

Regulation of muscle glycogenolytic flux during intense aerobic exercise after caffeine ingestion

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Chesley, Alan, Richard A. Howlett, George J. F. Heigenhauser, Eric Hultman, and Lawrence L. Spriet. Regulation of muscle glycogenolytic flux during intense aerobic exercise after caffeine ingestion. *Am. J. Physiol.* 275 (Regulatory Integrative Comp. Physiol. 44): R596–R603, 1998.—This study examined the effects of caffeine (Caf) ingestion on muscle glycogen use and the regulation of muscle glycogen phosphorylase (Phos) activity during intense aerobic exercise. In two separate trials, 12 untrained males ingested either placebo (Pl) or Caf (9 mg/kg body wt) 1 h before cycling at 80% maximum O₂ consumption ($\dot{V}O_{2max}$) for 15 min. Muscle biopsies were obtained from the vastus lateralis at 0, 3, and 15 min of exercise. In this study, glycogen “sparing” was defined as a 10% or greater reduction in muscle glycogen use during exercise after Caf ingestion compared with Pl. Muscle glycogen use decreased by 28% (Pl 255 ± 38 vs. Caf 184 ± 24 mmol/kg dry muscle) after Caf in six subjects [glycogen spacers (Sp)] but was unaffected by Caf in six other subjects [nonsparers (NSp), Pl 210 ± 35 vs. Caf 214 ± 37 mmol/kg dry muscle]. In both groups, Caf significantly increased resting free fatty acid concentration, significantly increased epinephrine concentration by twofold during exercise, and increased the Phos *a* mole fraction at 3 min of exercise compared with Pl, although not significantly. Caf improved the energy status of the muscle during exercise in the Sp group: muscle phosphocreatine (PCr) degradation was significantly reduced (Pl 47.9 ± 3.6 vs. Caf 40.4 ± 6.7 mmol/kg dry muscle at 3 min) and the accumulations of free ADP and free AMP (Pl 6.8 ± 1.3 vs. Caf 3.1 ± 1.4 μmol/kg dry muscle at 3 min; Pl 8.7 ± 0.8 vs. Caf 4.7 ± 1.1 μmol/kg dry muscle at 15 min) were significantly reduced. Caf had no effect on these measurements in the NSp group. It is concluded that the Caf-induced decrease in flux through Phos (glycogen-sparing effect) is mediated via an improved energy status of the muscle in the early stages of intense aerobic exercise. This may be related to an increased availability of fat and/or ability of mitochondria to oxidize fat during exercise preceded by Caf ingestion. It is presently unknown why the glycogen-sparing effect of Caf does not occur in all untrained individuals during intense aerobic exercise.

glycogen phosphorylase; cellular energy status; fat metabolism; variability

CAFFEINE (Caf) ingestion has been shown to decrease the rate of muscle glycogenolysis during moderate to intense aerobic exercise in endurance-trained subjects (11, 12, 22). It has been suggested that the glycogen-“sparing” effect of Caf is due to enhanced intramuscular and/or extramuscular fat mobilization and oxidation (7, 12, 22). Previous studies have provided support for this suggestion as muscle glycogen use was decreased during intense aerobic exercise when plasma free fatty acid (FFA) concentrations were elevated by Intralipid-

heparin infusion (9, 10). The decreased glycogen use in the high-FFA condition was associated with reductions in phosphocreatine (PCr) degradation and free P_i (P_{if}) and AMP (AMP_f) accumulations. Reductions in muscle glycogenolysis, PCr degradation, and P_{if} and AMP_f accumulations are also hallmark adaptations after endurance training when fat oxidation is increased (4, 15, 17). The results from these studies suggest that increased fat provision and an increased ability to oxidize fat improves the energy status of the cell at the onset of exercise and contributes to a reduction in muscle glycogenolysis during aerobic exercise.

Muscle glycogen is quantitatively the most important substrate for replenishing the ATP supply during intense aerobic exercise, and glycogen phosphorylase (Phos) is the rate-limiting enzyme for muscle glycogenolysis. Phos activity is regulated by a two-stage process. The first stage is transformation from the less active form of Phos *b* to the more active *a* form, which is mediated by Ca²⁺ and epinephrine (Epi) and may be regarded as a gross control mechanism that determines the potential upper limit for glycogenolytic flux. The second stage is posttransformational control of Phos by substrate availability (P_{if}) and allosteric modulation (AMP_f). This level of control fine tunes the glycogenolytic rate to the ATP demand and therefore to the energy state of the cell.

It is presently unknown if differences in cellular energy status account for caffeine's glycogen-sparing effects during intense aerobic exercise. Therefore, the purpose of this study was to examine how Caf regulates Phos activity during intense aerobic exercise in untrained subjects. We hypothesized that the sympathoadrenergic activation after Caf ingestion would increase the transformation of Phos *b* to *a* during exercise. However, despite the increased transformation, substrate and/or allosteric modulators (posttransformational factors) would account for a lower flux through Phos and a reduction in muscle glycogen use after Caf ingestion.

METHODS

Subjects. Twelve male subjects participated in the study. Their mean age, weight, and height were 23.9 ± 0.9 yr, 76.3 ± 3.6 kg, and 1.76 ± 0.02 m, respectively. None of the subjects was well trained, but many participated in some form of light physical activity. The experimental procedures were explained verbally and in writing, and informed consent was obtained from each subject. The experiment was approved by the University of Guelph Ethics Committee.

Experimental design. Subjects visited the laboratory on two occasions before commencing the study. On the first visit each subject performed an incremental maximal oxygen

uptake ($\dot{V}O_{2\max}$) test on a cycle ergometer. The mean $\dot{V}O_{2\max}$ for the group was 4.09 ± 0.13 l/min or 53.6 ± 2.8 ml·kg⁻¹·min⁻¹ (means \pm SE). During the second visit subjects performed a 15-min practice ride at 80% $\dot{V}O_{2\max}$. Subjects then reported to the laboratory on two occasions separated by at least 1 wk. During each trial subjects cycled at $\sim 80\%$ $\dot{V}O_{2\max}$ for 15 min. They received either Caf (9 mg/kg body wt) or placebo (Pl) in random fashion. This dose has been shown to spare muscle glycogen in trained subjects during intense aerobic exercise (22).

The subjects were instructed to maintain a high-carbohydrate diet for 2 days before each trial to ensure that muscle and liver glycogen stores were optimal, and they reported to the laboratory in the fed state. In addition, the subjects abstained from caffeine consumption 48 h before each trial. Before the study, six subjects were Caf users (>200 mg/day), two were light users (50–100 mg/day), and four were nonusers.

Experimental protocol. On arrival at the laboratory, subjects reclined on a bed and a catheter was placed in an antecubital vein and kept patent with a saline drip. A heating pad was placed over the catheterized forearm to arterialize the venous blood. A resting blood sample was obtained before the ingestion of Caf or Pl (–60 min) and the subjects then rested for 1 h (Fig. 1). Before the start of exercise three small incisions were made under local anesthesia in the skin overlying the vastus lateralis muscle. A resting biopsy sample was obtained, and a second resting blood sample was drawn at 0 min.

Subjects cycled for 15 min at $\sim 80\%$ $\dot{V}O_{2\max}$ (Fig. 1). Blood was sampled at 1.5, 5, 10, and 14 min of exercise, and muscle samples were obtained at 3 and 15 min. Expired gas samples were obtained from 6 to 8 and 12 to 14 min of exercise.

Analytic methods. Expired gas samples were analyzed online with a Sensor Medics 2900Z metabolic measurement cart (Yorba Linda, CA).

Muscle biopsies were immediately frozen in liquid N₂. The samples were freeze-dried, dissected free of visible connective tissue, and powdered. Two aliquots (3–4 mg each) were used for the enzymatic determination of muscle glycogen (16) on the 0- and 15-min exercise samples. A third aliquot (10–15 mg) was extracted with 0.5 M HClO₄ (with 1.0 mM EDTA) and neutralized with 2.2 M KHCO₃ for the determination of ATP, PCr, creatine (Cr), and lactate (La) (16).

A fourth aliquot of muscle obtained during exercise was analyzed for Phos activity in the direction of glycogen breakdown according to the method of Young et al. (28). For the determination of Phos activity, 3–4 mg of muscle were homogenized at –20°C in 0.2 ml of 100 mM Tris·HCl (pH 7.5) containing 60% glycerol, 50 mM potassium fluoride, and 10 mM EDTA. Homogenates were then diluted with 0.8 ml of the same buffer without glycerol and homogenized further at 0°C. Total (*a+b*) Phos activity (measured in the presence of 3 mM AMP) and Phos *a* activity (measured in the absence of added

AMP) were measured at 30°C with a spectrophotometer at 340 nm. Phos activity was measured with 20 mmol/l P_i, and the maximum velocity (V_{\max}) *a* and V_{\max} (*a+b*) were calculated using Michaelis constant (K_m) values for P_i of 26.2 and 6.8 mmol/l, respectively (average of values in Refs. 3, 5). V_{\max} was derived from the equation of Lineweaver and Burk (18): $1/V = (K_m/V_{\max})(1/S) + (1/V_{\max})$, where *V* is initial reaction rate expressed as millimoles per kilogram dry muscle per minute, *S* is P_i concentration in millimoles per liter, and K_m is either 26.2 or 6.8 millimoles per liter. The mole fraction of Phos *a* is presented as a percentage and calculated from V_{\max} *a*/ V_{\max} (*a+b*) $\times 100$.

A fifth aliquot of muscle from one biopsy sample was assayed in duplicate for citrate synthase (CS) activity according to the method of Srere (23).

The free contents of ADP (ADP_f) and AMP_f were estimated on the basis of the near equilibrium nature of the Cr phosphokinase and adenylate kinase reactions, respectively. ADP_f was calculated using the measured values of ATP, PCr, Cr, and the calculated H⁺ concentration. Intramuscular H⁺ concentrations were estimated from the muscle La contents according to the regression equation of Sahlin et al. (21), and a K_{obs} value of 1.66×10^{-9} M was employed. AMP_f was calculated from the ATP content, the calculated ADP_f value, and a K_{obs} value of 1.05 M (8). P_i_f at rest was assumed to be 10.8 mmol/kg dry muscle (3). This value was added to the difference between resting and exercise PCr contents to estimate P_i_f during exercise (8).

All muscle metabolite contents and Phos and CS activities data were corrected to the highest total Cr content for an individual's biopsies. Muscle metabolite data are expressed as millimoles per kilogram dry muscle, except ADP_f and AMP_f, which are micromoles per kilogram dry muscle. Phos and CS activities are expressed as millimoles per kilogram dry muscle per minute.

To determine plasma Epi, 7 ml of blood were added to a heparinized vacutainer with 120 μ l of 0.24 M EGTA-reduced glutathione. The tubes were centrifuged for 15 min, and the plasma was stored at –80°C until analysis. Plasma Epi and resting plasma Caf levels were determined by high-performance liquid chromatography, as described previously (14, 25).

A 200- μ l aliquot of heparinized blood was deproteinized with 1.0 ml of 0.6 N HClO₄, and the supernatant was used for the enzymatic determination of whole blood glucose and La (1). For plasma FFA determinations, 1 ml of heparinized blood was rapidly centrifuged and 400 μ l of plasma was added to 100 μ l 5 M NaCl and heated at 56°C for 30 min to inactivate lipoprotein lipase (10). Plasma was stored at –80°C and analyzed with an enzymatic colorimetric kit (Wako NEFA C kit; Wako Chemicals, Dallas, TX).

Statistical analysis. All data are means \pm SE. Unpaired *t*-tests were used to compare $\dot{V}O_{2\max}$, plasma Caf levels, and total Phos, and CS activities between sparsers (Sp) and nonsparers (NSp).

On the remaining data, no statistical comparisons were made between the Sp and NSp groups. Oxygen uptake (% $\dot{V}O_{2\max}$), muscle and blood metabolites, Phos *a* mole fractions, and plasma Epi concentrations were analyzed independently in the Sp group and in the NSp group using two-way repeated-measures ANOVA (trial \times time). When a significant *F* ratio was found, a least-significant difference post hoc test was employed to locate differences between specific means. Statistical significance was accepted at $P \leq 0.05$.

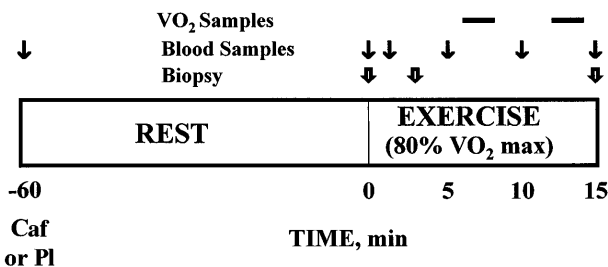


Fig. 1. Experimental protocol. Caf/Pl, caffeine or placebo ingestion. $\dot{V}O_2$, O₂ consumption; $\dot{V}O_{2\max}$, maximum $\dot{V}O_2$.

RESULTS

Glycogen-sparing effect of Caf. Glycogen sparing was defined in the present study as a 10% or greater reduction in muscle glycogen use during 15 min of cycling at $\sim 80\%$ $\dot{V}O_{2\max}$ after Caf ingestion compared with the Pl condition. Previous work in our laboratory demonstrated a reliability of 3–10% for muscle glycogen measured in muscle samples from repeat needle biopsies (26). Of the 12 subjects in the present study, six demonstrated muscle glycogen sparing after Caf ingestion. The average sparing was a $28 \pm 2.6\%$ reduction in muscle glycogen use, with a range of 21–38% (Table 1). The remaining six subjects did not spare muscle glycogen after Caf ingestion and used an average $2.8 \pm 3.1\%$ more glycogen in the Caf trial (Table 1). The preexercise muscle glycogen contents were similar in the Pl and Caf trials both within and between the Sp and NSp groups (Table 1). In the Sp group, muscle glycogenolysis was 255 ± 38 mmol/kg dry muscle in the Pl trial compared with 184 ± 24 mmol/kg dry muscle during the Caf trial. In contrast, muscle glycogenolysis was unaffected by Caf in the NSp group (Pl 210 ± 35 vs. Caf 214 ± 37 mmol/kg dry muscle).

Characteristics of glycogen Sp and NSp. The mean $\dot{V}O_{2\max}$ was not significantly different between groups (Sp 54.6 ± 3.5 vs. NSp 54.8 ± 4.4 ml·kg⁻¹·min⁻¹). Mean CS activities were also similar (Sp 45.1 ± 7.2 vs. NSp 46.0 ± 4.2 mmol·kg dry muscle⁻¹·min⁻¹). The calculated maximal Phos *a+b* activities (V_{\max} *a+b*) were not different between groups or affected by Caf (Sp: Pl 137.4 ± 8.1 vs. Caf 134.7 ± 9.8 ; NSp: Pl 145.9 ± 11.4 vs. Caf 139.9 ± 8.3 mmol·kg⁻¹·min⁻¹).

Plasma Caf concentrations were not different between groups (Sp 48.2 ± 9.8 vs. NSp 52.7 ± 10.3 μM) at the onset of exercise (1 h after Caf ingestion). $\dot{V}O_2$ was

Table 1. Individual muscle glycogen data for glycogen sparsers and nonsparers

Subject	Placebo			Caffeine		
	0 min	15 min	Delta	0 min	15 min	Delta
<i>Sparers</i>						
JM	480.1	235.2	244.9	357.0	162.7	194.3
AC	352.3	177.2	175.1	628.9	491.1	137.8
IN	588.9	265.8	323.1	443.5	192.5	251.0
ATC	905.7	496.4	409.3	768.8	514.6	254.2
DR	386.6	205.6	181.0	450.6	317.0	133.6
MT	456.1	259.5	196.6	469.2	336.2	133.0
Means	528.3	273.3	255.0	519.7	335.7	184.0
\pm SE	82.6	46.7	38.2	61.5	59.7	23.7
<i>Nonsparers</i>						
MW	506.4	328.5	177.9	601.4	411.8	189.6
RS	563.4	261.6	301.8	687.7	343.4	344.3
TO	644.0	455.0	189.0	639.8	446.0	193.8
DP	734.2	449.0	285.2	599.1	331.8	267.3
TP	644.9	406.6	238.3	628.8	407.6	221.2
GD	412.9	347.7	65.2	407.1	337.1	70.0
Means	584.3	374.7	209.6	594.0	379.6	214.4
\pm SE	46.8	30.9	35.3	39.6	19.7	37.2

Values are mmol/kg dry muscle.

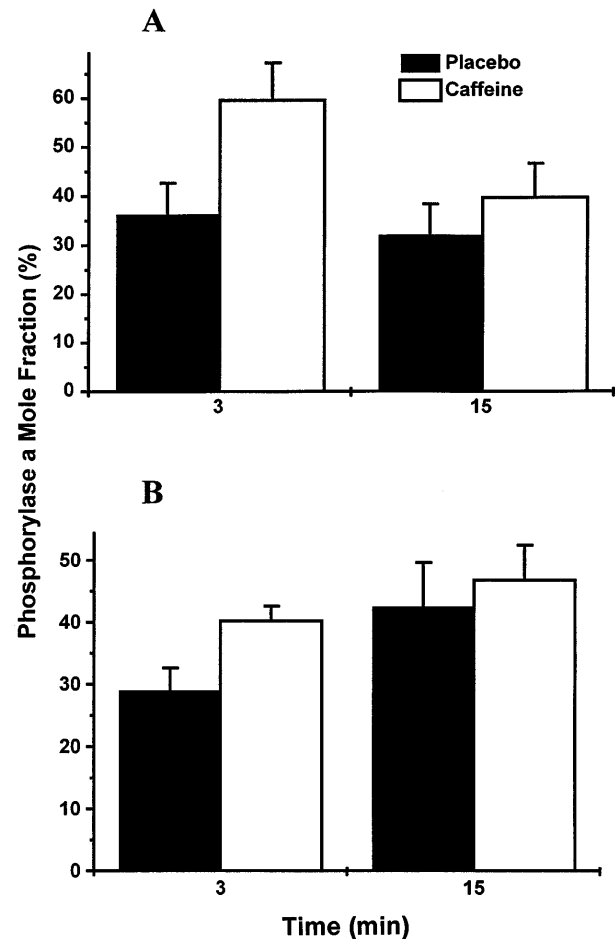


Fig. 2. Phosphorylase (Phos) *a* mole fraction in glycogen sparsers (A) and nonsparers (B) at 3 and 15 min of exercise. There was a trend for Caf to increase the Phos *a* mole fraction in both sparsers ($P = 0.0669$) and nonsparers ($P = 0.0997$).

similar at 6 and 12 min of exercise in all trials and groups and averaged 81.2 ± 2.2 and $83.5 \pm 2.6\%$ in the Pl and Caf trials for the Sp and 78.3 ± 3.2 and $79.2 \pm 3.2\%$ in the Pl and Caf trials for the NSp.

Phos transformation during exercise. In both Sp and NSp, Caf increased the Phos *a* mole fraction at 3 min ($P = 0.067$) and to a lesser extent at 15 min ($P = 0.100$) of exercise compared with Pl, but the increases were not statistically significant (Fig. 2, A and B).

Muscle metabolites. ATP contents were similar between Caf and Pl trials in the Sp and NSp groups, with the exception that ATP was significantly lower at 15 min of exercise in the Caf trial of the NSp group (Table 2). Muscle La accumulation increased with exercise in Sp and NSp, but caffeine had no effect on La accumulation in either group (Table 2).

At 3 min of exercise, Sp had significantly more PCr available in the Caf compared with the Pl trial (Fig. 3A), whereas available PCr was unchanged by Caf in NSp (Fig. 3B). This difference in PCr degradation at 3 min of exercise translated into an 11% reduction in the calculated P_{if} content in Sp, whereas Caf had no effect on P_{if} concentration throughout exercise in NSp (Table 3).

Table 2. Muscle ATP and lactate contents at rest and during exercise in glycogen sparsers and nonsparers

	0 min	3 min	15 min
ATP			
Sparers			
PI	28.78 ± 2.34	28.81 ± 1.68	24.95 ± 0.68*
Caf	29.30 ± 4.05	26.53 ± 2.56	23.82 ± 1.89*
Nonsparers			
PI	27.77 ± 1.90	24.22 ± 1.52	24.74 ± 1.60
Caf	27.65 ± 2.18	23.28 ± 2.40	20.01 ± 2.39*†
La			
Sparers			
PI	4.3 ± 1.0	48.6 ± 6.9*	68.8 ± 10.8*
Caf	5.2 ± 1.0	43.5 ± 7.4*	64.5 ± 6.8*
Nonsparers			
PI	5.3 ± 1.7	70.2 ± 8.5*	73.0 ± 5.5*
Caf	7.0 ± 1.4	82.7 ± 10.1*	85.7 ± 13.4*

Values are means ± SE in mmol/kg dry muscle. PI, placebo trial; Caf, caffeine trial; La, lactate. *Significantly different from 0 min PI and Caf time points; †significantly different from corresponding PI mean.

Table 3. Calculated free P_i contents during exercise in glycogen sparsers and nonsparers

	3 min	15 min
Sparers		
PI	57.5 ± 3.9	66.6 ± 5.3
Caf	50.6 ± 6.6	63.3 ± 7.0
Nonsparers		
PI	57.1 ± 5.2	67.0 ± 2.5
Caf	69.2 ± 6.9	72.8 ± 3.9

Values are means ± SE in mmol/kg dry muscle.

The pattern of ADP_f and AMP_f accumulation was also different between Sp and NSp. Caf significantly reduced the accumulations of ADP_f and AMP_f at 3 and 15 min of exercise compared with PI in the Sp group (Figs. 4A and 5A). In NSp, Caf did not blunt the accumulations of ADP_f or AMP_f compared with PI (Figs. 4B and 5B).

Plasma Epi and blood metabolites. In Sp, Caf produced an approximately twofold increase in plasma Epi concentration compared with PI, starting at 1.5 min of exercise (Fig. 6A). This response was also observed in NSp, but did not begin until 5 min of exercise (Fig. 6B).

Caf significantly increased blood glucose levels during exercise in Sp and NSp compared with PI (Tables 4

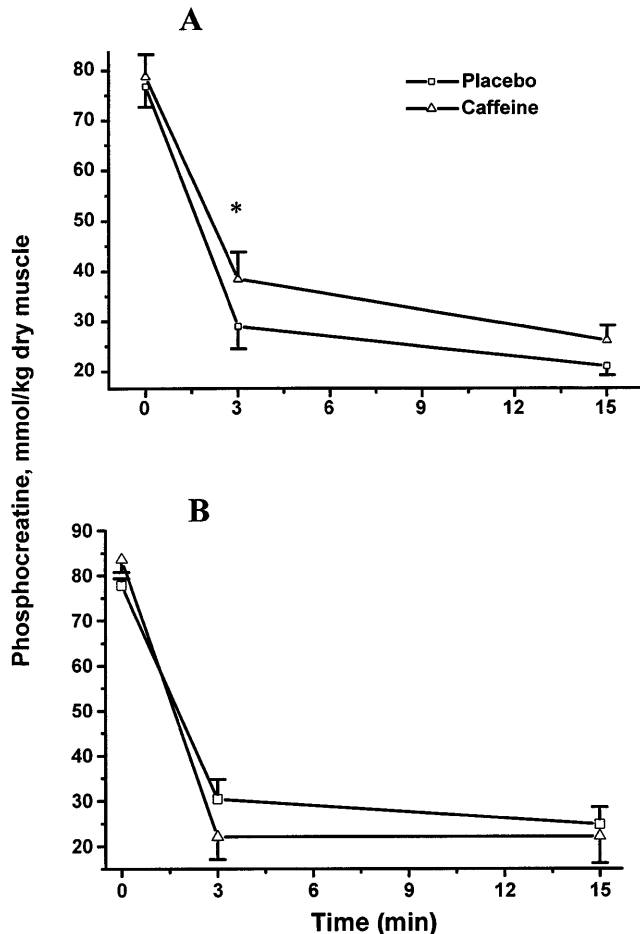


Fig. 3. Muscle phosphocreatine (PCr) contents in glycogen sparsers (A) and nonsparers (B) during rest and exercise. *Significantly different from corresponding PI time point. Significant reductions in PCr were found across time in both groups.

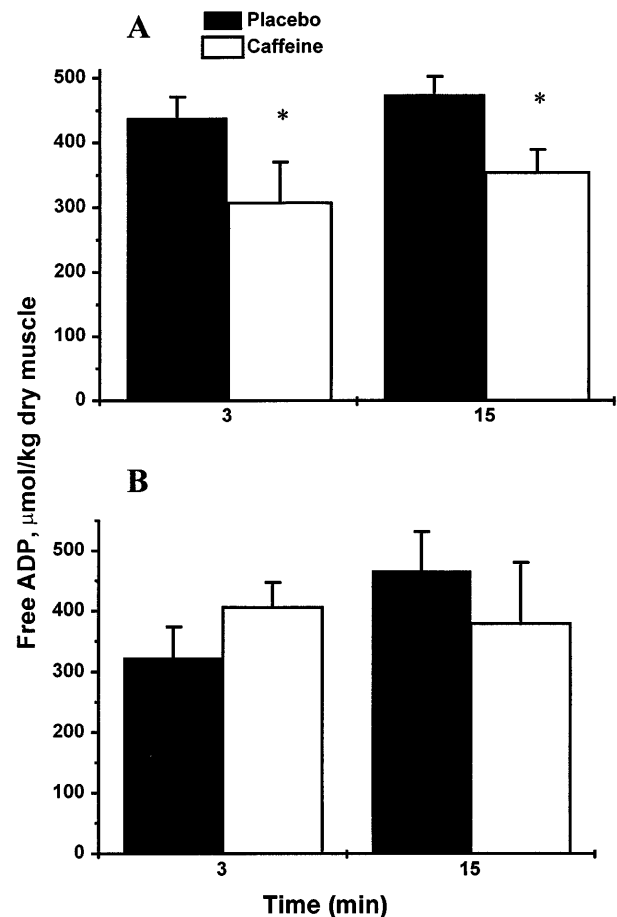


Fig. 4. Calculated free ADP contents in glycogen sparsers (A) and nonsparers (B) during exercise. *Significantly different from corresponding PI time points.

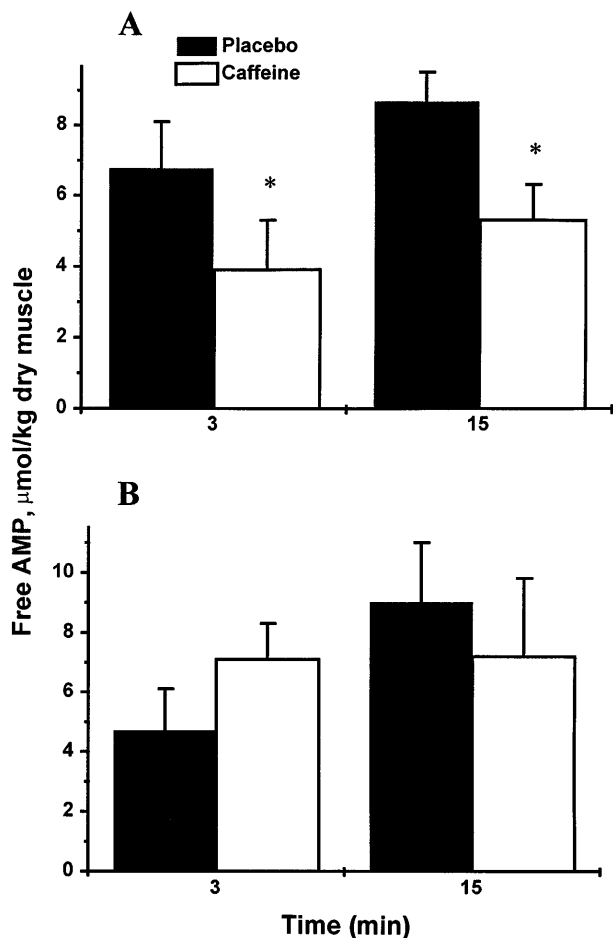


Fig. 5. Calculated free AMP contents in glycogen spacers (A) and nonsparers (B) during exercise. *Significantly different from corresponding PI time points.

and 5). Blood La levels increased during exercise in both groups. Caf had no effect on blood La concentration compared with the PI trial in Sp (Table 4), but increased La levels at 10 and 14 min of exercise in NSp (Table 4). Resting plasma FFA concentrations were significantly increased 1 h after Caf ingestion in the Sp (59%) and NSp (68%) groups (Tables 4 and 5). FFA concentrations were similar between the Caf and PI trials throughout exercise in the Sp and NSp.

DISCUSSION

The purpose of this study was to examine the mechanisms involved in the glycogen-sparing effect of ingesting Caf before intense aerobic exercise. The transformation state of glycogen Phos and the posttransformational regulators of this enzyme were measured in PI and Caf trials. However, Caf ingestion produced a variable glycogen-sparing response in untrained subjects performing 15 min of cycling at $\sim 80\%$ $\dot{V}O_{2\max}$ (Table 1). A group of six subjects used 21–38% (mean = $28 \pm 2.6\%$) less glycogen in the Caf trial (Sp), whereas Caf had no effect on muscle glycogenolysis in six other subjects (NSp). Glycogen sparing was defined as a decrease in glycogen use of 10% or greater, as our previous work demonstrated a reliability of 3–10% for the measure-

ment of muscle glycogen in repeat needle biopsy samples (26). Therefore, we were able to test our original hypotheses in a group of six subjects. In addition, the data from the NSp group allowed us to examine the hypotheses from a different perspective. The changes that we predicted in the group that experienced Caf-induced glycogen sparing would not be expected in the group where no sparing occurred.

The mean preexercise muscle glycogen contents were similar between trials in both the Sp and NSp. However, on an individual basis, muscle glycogenolysis was significantly correlated with the preexercise glycogen content in the PI ($r^2 = 0.56$) and Caf ($r^2 = 0.76$) trials for the NSp group and in the PI trial for the Sp group ($r^2 = 0.94$). Only in the Caf trial for the Sp group was there no relationship ($r^2 = 0.07$). These findings suggest that the rate of glycogenolysis is governed by factors other than the preexercise glycogen content in the Sp group during the Caf trial.

The subjects who spared muscle glycogen in the Caf trial degraded less PCr during the initial 3 min of exercise and accumulated less ADP_f and AMP_f . In contrast, the NSp degraded similar amounts of PCr and had similar accumulations of ADP_f and AMP_f in both

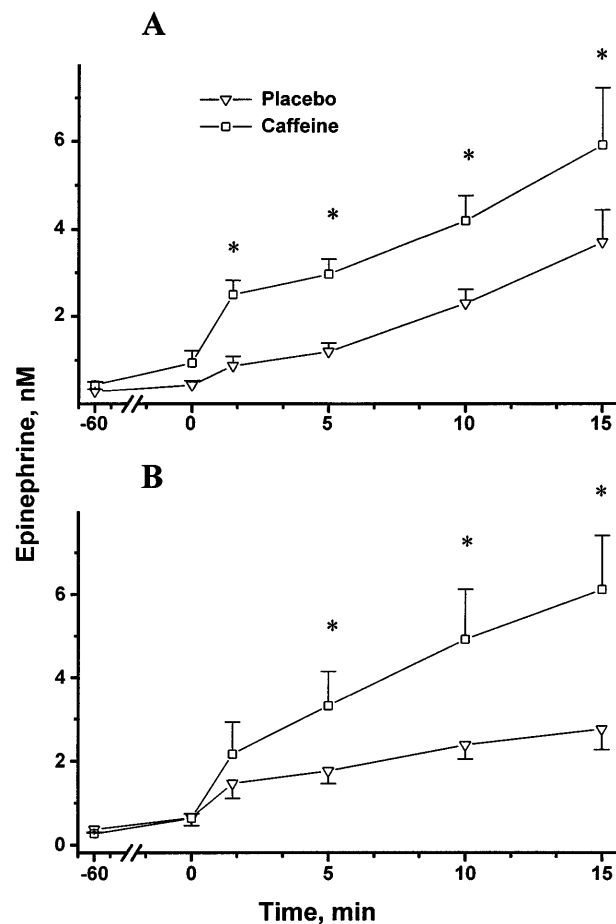


Fig. 6. Plasma epinephrine concentrations in glycogen spacers (A) and nonsparers (B) at rest and during exercise. There were significant main effects for trial and time and a trial \times time interaction was also present in both spacers and nonsparers. *Significantly different from corresponding PI time point.

Table 4. Blood glucose, lactate, and plasma FFA concentrations at rest and during exercise in glycogen sparrers

Time, min	Glucose		Lactate		FFA	
	PI	Caf	PI	Caf	PI	Caf
-60	4.28 ± 0.30	3.96 ± 0.26	0.58 ± 0.10	0.64 ± 0.06	0.35 ± 0.09	0.39 ± 0.08
0	4.28 ± 0.18	4.88 ± 0.14*	0.59 ± 0.15	1.13 ± 0.17	0.42 ± 0.11	0.62 ± 0.17†
1.5	4.15 ± 0.12	4.89 ± 0.10*	2.33 ± 0.67	2.73 ± 0.66	ND	ND
5	4.00 ± 0.16	4.80 ± 0.23*	5.69 ± 1.19	6.13 ± 1.31	0.30 ± 0.04	0.45 ± 0.13
10	3.68 ± 0.20	4.53 ± 0.26*	9.04 ± 1.34	8.89 ± 1.40	ND	ND
14	3.78 ± 0.28	4.47 ± 0.25*	9.45 ± 1.47	11.10 ± 1.34	0.30 ± 0.07	0.25 ± 0.04

Values are means ± SE in mM. ND, no determination; FFA, free fatty acid. *Significantly different from corresponding PI value; †significantly different from -60 min. A significant main effect for time was found for lactate.

trials. Transformation of Phos to the more active *a* form was increased by Caf in both groups, but despite this, glycogenolytic flux was reduced in the Sp group and was unaffected in the NSp group. These results suggest that glycogenolytic flux during intense aerobic exercise after Caf ingestion is unaffected by changes in the transformational state of Phos and is regulated by posttransformational mechanisms related to the energy status of the cell.

Flux through muscle glycogen Phos is regulated by a two-stage control process during contractile activity. The first stage is the transformation of Phos from a less active *b* form to a more active *a* form, which sets the potential upper limit for glycogenolysis. The second stage is posttransformational control of Phos by substrate availability (P_{if}) and allosteric modulation (AMP_f). This stage acts to fine tune glycogenolysis to the demand for ATP via regulators intimately linked to the energy status of the cell. For example, the K_m of Phos *a* for P_{if} is in the physiological range and increases in AMP_f decrease the K_m of Phos *a* for P_{if} (3, 19, 20). Many studies have suggested that these regulators are major determinants of the glycogenolytic rate during intense static and dynamic exercise (3-5, 9, 19, 20).

Phos *b* to *a* transformation is mediated by increases in intracellular calcium and Epi-induced increases in cAMP (2). In this study, there was a strong trend for a Caf-mediated increase in the fraction of Phos in the *a* form at 3 min of exercise in both the Sp and NSp groups. This increase was likely due to the elevated plasma Epi levels observed in both Sp and NSp that occurred as early as 1.5 min of exercise. This effect was previously observed during intense aerobic exercise

when Epi was infused to levels normally found after Caf ingestion (5).

A central finding in this study was that, despite no significant change in the Phos *a* mole fraction (a strong trend for an increase) in the Caf trials, glycogenolytic flux decreased in the Sp and was unaffected in the NSp. The dissociation between Phos transformation state and muscle glycogenolysis in both groups demonstrates the importance of posttransformational control mechanisms (substrate availability and allosteric modulators) in regulating glycogenolytic flux. When Caf ingestion blunted the use of PCr (and increase in P_i) and the accumulation of AMP_f during exercise, glycogen sparing occurred, and when these changes were absent, no sparing occurred. These factors also appeared to override the normal strong relationship between preexercise glycogen content (a second substrate for Phos) and glycogenolysis during intense aerobic exercise.

The reductions in PCr degradation and AMP_f accumulation observed in Sp are consistent with an enhanced oxidation of FFA derived from plasma or intramuscular triglyceride (TG) stores. Previous studies have provided evidence that fat mobilization and oxidation are increased during aerobic exercise preceded by Caf ingestion (7, 12, 22). In the present study, the source of the FFA remains unknown. One hour after caffeine ingestion, the mean resting plasma FFA concentrations increased by 59 and 68%, respectively, in the Sp and NSp groups (Tables 4 and 5). It is possible that Sp were able to oxidize a larger portion of these FFA compared with NSp in the early minutes of exercise. However, this hypothesis requires more rigorous testing because static measures of plasma FFA do not provide informa-

Table 5. Blood glucose, lactate, and plasma FFA concentrations at rest and during exercise in glycogen nonsparers

Time, min	Glucose		Lactate		FFA	
	PI	Caf	PI	Caf	PI	Caf
-60	3.96 ± 0.35	4.31 ± 0.16	1.14 ± 0.16	0.67 ± 0.17	0.22 ± 0.04	0.33 ± 0.11
0	3.88 ± 0.20	4.26 ± 0.31	0.88 ± 0.26	1.39 ± 0.11	0.26 ± 0.04	0.59 ± 0.17†
1.5	4.04 ± 0.28	4.73 ± 0.16*	2.51 ± 0.39	3.71 ± 0.52	ND	ND
5	3.85 ± 0.29	4.69 ± 0.26*	5.85 ± 0.99	6.85 ± 1.38	0.22 ± 0.04	0.45 ± 0.14
10	3.74 ± 0.24	4.67 ± 0.27*	8.29 ± 1.78	10.38 ± 1.83*	ND	ND
14	3.52 ± 0.52	4.63 ± 0.48*	7.13 ± 1.63	10.37 ± 2.03*	0.26 ± 0.10	0.43 ± 0.12

Values are means ± SE in mM. *Significantly different from corresponding PI value; †significantly different from -60 min. A significant main effect of time was found for blood lactate.

tion about fat oxidation. A second possibility is that Caf induced an increase in intramuscular TG hydrolysis in Sp compared with NSp. An earlier study demonstrated that Caf enhanced intramuscular TG hydrolysis by 150% compared with PI during 30 min of aerobic exercise at 70% $\dot{V}O_{2\max}$ (12). However, these findings do not explain why Caf would enhance endogenous fat use in some subjects and not in others (see below).

In addition to Caf ingestion, acute fat provision (6, 9, 10, 24) and endurance training (4, 15, 17) are other experimental manipulations that result in muscle glycogen sparing during intense aerobic exercise. These conditions, like Caf, produced a tighter coupling between energy demand and supply as assessed by reductions in the use of PCr and accumulation of ADP_f and AMP_f during exercise (4, 9, 10, 15). All three conditions appear to reduce muscle glycogenolysis via mechanisms related to posttransformational control of Phos. It should be noted that Caf ingestion and acute fat provision appear to promote fat oxidation and produce a tighter coupling without changes in mitochondrial density. However, aerobic training produces mitochondrial biogenesis and increases the capacity of the pathways involved in the oxidation of fat.

In common with the present findings, a variable glycogen sparing response has been observed during intense aerobic exercise with acute fat provision (Intralipid-heparin infusion) in untrained subjects (9). In NSp, Intralipid infusion did not improve the match between energy demand and supply compared with glycogen Sp despite similar elevations in plasma FFA concentration. It is possible that a limitation in fat uptake or oxidation existed in NSp compared with Sp in these studies.

In this Caf study and previous work (4, 9), we have proposed that increased provision and oxidation of FFA improve the match between energy demand and supply during aerobic exercise at $\geq 80\%$ $\dot{V}O_{2\max}$. This acts to dampen the increases in ADP_f , AMP_f , and P_{if} during the initial stages of exercise at this intensity. A critical question is how the presence of extra FFA during the rest period before and/or during the early stages of intense aerobic exercise improves the energy status of the cell.

The rate of mitochondrial ATP production is regulated by O_2 availability and the NAD^+ -to- $NADH$ concentration and ATP-to- $ADP \times P_i$ concentration ratios (27). It is assumed that the availability of O_2 was not limiting in either the Caf or PI trials in the present study, leaving two factors to regulate mitochondrial ATP production. Enhanced fat availability and oxidation at rest and/or the onset of exercise in the Caf trial of the glycogen sparer group may have increased mitochondrial $NADH$ concentration. This could mean that lower accumulations of ADP_f and P_{if} would be needed to achieve the required rate of ATP production during exercise. The experimental findings of From et al. (13) using Langendorff perfused rat hearts support this suggestion. They reported that the provision of short chain fatty acids, which rapidly cross cell membranes, resulted in lower accumulations of cytosolic

ADP_f and P_{if} at a given rate of contraction and mitochondrial ATP production. They also speculated that increases in mitochondrial $NADH$ concentration were responsible for the improved phosphorylation state (13). Normally, the provision of FFA into the muscle cell and/or mitochondria appears to limit fat oxidation during intense exercise. However, the extra FFA provision during situations such as acute fat infusion, Caf ingestion, and after aerobic training may partially circumvent this problem. Last, it is important to note that the improved phosphorylation state reduces the need for glycolytically derived reducing equivalents for mitochondrial respiration. The lower accumulations of AMP_f and P_{if} decrease muscle glycogenolysis at the level of glycogen Phos.

The mechanism for the ability of Caf to spare muscle glycogen in some untrained individuals but not in others is unresolved. $\dot{V}O_{2\max}$ and CS activities were similar between Sp and NSp, suggesting that whole body and muscle oxidative potential were not the factors accounting for differences in glycogenolytic flux. However, additional measurements of muscle β -oxidation and respiratory chain capacities and intramuscular TG contents would be helpful in determining if Sp had a greater potential for fat oxidation. Preexercise glycogen contents were also not different between the two groups.

Furthermore, there were no differences in habitual Caf use between the Sp and NSp groups, as four subjects were Caf users and two subjects were nonusers in both groups. Mean plasma Caf levels were also not different between groups, although this does not provide information about tissue sensitivity to Caf in the two groups. Future work examining the effects of Caf on muscle glycogen metabolism should be directed toward fat uptake and oxidation. The recent observation that increased plasma FFA availability does not always result in muscle glycogen sparing during intense aerobic exercise in untrained subjects points to possible limitations for fat uptake at the muscle and/or mitochondrial membranes or a limitation in the ability to oxidize fat (9). It is also important to point out that the mechanism for the Caf-induced muscle glycogen sparing reported to occur in trained subjects (11, 12, 22) has not been examined. The proposed mechanism for glycogen sparing for the select group of untrained subjects in this paper could be tested in trained subjects.

In summary, the present findings demonstrate that Caf resulted in a sparing of muscle glycogen in some untrained subjects and not in others during 15 min of intense aerobic exercise. In the group that spared muscle glycogen, the decreased flux through Phos *a* was unaffected by changes in the transformational state of the enzyme and was related to posttransformational factors associated with the energy status of the cell (less PCr use and lower AMP_f accumulation). These findings suggest that enhanced fat oxidation by the working muscles early in exercise may be responsible for the reduction in glycogenolytic flux in glycogen Sp compared with NSp.

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