W. This difference may be explained by a lower volume of drink and amount of CHO ingested during the present time trial: 6 ml/kg BW or 0.5 g CHO/min vs. 11 ml/kg BW or 1 g CHO/min in the study of Jeukendrup et al. (22).

To our knowledge, the excretion of Caf with sweat during exercise has not been analyzed until now. It is known that only 0.5–3% of Caf ingested is excreted in the urine, the majority being metabolized in the liver. In the present study, it has been shown that Caf concentrations in sweat are higher than in early postexercise urine. Although sweat was collected by a regional sampling method and this method may underestimate whole body excretion values, the results reveal that a considerable amount of Caf is excreted with sweat. Further studies are needed to quantify more precisely the exact amounts of Caf that are excreted with sweat.

A substantial number of studies have shown that the mean urinary Caf levels were always below the IOC limit of 12 µg/ml with Caf dosages up to 9 mg/kg BW (13, 19, 28, 34, 37). However, in single individuals a dosage of 9 mg/kg BW may lead to urinary Caf concentrations above the limit accepted by the IOC, whereas a lower dosage of 5 mg/kg BW has generally been suggested to be safe (19, 28). In the present study, a CES intake in a mean volume of 1,026 ml with 150, 225, and 320 mg Caf/l supplying amounts of Caf of 2.1, 3.2, and 4.5 mg/kg BW, respectively, did not result in any urinary Caf level near or above the IOC doping limit. Urinary Caf concentrations after exercise were surprisingly very low. With the highest Caf dosage of 4.5 mg/kg

BW, a mean urinary Caf concentration of 2.5 µg/ml was obtained. Other studies observed a postexercise mean urinary Caf concentration of 4.8 µg/ml in well-trained Caf users after ingestion of 5 mg/kg BW Caf (28) and 6.1 and 5.8 µg/ml in nontrained Caf users and nonusers, respectively (36). It is supposed that urinary Caf levels in the present study were so low because of spreading out the Caf dose throughout the experiment instead of using one single-bolus administration.

Despite the low level, the interindividual variations in the postexercise urinary Caf concentration were large (Fig. 4). Several subjects always had relatively high urinary Caf levels, whereas others always had relatively low urinary Caf levels. This was the case for all dosages given. These differences among subjects may be explained by individual variations in the rate at which Caf is metabolized by the liver.

Although it has been shown that Caf is a diuretic under resting conditions (18, 40), no Caf-induced diuresis has been observed during exercise (40). Also, in the present study no significant increase in urine volume was found as a result of Caf ingestion. It is possible that during exercise the diuretic effect of Caf may be counteracted by action of catecholamines, which induce constriction of renal arterioles and reduce glomerular filtration rate (6). Additionally, catecholamines may increase Na<sup>+</sup> and Cl<sup>-</sup> reabsorption in the proximal and distal tubules by affecting aldosterone and/or antidiuretic hormone, resulting in water conservation (2, 10). Additionally, Caf intake during exercise appears to have no effect on sweat loss, body temperature, and plasma volume (15). For this reason, the suggestion that Caf intake may result in a poor hydration status and thereby affect performance negatively is unfounded.

From the present data it is concluded that a relatively low dose of Caf added to CES supports 1-h time trial cycling performance. Furthermore, an ingestion of 4.5 mg Caf/kg BW did not result in further performance improvement compared with 3.2 mg Caf/kg BW. These ergogenic effects cannot be explained by differences in fat metabolism. No effects on diuresis were observed, and urinary Caf levels remained widely below the IOC doping limit in all individuals.

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