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Comparison of caffeine and theophylline ingestion: exercise metabolism and endurance

F. GREER, D. FRIARS, AND T. E. GRAHAM
Department of Human Biology and Nutritional Sciences,
University of Guelph, Guelph, Ontario, Canada N1G 2W1

Greer, F., D. Friars, and T. E. Graham. Comparison of caffeine and theophylline ingestion: exercise metabolism and endurance. *J Appl Physiol* 89: 1837–1844, 2000.— This two-part investigation compared the ergogenic and metabolic effects of theophylline and caffeine. Initially (*part A*), the ergogenic potential of theophylline on endurance exercise was investigated. Eight men cycled at 80% maximum O_2 consumption to exhaustion 90 min after ingesting either placebo (dextrose), caffeine (6 mg/kg; Caff), or theophylline (4.5 mg/kg Theolair; Theo). There was a significant increase in time to exhaustion in both the Caff (41.2 ± 4.8 min) and Theo (37.4 ± 5.0 min) trials compared with placebo (32.6 ± 3.4 min) ($P < 0.05$). In *part B*, the effects of Theo on muscle metabolism were investigated and compared with Caff. Seven men cycled for 45 min at 70% maximum O_2 consumption (identical treatment protocol as in *part A*). Neither methylxanthines (MX) affected muscle glycogen utilization ($P > 0.05$). Only Caff increased plasma epinephrine ($P < 0.05$), but both MX increased blood glycerol levels ($P < 0.05$). Muscle cAMP was increased ($P < 0.05$) by both MX at 15 min and remained elevated at 45 min with Theo. This demonstrates that both MX are ergogenic and that this can be independent of muscle glycogen.

adenosine antagonism; ergogenic; methylxanthines; adenosine 3′,5′-cyclic monophosphate; catecholamines

MANY STUDIES EXAMINING THE mechanism behind the ergogenic effect of caffeine have focused on the methylxanthine-induced increase in circulating plasma epinephrine and the metabolic changes that occur with exercise (12, 15, 19). However, recent studies have provided evidence that the original hypothesis by Costill et al. (12) may not be the critical mechanism behind the effects of caffeine in all exercise conditions. For example, an ergogenic effect of caffeine has been demonstrated without an increase in plasma epinephrine (20, 35). In addition, an increase in free fatty acid (FFA) mobilization without a corresponding catecholamine response has been demonstrated, indicating a direct effect of caffeine on fat cells (28, 35). Carbohydrate metabolism has been shown to be unaltered when epinephrine was infused to mimic the caffeine-induced epinephrine response (10). Glycogen sparing has not always been found during exercise after caffeine ingestion (9), and an increase in performance has been observed in exercise situations in which muscle glycogen is not the limiting factor and when no glycogen sparing was observed (23). A direct effect of caffeine on the central nervous system has also been postulated to explain the ergogenic effect of this methylxanthine; however, it has been demonstrated that this is not a critical mechanism (28).

Three possible mechanisms through which the methylxanthines may exert their metabolic effects include increased intracellular Ca^{2+} release (4), inhibition of cAMP phosphodiesterase (6), and antagonism of adenosine receptors (32). It is now established that adenosine receptor antagonism is the most relevant mechanism in vivo (17) because pharmacological doses of methylxanthines (mM) rather than physiological doses (μM) are needed to elicit a Ca^{2+} or phosphodiesterase inhibition effect (7, 33).

Methylxanthines are nonselective adenosine receptor antagonists at A_1 and A_2 receptors, and in vitro theophylline, a dimethyxanthine, is a more potent adenosine receptor antagonist than caffeine (5). Biaggioni et al. (3) demonstrated an attenuation of adenosine-induced cardiovascular effects after theophylline administration. In addition, Costa and Biaggioni (11) reported that adenosine administration increases muscle sympathetic nervous system activity and that this increase was dampened by theophylline infusion, providing evidence of a methylxanthine-induced antagonism of adenosine receptors in various human tissues.

Recently, Vergauwen et al. (37) found that adenosine receptor antagonism by caffeine stimulated rather then inhibited net glycogenolysis in a contracting isolated rat hindlimb perfusion. This study demonstrated that adenosine inhibits glycogenolysis in contracting oxidative muscle fibers and may be a potential modulator of carbohydrate metabolism. Furthermore, Raguso et al. (30) used stable isotopes and indirect calorimetry to determine the effect of theophylline on substrate metabolism during 30 min of moderate submaximal exercise. Glucose rate of disappearance was less after theophylline administration, suggesting that

adenosine antagonism decreased glucose uptake during exercise. Estimation of muscle glycogen utilization, based on respiratory exchange ratio (RER) data, led to the conclusion that adenosine may play a role in regulating carbohydrate metabolism by decreasing glycogenolysis in contracting skeletal muscle. These studies suggest that methylxanthines should increase muscle glycogen use via adenosine antagonism, yet previous investigations of exercising humans demonstrated a glycogen sparing effect of caffeine (14, 15, 34).

The purpose of this study was twofold: *1*) to investigate the ergogenic potential of theophylline on endurance exercise (*part A*) and *2*) to examine the effects of this compound on muscle metabolism and compare it to the effects of caffeine (*part B*). We hypothesized that theophylline would elicit an ergogenic effect and would stimulate a greater net glycogenolysis compared with placebo and caffeine because of its higher affinity for adenosine receptors.

METHODS

Subjects

Eight healthy, active male volunteers [mean maximum O_2 consumption ($\dot{V}O_{2\,max}$) = 57.5 ± 2.7 ml·kg^{-1}·min^{-1}] served as subjects for *part A*, whereas seven male subjects (mean $\dot{V}O_{2\,max}$ = 56.3 ± 3.5 ml·kg^{-1}·min^{-1}) volunteered for *part B*. All were physically active with no history of respiratory problems. The experimental procedures and possible risks of the study were explained, and informed written consents were given by all subjects. The study was approved by the University of Guelph Ethics Committee.

Preexperimental Protocol

Each subject reported to the laboratory on two occasions before the actual experimental trials. On the first visit, the subjects performed an incremental $\dot{V}O_{2\,max}$ test on an electrically braked cycle ergometer (Quinton Excalibur Sport). The second visit served as a practice ride for the subjects. For *part A*, subjects cycled for ~15–20 min at a power output selected to elicit ~80–85% $\dot{V}O_{2\,max}$. Subjects involved in *part B* cycled for 30–40 min at a power output selected to elicit ~65–70% $\dot{V}O_{2\,max}$. These sessions served to habituate the subject to the cycle ergometer and to confirm that the chosen power output was appropriate.

Experimental Protocol

Subjects reported to the laboratory three times in a postprandial state. Each trial was separated by ~1 wk. For a given subject, all trials were conducted at the same time of day. Subjects were asked to maintain their normal training schedule and diet throughout the duration of the study and were instructed to prepare for each trial as they would for competition, i.e., have a high-carbohydrate meal and be well rested. In addition, subjects were asked to abstain from all sources of caffeine and theophylline for 48 h before each testing session.

At the start of each test, a catheter was placed into the medial anticubital vein, and a normal saline drip was started to maintain catheter patency. A resting blood sample was obtained (referred to as −90 min), and the subject ingested a gelatin capsule containing either placebo (dextrose; Pl), caffeine (6 mg/kg; Caff), or theophylline (4.5 mg/kg Theolair; Theo). Preliminary work demonstrated that these doses of caffeine and theophylline resulted in similar plasma methylxanthine concentrations. Treatment was administered in a random, double-blind fashion. Peak blood levels of theophylline are reached 90 min after the ingestion of Theolair (31); thus after 90 min of resting quietly a second blood sample was obtained (0 min). Subjects warmed up with light stretching and cycling. The warm-up was accurately recorded during the first trial, and subjects performed the same routine in each subsequent trial.

Subjects involved in *part A* then cycled at a power output that required ~80–85% $\dot{V}O_{2\,max}$ to a point of voluntary exhaustion (Exh) (inability to maintain pedal cadence above 40 rpm, i.e., the lower limit of the electronically braked bicycle). Blood and expiratory gas samples were obtained every 15 min and within 5 min of Exh. All samples were taken while subjects were exercising. During the trials, subjects were given no external cues regarding time and were verbally encouraged to cycle to Exh in all trials.

Subjects involved in *part B* of this study were required to cycle for 45 min at ~65–70% $\dot{V}O_{2\,max}$. Blood samples and expiratory gas measurements were taken at 15, 30, and 45 min, and muscle biopsies were taken from the vastus lateralis immediately before exercise, 15 min into exercise, and at the end of the 45-min exercise period. The exercise intensity used in *part A* was chosen so that the results found in this study could be compared with previous work from our laboratory (20). In *part A*, Exh occurred at a different time for each trial, making comparison of metabolism difficult; therefore, a workload of 65–70% $\dot{V}O_{2\,max}$ was used in *part B* to ensure steady-state metabolism to permit comparisons at defined time points and for comparison with the work conducted by Raguso et al. (30).

Analyses

Expired air samples were analyzed for fractions of O_2 and CO_2 with an Applied Electrochemical S-3A O_2 analyzer and a Sensormedics LB-2 CO_2 detector, respectively. The analyzers were calibrated with gases of known concentrations. Blood samples were immediately separated into two aliquots: 3 ml were transferred to a nontreated tube for serum, and 7 ml were transferred to a sodium-heparinized tube. Hematocrit was measured in duplicate from the latter sample by using high-speed centrifugation. A modest hemoconcentration occurred in the exercise samples, but there was no difference among trials. A 100-μl aliquot of blood was added to 500 μl of 0.6 M perchloric acid. A solution of 120 μl of 0.24 M EGTA and reduced glutathione was then added to the remaining blood.

In *part B*, The EGTA- and glutathione-treated plasma was analyzed in duplicate for epinephrine and norepinephrine concentrations by HPLC (Waters) as described by Weiker (39). Plasma caffeine, paraxanthine, theophylline, and theobromine were measured by using fully automated HPLC (Waters). An aliquot of 150 μl of plasma was added to ~40 mg ammonium sulfate and 50 μl 0.05% acetic acid. After the addition of 25 μl of an internal standard solution [7-(β-hydroxypropyl)theophylline] and 3 ml chloroform-isopropyl alcohol (85:15 vol/vol) extracting solvent, the mixture was vortexed for 30 s and centrifuged for 10 min at 2,500 rpm. The organic phase was transferred and dried under oxygen-free N_2 and resuspended in HPLC mobile-phase solvent (3% isopropanol-0.05% acetic acid-0.5% methanol), and 100 μl were injected into a Beckman Ultrasphere IP C_{18} 5-μl column. Methylxanthines were measured at 282-nm wavelength. Reagents for standards were obtained from Sigma Chemical. The blood-acid extracts were analyzed enzymati-

cally in duplicate for lactate, glucose, and glycerol (2). Serum was analyzed enzymatically in duplicate for FFA (26).

The muscle biopsy samples in *part B* were immediately frozen in liquid N_2, removed from the needle, and stored at $-80°C$. The entire biopsy was then freeze dried and powdered to dissect out all nonmuscular elements. Two aliquots of powered muscle (3–4 mg) were used for the enzymatic determination of glycogen (22). An additional aliquot was extracted with 0.5 M perchloric acid (1.0 mM EDTA), neutralized with 2.2 M $KHCO_3$, and analyzed enzymatically for muscle citrate, lactate, and glucose 6-phosphate (G-6-P) (2). Muscle was extracted and analyzed for acetyl-CoA by using a radiolabeled technique (8). A final aliquot of muscle was extracted and analyzed for AMP concentration by using a radioimmunoassay (18). A portion of the biopsy was also extracted for the determination of total creatine on each muscle sample (16). All muscle metabolite contents were corrected to the highest total creatine content for each individual's biopsies. All muscle data are expressed per kilogram dry muscle (dm) weight.

Statistics

RER data, performance time to Exh, and the change in muscle glycogen levels between time points were compared by using a one-way ANOVA for repeated measures. All blood and muscle data were analyzed by using a two-way ANOVA (time by treatment) for repeated measures. If significance was found ($P < 0.05$), the Tukey test was performed as the post hoc analysis. Data are reported as means $\pm$ SE.

RESULTS

Part A

Performance. The most important finding in *part A* of the study was an increase ($P < 0.05$) in time to Exh in both the Caff and Theo trials compared with Pl (Fig. 1). All eight subjects rode longer after Caff ingestion vs. Pl, whereas six of the eight subjects rode longer after Theo ingestion vs. Pl. This represents a 22% increase in Exh after Caff ingestion and a 14% increase after Theo administration. There was no significant difference ($P > 0.05$) in Exh between the Caff and Theo trials. Subjectively, two subjects complained of nausea
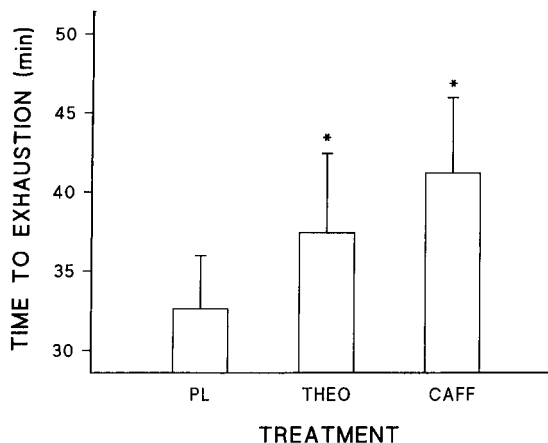

Fig. 1. Performance time to exhaustion cycling at ~80% maximum O_2 consumption ($\dot{V}O_{2\,max}$) after placebo (Pl), caffeine (Caff), or theophylline (Theo) ingestion. *Statistically significant from Pl ($P < 0.05$).

Table 1. *Summary of blood metabolite data for part A*

Treatment, mM	Time, min			
	−90	0	15	Exhaustion
Glucose				
Pl	3.88 ± 0.19^a	4.24 ± 0.19^a	4.11 ± 0.21^a	4.86 ± 0.32^a
Theo	4.16 ± 0.27^a	4.79 ± 0.35^a	4.48 ± 0.33^a	$5.37 \pm 0.45^{a*}$
Caff	3.90 ± 0.22^a	4.36 ± 0.41^a	4.55 ± 0.43^a	$5.49 \pm 0.36^{a*}$
Lactate				
Pl	1.06 ± 0.12^a	0.89 ± 0.10^a	6.63 ± 0.81^b	10.16 ± 0.83^c
Theo	1.14 ± 0.14^a	1.36 ± 0.20^a	$8.61 \pm 1.21^{b*}$	$11.92 \pm 1.33^{c*}$
Caff	1.23 ± 0.10^a	1.20 ± 1.14^a	7.54 ± 0.65^b	$11.78 \pm 0.68^{c*}$
FFA				
Pl	0.33 ± 0.03^a	0.33 ± 0.03^a	0.35 ± 0.04^a	0.38 ± 0.03^a
Theo	0.28 ± 0.03^a	0.38 ± 0.06^a	0.32 ± 0.03^a	0.41 ± 0.08^a
Caff	0.31 ± 0.03^a	0.35 ± 0.05^a	0.35 ± 0.05^a	0.32 ± 0.04^a
Glycerol				
Pl	0.41 ± 0.05^a	0.46 ± 0.07^a	0.68 ± 0.11^a	1.23 ± 0.13^b
Theo	0.48 ± 0.07^a	0.63 ± 0.07^a	0.82 ± 0.12^a	1.28 ± 0.10^b
Caff	0.44 ± 0.11^a	0.52 ± 0.12^a	0.57 ± 0.04^a	1.15 ± 0.08^b

Values are means $\pm$ SE. Treatment is indicated as placebo (Pl), theophylline (Theo), or caffeine (Caff). FFA, free fatty acid. Superscripted letters represent significance between time points. Values with the same letter are not significantly different from each other ($P > 0.05$). *Significantly different from Pl ($P < 0.05$).

and dizziness after Theo ingestion. This is a common complaint of individuals who are sensitive to this drug.

Plasma methylxanthines. Subjects had low levels of caffeine (0.2 ± 0.1 μM) and undetectable levels of theophylline before each trial, confirming their compliance to abstain from methylxanthine-containing substances. A dose of 4.5 mg/kg Theolair administered in the Theo trial resulted in a plasma theophylline concentration of 34.1 ± 1.5 μM with low levels of caffeine (0.18 ± 0.1 μM), paraxanthine (0.03 μM), and theobromine (2.89 ± 1.7 μM) when measured 15 min into the exercise session. At the same time point, after the ingestion of 6 mg/kg of Caff, plasma caffeine levels were 33.7 ± 2.2 μM with the following dimethyxanthine concentrations: 3.6 ± 0.5 μM paraxanthine, 3.3 ± 1.9 μM theobromine, and 0.62 ± 0.1 μM theophylline. Thus plasma theophylline levels in the Theo trial were almost identical to plasma caffeine levels in the Caff trial.

Pulmonary data. Mean RER data were not different ($P > 0.05$) in any of the three experimental conditions: Pl, 1.0 ± 0.03; Theo, 0.99 ± 0.02; and Caff, 1.0 ± 0.02. Similarly, mean O_2 consumption was also similar among treatments: Pl, $80.3 \pm 0.5\%$ $\dot{V}O_{2\,max}$; Theo, $81.4 \pm 0.4\%$ $\dot{V}O_{2\,max}$; and Caff, $79.7 \pm 0.7\%$ $\dot{V}O_{2\,max}$.

Blood-borne metabolites. A summary of the data for blood glucose, lactate, glycerol, and serum FFA are presented in Table 1. Blood glucose concentrations were similar at rest in all three treatments and increased slightly ($P > 0.05$) during exercise. Although there was a significant increase ($P < 0.05$) in blood glucose concentrations at Exh in the Caff and Theo trials compared with Pl, this is difficult to interpret physiologically as Exh represents different time points for each trial.

Theo ingestion resulted in a significant elevation ($P < 0.05$) in blood lactate throughout the exercise period compared with Pl. Blood lactate concentrations were significantly higher ($P < 0.05$) in the Caff trial compared with the Pl trial at Exh only. As expected, blood lactate increased progressively throughout the exercise period.

Neither Caff or Theo ingestion resulted in any change in serum FFA levels or blood glycerol concentrations. Serum FFA concentrations remained relatively stable throughout the duration of the exercise period, whereas blood glycerol concentrations were significantly higher ($P < 0.05$) at Exh than at either rest or 15 min of exercise in all three treatment conditions.

Part B

Plasma methylxanthines. As in *part A*, subjects arrived at the laboratory with low methylxanthine concentrations before each trial. Fifteen minutes into exercise in the Theo trial, plasma levels of theophylline were 30.3 ± 1.6 µM. Plasma caffeine concentrations in the Caff trial were 32.0 ± 2.9 µM.

Plasma catecholamines. Before capsule ingestion, plasma epinephrine levels were similar in all trials (Fig. 2). However, 90 min after the ingestion of Caff, plasma epinephrine concentration was significantly higher ($P < 0.05$) compared with that for Pl and Theo. Theo ingestion resulted in concentrations very similar to those with Pl. At 30 and 45 min of exercise, epinephrine concentration was statistically higher in the Caff trial compared with Pl. Although there was a trend for higher epinephrine values after Caff ingestion at these time points compared with Theo, this was not statistically significant ($P > 0.05$). As expected, plasma epinephrine levels increased ($P < 0.05$) in all trials as a result of exercise. There was no effect of Caff or Theo ingestion on plasma norepinephrine levels compared with that of Pl. At the onset of exercise, norepinephrine
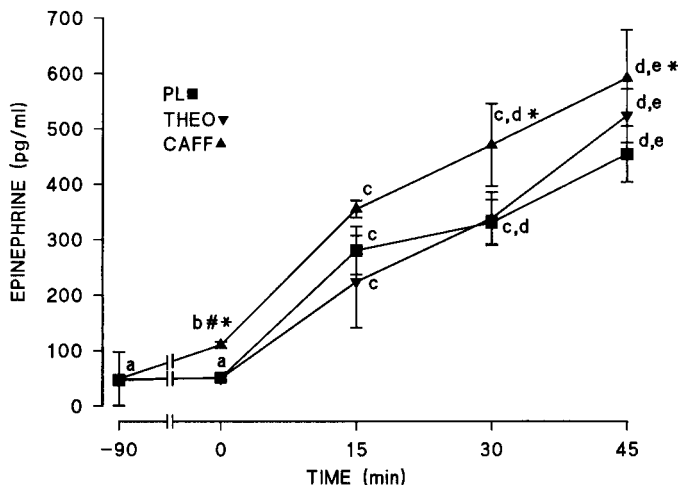

Fig. 3. Plasma norepinephrine concentration during cycling at ~70% $\dot{V}O_{2\,max}$ after Pl, Caff, or Theo ingestion. Values with the same superscripted letter are not significantly different from each other ($P > 0.05$).

concentration increased ($P < 0.05$) and remained elevated throughout the exercise session in all trials (Fig. 3).

Muscle glycogen and blood glucose. There was no effect ($P > 0.05$) of Theo or Caff on muscle glycogen net utilization at any time point throughout the exercise protocol (Fig. 4). Glycogen levels decreased ($P < 0.05$) by 102 ± 19 mmol glycosyl units/kg dm in the Pl trial within the first 15 min of exercise. Similar reductions also occurred in the Theo trial (115 ± 22 mmol glycosyl units/kg dm) and Caff trial (116 ± 31 mmol glycosyl units/kg dm). As expected, the net rate of glycogenolysis was reduced ($P < 0.05$) in the subsequent 30 min by about 50%. Overall net glycogen utilization was also similar among trials. Neither Theo or Caff ingestion had any statistically significant effect ($P > 0.05$) on blood glucose at any time throughout the exercise protocol compared with the Pl trial (Table 2).

Fig. 2. Plasma epinephrine concentration during cycling at ~70% $\dot{V}O_{2\,max}$ after Pl, Caff, or Theo ingestion. Values with the same superscripted letter are not significantly different from each other ($P > 0.05$). *Significantly different from Pl ($P < 0.05$). #Significantly different from Theo ($P < 0.05$).
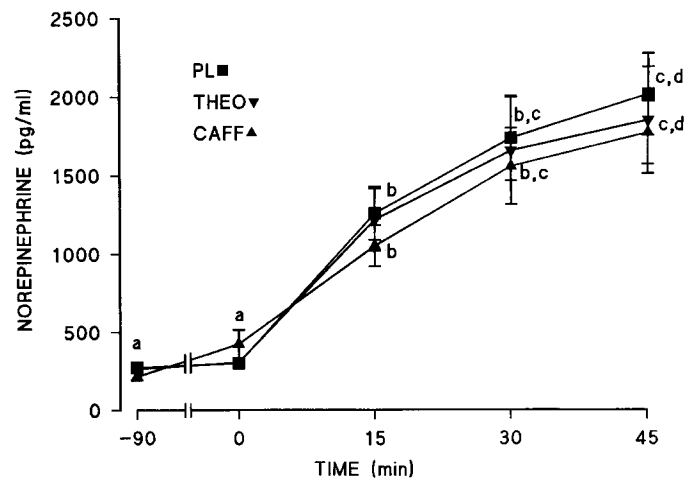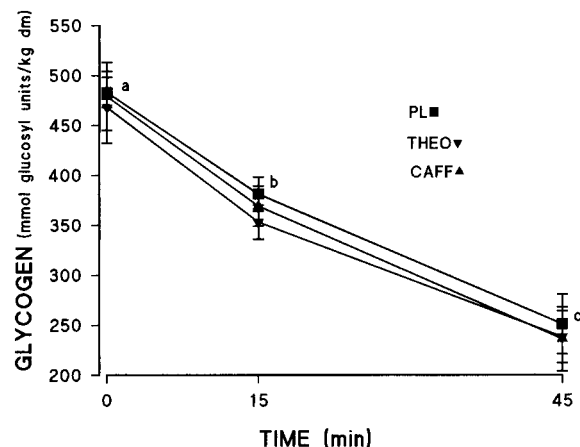

Fig. 4. Muscle glycogen contents during cycling at ~70% $\dot{V}O_{2\,max}$ after Pl, Caff, or Theo ingestion. dm, Dry muscle. Within a treatment, values with the same superscripted letter are not significantly different from each other ($P > 0.05$).

Table 2. *Summary of blood metabolite data for part B*

Treatment, mM	Time, min				
	−90	0	15	30	45
Glucose					
Pl	4.33 ± 0.42^a	4.35 ± 0.23^a	3.49 ± 0.24^a	3.84 ± 0.14^a	3.65 ± 0.25^a
Theo	4.23 ± 0.35^a	3.96 ± 0.45^a	3.98 ± 0.28^a	3.54 ± 0.35^a	3.87 ± 0.24^a
Caff	4.72 ± 0.33^a	4.52 ± 0.29^a	4.03 ± 0.28^a	3.85 ± 0.27^a	4.17 ± 0.24^a
FFA					
Pl	0.27 ± 0.04^a	0.23 ± 0.04^a	0.26 ± 0.05^a	0.36 ± 0.06^a	0.39 ± 0.05^b
Theo	0.20 ± 0.03^a	0.35 ± 0.07^a	0.38 ± 0.07^a	0.47 ± 0.04^a	0.65 ± 0.09^b
Caff	0.37 ± 0.11^a	0.46 ± 0.14^a	0.39 ± 0.08^a	0.45 ± 0.09^a	0.53 ± 0.09^b
Glycerol					
Pl	0.30 ± 0.02^a	0.32 ± 0.03^a	0.45 ± 0.05^a	0.63 ± 0.1^a	0.77 ± 0.11^a
Theo	0.32 ± 0.03^a	0.53 ± 0.08^a	$0.74 \pm 0.09^{b*}$	$0.96 \pm 0.12^{b*}$	$1.21 \pm 0.16^{c*\dagger}$
Caff	0.38 ± 0.05^a	0.48 ± 0.06^a	$0.66 \pm 0.09^{b*}$	$0.80 \pm 0.14^{b*}$	$1.04 \pm 0.21^{b*}$
Lactate					
Pl	0.91 ± 0.15^a	0.73 ± 0.14^a	3.81 ± 0.56^b	3.78 ± 0.59^b	3.82 ± 0.42^b
Theo	1.05 ± 0.1^a	1.05 ± 0.18^a	4.31 ± 0.37^b	4.27 ± 0.62^b	4.19 ± 0.25^b
Caff	0.93 ± 0.14^a	0.99 ± 0.17^a	4.25 ± 0.55^b	4.14 ± 0.65^b	4.10 ± 0.63^b

Values are means ± SE. Superscripted letters represent significance between time points. Values with the same letter are not significantly different from each other ($P > 0.05$). *Significantly different from Pl ($P < 0.05$). †Significantly different from Caff ($P < 0.05$).

Muscle and blood lactate. Muscle lactate concentration (Table 3) was not significantly ($P > 0.05$) affected by methylxanthine ingestion. However, at 15 min of exercise, there was a trend ($P = 0.07$) for higher lactate levels in the Caff and Theo trials compared with Pl. This trend was maintained at 45 min in the Theo trial. As expected, blood lactate levels increased ($P < 0.05$) with exercise; however, there was no effect of treatment on this metabolite (Table 2).

Muscle G-6-P. There was no effect of Caff or Theo ingestion on G-6-*P* concentrations compared with Pl (Table 3). Intramuscular G-6-*P* concentrations were significantly higher ($P < 0.05$) at 15 and 45 min of exercise compared with rest.

Muscle citrate. There was no statistically significant effect of Theo or Caff on citrate values compared with Pl at any time point throughout the protocol (Table 3). Exercise resulted in an increase ($P < 0.05$) in citrate levels by 15 min, which was maintained throughout the end of exercise when compared with resting concentrations.

Acetyl-CoA. Neither Caff nor Theo had any effect on acetyl-CoA concentrations at rest or during exercise compared with Pl (Table 3). In all three trials, acetyl CoA concentrations increased significantly ($P < 0.05$) over resting values during exercise.

Muscle cAMP. Caff and Theo ingestion resulted in a significant increase ($P < 0.05$) in muscle cAMP concentration 15 min into the exercise protocol compared with Pl (Fig. 5). This elevation in cAMP levels was maintained in the Theo trial so that by 45 min, cAMP concentration in the Theo trial was significantly higher ($P < 0.05$) compared with that in the Caff and Pl trials.

Table 3. *Summary of muscle metabolite data for part B*

Treatment, mmol/kg dm	Time, min		
	0	15	45
G-6-*P*			
Pl	0.82 ± 0.08^a	1.99 ± 0.32^b	2.75 ± 0.28^b
Theo	0.88 ± 0.18^a	2.59 ± 0.25^b	3.47 ± 0.43^b
Caff	0.90 ± 0.18^a	2.54 ± 0.28^b	2.53 ± 0.42^b
Citrate			
Pl	0.24 ± 0.04^a	0.56 ± 0.04^b	0.68 ± 0.06^b
Theo	0.39 ± 0.04^a	0.73 ± 0.08^b	0.74 ± 0.08^b
Caff	0.39 ± 0.07^a	0.69 ± 0.07^b	0.76 ± 0.12^b
Acetyl-CoA			
Pl	13.08 ± 2.23^a	28.33 ± 3.36^b	34.05 ± 4.67^b
Theo	16.25 ± 1.03^a	33.97 ± 4.76^b	31.51 ± 3.73^b
Caff	14.95 ± 2.02^a	29.62 ± 3.21^b	31.73 ± 3.82
Lactate			
Pl	5.01 ± 1.05^a	14.69 ± 2.28^b	15.8 ± 1.67^b
Theo	5.07 ± 0.61^a	22.45 ± 2.95^b	20.1 ± 4.04^b
Caff	5.03 ± 0.74^a	19.35 ± 1.58^b	15.14 ± 2.99^b

Values are means ± SE. dm, Dry muscle; G-6-*P*, glucose 6-phosphate; superscripted letters represent significance between time points. Values with the same letter are not significantly different from each other ($P > 0.05$).
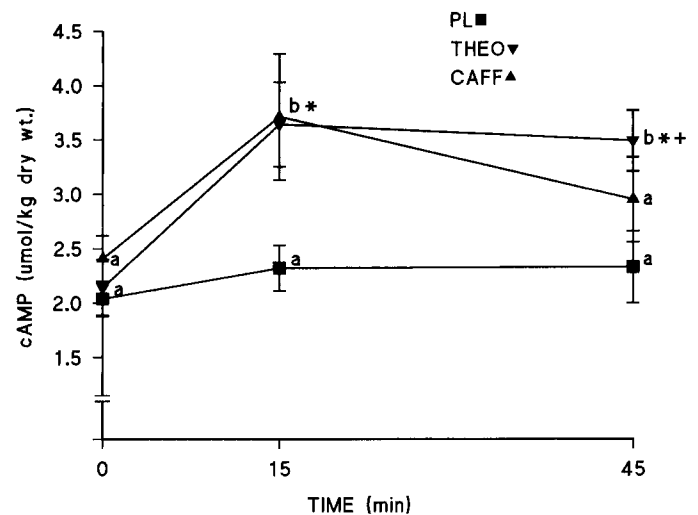

Fig. 5. Muscle cAMP concentration during cycling at ~70% $\dot{V}_{O_{2\,max}}$ after Pl, Caff, or Theo ingestion. Values with the same superscripted letter are not significantly different from each other ($P > 0.05$). *Statistically significant from Pl ($P < 0.05$). +Statistically significant from Caff ($P < 0.05$).

Respiratory data. RER was significantly higher ($P <$ 0.05) in the Caff trial compared with Theo (0.91 ± 0.02 vs. 0.87 ± 0.02), whereas neither treatment was different ($P > 0.05$) compared with Pl (0.90 ± 0.02). This represented a 13% decrease in carbohydrate utilization in the Theo compared with Caff trial. As in *part A*, the mean O_2 consumption was not different ($P > 0.05$) for any of the experimental conditions: Pl, $67.9 \pm 0.5\%$ $\dot{V}O_{2\,max}$, Theo, $69.5 \pm 0.7\%$ $\dot{V}O_{2\,max}$, and Caff, $68.1 \pm 0.6\%$ $\dot{V}O_{2\,max}$.

Blood glycerol and FFAs. There was a trend ($P = 0.08$) for higher blood glycerol concentrations 90 min after Theo ingestion vs. Pl (0.53 ± 0.08 vs. 0.32 ± 0.03 mM) (Table 2). Once exercise began, blood glycerol levels were significantly higher ($P < 0.05$) in both the Theo and Caff trials compared with Pl and remained elevated over Pl levels until the end of the exercise period. In addition, blood glycerol concentrations were significantly higher ($P < 0.05$) in the Theo trial compared with the Caff trial at 45 min. There was no effect of treatment on plasma FFA concentrations. At the end of exercise, FFA levels were significantly higher ($P < 0.05$) compared with other time points at rest and during exercise. (Table 2).

DISCUSSION

This two-part study examined the effect of theophylline on whole body endurance exercise performance and skeletal muscle metabolism. The major finding in *part A* of this investigation was an ergogenic benefit of theophylline on exercise performance. These results are in agreement with the only other study to examine the effect of theophylline on skeletal muscle endurance and metabolism (25). In a limited study ($n = 3$), Marsh et al. (25) investigated the effect of theophylline on the metabolism of wrist flexor muscles during a progressive $\dot{V}O_{2\,max}$ exercise test (~16 min) and concluded that a therapeutic dose of theophylline significantly increased the endurance of forearm musculature. The present study is the first to examine the potential ergogenic effect of theophylline on whole body endurance exercise and the first to compare its effects to those elicited by caffeine. Subjects rode on average 5 min longer in the Theo trial vs. Pl and 9 min longer after Caff ingestion compared with Pl. The dose of Theo used in the present study (4.5 mg/kg Theolair, resulting in plasma theophylline levels of 34.1 ± 1.5 μM) was chosen to be at the low end of the therapeutic range (31). However, even at this low dose of Theo, two of the eight subjects in *part A* experienced nausea and dizziness. Subsequently, one of these two subjects rode longer on the Pl vs. Theo. None of the eight subjects reported any adverse effects after Caff ingestion. These results do support our hypothesis that theophylline is an ergogenic aid, and, as discussed below, some of the data from *part B* support the theory that theophylline is a more potent adenosine receptor antagonist compared with caffeine.

A major finding of *part B* was the lack of a glycogen-sparing effect of Theo or Caff compared with Pl during 45 min of cycling at 70% $\dot{V}O_{2\,max}$. Chesley et al. (9) found no influence of caffeine on net glycogenolysis during 15 min of cycling at 85% $\dot{V}O_{2\,max}$ in 13 untrained men. This is in contrast to several earlier investigators (15, 16, 34), who reported glycogen sparing after caffeine ingestion during endurance exercise. However, other investigations have reported an increase in the rate of glycogenolysis in active skeletal muscle after methylxanthine administration (30, 37). Whereas the results of Vergauwen et al. (37) may not be directly applicable to the present data, as they used a perfused rat hindlimb preparation, Raguso et al. (30) suggested similar findings in human subjects.

Under similar exercise conditions (subjects cycled for 30 min at ~70–75% $\dot{V}O_{2\,max}$) and plasma theophylline concentrations (~30 μM), Raguso et al. (30) observed a reduction in the glucose rate of disappearance with no change in RER and concluded that adenosine antagonism may play a role in regulating glucose uptake and glycogenolysis in contracting skeletal muscle. The increase in muscle glycogen utilization reported by Raguso et al. is in contrast to the negligible effect of methylxanthines on carbohydrate metabolism found in the present study. It is quite apparent from the conflicting results in the literature that there must be interacting factors that determine the effect of methylxanthines on muscle carbohydrate metabolism, and, as will be discussed below, the specific distribution of adenosine receptors may be critical.

One possible explanation for the conflicting results for the glycogen sparing effects of caffeine could be different fiber types used in different exercise intensities. Vergauwen et al. (36, 37) provide functional evidence for the existence of the adenosine receptor subtype A_1-dependent mechanism of action in stimulating insulin-dependent glucose transport in slow-twitch skeletal muscle, whereas our laboratory has presented preliminary evidence demonstrating the physical presence of A_2 adenosine receptors in rat slow-twitch skeletal muscle (21). Furthermore, skinned, slow-twitch human fibers have been shown to be about two to three times more sensitive to caffeine compared with fast-twitch fibers (27).

To our knowledge, this is the first study to measure the effects of methylxanthines on cAMP concentration in human muscle. The low dose of caffeine used in the present experiment exerts most, if not all, of its physiological action via inhibition of cellular A_1 and A_2 receptors (17). This implies that A_1-receptor antagonism by Caff and Theo resulted in an elevation of muscle cAMP concentration during exercise. Both methylxanthines resulted in elevated cAMP 15 min into exercise compared with Pl. This elevation was maintained in the Theo trial so that, by 45 min, cAMP concentration was significantly higher compared with that in the Caff and Pl trials. This is consistent with theophylline being a more potent adenosine receptor antagonist compared with caffeine (5). These data demonstrating an elevation in cAMP levels after adenosine receptor antagonism are consistent with what has previously been reported (36) and provide addi-

tional functional evidence for the existence of A_1 receptors in skeletal muscle.

The negligible effect of theophylline ingestion on epinephrine is similar to the study by Raguso et al. (30) yet is in contrast to other studies (38). The caffeine-induced increase in plasma epinephrine is similar to what has previously been reported (20). However, these investigators concluded that the increase in plasma epinephrine levels as a result of caffeine ingestion had no detectable effect on metabolism. This is consistent with van Soeren et al. (35) and Mohr et al. (28), who reported caffeine-induced metabolic effects without an increase in catecholamines in tetraplegic patients. Furthermore, the data in the present study suggest that theophylline and caffeine affect central nervous system regulation of the sympathetic nervous system differently.

Inherent in the mechanisms that have been proposed to explain the glycogen-sparing effect of caffeine is the assumption that fat oxidation is increased after caffeine ingestion. Because adenosine is a potent inhibitor of lipolysis in vivo (29), adenosine antagonism by methylxanthines should stimulate lipolysis. Our data from *part B* are in agreement with this hypothesis because both caffeine and theophylline resulted in increased blood glycerol levels. Furthermore, 45 min into exercise in *part B*, blood glycerol concentration was higher in the Theo trial vs. Caff and Pl. This supports further the hypothesis that adenosine may be involved in the regulation of lipid metabolism, because theophylline is a more potent adenosine receptor antagonist than caffeine (5). These data provide additional support for the argument that various metabolic responses to methylxanthines are not always related to a catecholamine-induced mechanism.

It has been suggested that increases in skeletal muscle citrate and acetyl-CoA concentrations may inhibit phosphofructokinase and pyruvate dehydrogenase reactions, resulting in decreased muscle glycogenolysis (13, 40), and that this could be enhanced by methylxanthines as a result of an increase in fat metabolism. In the present study, there was no significant effect of methylxanthine ingestion on intramuscular acetyl-CoA, citrate, or G-6-*P* concentrations, which is internally consistent with the lack of glycogen sparing observed. These data are also in general agreement with Spriet et al. (34). Although they reported a glycogen sparing effect of caffeine early in the exercise, muscle citrate and acetyl CoA concentrations remained similar between treatments.

Theo ingestion resulted in an increase in blood lactate levels at all time points during exercise compared with Pl when subjects were cycling at 80% $\dot{V}O_{2\,max}$ (*part A*), whereas the only effect of Caff on blood lactate concentration vs. Pl was at Exh. Only one study has examined the effect of caffeine on muscle lactate levels (23). That study reported an increase in muscle lactate concentration during exercise bouts of fixed power output (100% $\dot{V}O_{2\,max}$) and duration (2 min) after caffeine ingestion. Muscle lactate in the present study was not significantly affected by methylxanthine ingestion;

however, the exercise intensity was much lower (~70% $\dot{V}O_{2\,max}$).

The significant increase in muscle cAMP and the tendency for an increase in muscle lactate illustrated in *part B* both imply that muscle glycogenolysis may have been enhanced, even though our glycogen data do not support this. Given the variability (~10%) (1) encountered when muscle glycogen utilization is quantified by using the muscle biopsy technique, a small, but perhaps significant, effect of Theo and/or Caff on glycogen use could have been missed. Furthermore, it is possible that the exercise intensity used in *part B* (~70% $\dot{V}O_{2\,max}$) was too low to show an effect of adenosine receptor antagonism on muscle glycogenolysis. That is, antagonizing adenosine receptors and tighter coupling between the activation of β-receptors and cAMP production may only affect muscle glycogen use when catecholamine (epinephrine) and adenosine concentrations are especially high, such as in intense exercise. This possibility is supported by the observation that blood lactate levels were significantly elevated by Theo when subjects were cycling at ~80% $\dot{V}O_{2\,max}$ (*part A*).

In conclusion, the ingestion of Theo resulted in a 14% increase in performance during whole body cycling exercise at 80% $\dot{V}O_{2\,max}$. One practical application of this finding is that theophylline should be considered an ergogenic aid. Presently, theophylline is not a banned or controlled substance by the International Olympic Committee; however, our results support the hypothesis that theophylline is an ergogenic aid. These data also illustrate that muscle glycogen sparing is not always observed after the ingestion of caffeine or theophylline. Furthermore, the lack of epinephrine increase as a result of theophylline ingestion demonstrates that methylxanthines do not always assert their metabolic effects through catecholamine-induced mechanisms. In addition, adenosine receptor antagonism in both adipose tissue and skeletal muscle by the methylxanthines may be involved in the regulation of substrate metabolism during exercise. The former is evidenced by an increase in glycerol, and the latter by an increase in muscle cAMP. These data indicate that adenosine is an important regulator of metabolism in these tissues.

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