

Caffeine ingestion does not impede the resynthesis of proglycogen and macroglycogen after prolonged exercise and carbohydrate supplementation in humans

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Submitted 17 July 2003; accepted in final form 7 November 2003

Battram, D. S., J. Shearer, D. Robinson, and T. E. Graham. Caffeine ingestion does not impede the resynthesis of proglycogen and macroglycogen after prolonged exercise and carbohydrate supplementation in humans. *J Appl Physiol* 96: 943–950, 2004. First published November 14, 2003; 10.1152/jappphysiol.00745.2003.—The purpose of this study was to examine the effects of caffeine (Caf) ingestion on pro- (PG) and macroglycogen (MG) resynthesis in 10 healthy men. Subjects completed two trials, consisting of a glycogen-depleting exercise, while ingesting either Caf or placebo capsules. Throughout recovery, biopsies were taken at 0 (exhaustion), 30, 120, and 300 min, and 75 g of carbohydrate were ingested at 0, 60, 120, 180, and 240 min. Whereas Caf ingestion resulted in a higher blood glucose concentration and decreased glycogen synthase fractional velocity ($P \leq 0.05$), no effect was observed in either the amount or rate of PG and MG resynthesis. PG concentration increased significantly at each time point during recovery, whereas MG concentration remained unchanged until 120 min. The net rate of PG resynthesis was 115 mmol·kg dw⁻¹·h⁻¹ during the first 30 min of recovery, and then it significantly decreased by 62% throughout the remaining 4.5 h of recovery. The net rate of MG resynthesis was 77% lower than the net rate of PG resynthesis during the first 30 min of recovery and remained constant throughout 5 h of recovery despite increasing levels of insulin. In conclusion, Caf ingestion does not impede the resynthesis of PG or MG after an extensive depletion of muscle glycogen and with the provision of exogenous dietary carbohydrate. glycogen synthase; insulin; recovery

THE PROCESS OF GLYCOGEN RESYNTHESIS is often described as occurring in two phases: an early, rapid phase that is termed insulin independent followed by a slower insulin-dependent phase (24, 33). Whereas it was once believed that the nonequilibrium enzyme glycogen synthase (GS) determined the rate of glycogen resynthesis, we now know that both *glucose transport and uptake* and glycogen concentration can effect the glycogen resynthesis process (4). In addition to these factors, it is well established that the rate of glycogen resynthesis can be enhanced by the provision of exogenous carbohydrate (CHO) (7, 21, 23, 26, 38). Ivy et al. (23) have reported that not only the amount but also the timing and frequency of CHO ingested can play a role in determining the rate and amount of glycogen that can be resynthesized throughout recovery.

CHO is not the only dietary factor known to affect glucose metabolism and hence glycogen resynthesis. Studies on rodents and humans have noted a caffeine-induced impairment on various aspects of resting glucose metabolism (8, 12, 13, 18,

25, 36). In human subjects, caffeine ingestion before either an oral glucose tolerance test or a hyperinsulinemic-euglycemic clamp resulted in significant impairments in glucose disposal (~30%) (12, 13, 36). More recently, Thong et al. (36), using an one-legged knee extensor model, direct Fick measurements, and a hyperinsulinemic-euglycemic clamp, observed a 50% decrease in leg glucose uptake and a ~37% decrease in GS activity in both the rested and previously exercised legs. Despite these decreases, no differences in total glycogen concentrations were observed between the placebo and caffeine treatments. We suspect that the lack of a caffeine effect on glycogen concentration was due to 1) a moderate level of glycogen at exhaustion [~280 mmol/kg dry weight (dw)] and 2) a short period of observation (100 min). Both of these conditions could result in a low rate of glycogen resynthesis and minimal changes in glycogen concentration within their trials and potentially may have masked caffeine's effect on glycogen concentration. Therefore, it is possible that if the rate of glycogen resynthesis is increased and the change in glycogen concentration is greater throughout recovery, the caffeine-induced impairments on glucose uptake and GS may then be detected in the glycogen concentrations.

Glycogen synthesis rates have been extensively studied under a variety of postexercise conditions (6, 7, 23, 32, 33, 37, 38). To date, however, no study utilizing biopsies and a CHO-feeding regime have taken their first biopsy sample <2 h postexercise. In addition, studies utilizing ¹³C-nuclear magnetic resonance (NMR) spectroscopy to examine glycogen resynthesis rates during the first 2 h of recovery typically have not included a CHO-supplementation protocol (33, 37). Only in a recent study by Ivy et al. (22) was NMR spectroscopy combined with CHO supplementation. That study, however, as well as others involving NMR spectroscopy, reported total glycogen resynthesis rates and did not separate muscle glycogen into fractions. Muscle glycogen can be separated into two fractions on the basis of acid solubility. The smaller particles [proglycogen (PG)] precipitate into acid because of the higher protein-to-CHO ratio, whereas the larger particles [macroglycogen (MG)] remain acid soluble. These fractions do not represent two distinct pools of glycogen, but rather they represent a continuum of glycogen particles (29). Nevertheless, several studies have demonstrated that these two fractions do not respond identically during times of glycogen catabolism and anabolism (2, 10, 35). Adamo et al. (2) reported that PG is

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the predominant fraction synthesized during the first 4 h of recovery from exhaustive exercise in humans and noted that PG resynthesis was more sensitive to CHO availability than the MG fraction. Although this study was the first to examine PG and MG resynthesis in humans, it is likely that the net rate of PG resynthesis was underestimated because of the fact that the first biopsy was taken at 4 h postexercise.

Therefore, the purpose of this study was to examine in humans 1) the effects of caffeine ingestion during exercise on the net resynthesis of PG and MG during early recovery and 2) the pattern of PG and MG resynthesis after exhaustive exercise with early sampling (<2 h) and the ingestion of a large, immediate and frequent amount of CHO. It is hypothesized that caffeine will have a negative impact on glycogen resynthesis and that the largest impact will be on PG pool because of the fact that it is the predominant pool synthesized during the first few hours of recovery. It is also hypothesized that the initial rates of PG resynthesis will be higher than previously reported within the literature.

METHODS

Subjects

This study was approved by the University of Guelph's Human Subjects Committee. Ten male subjects volunteered to participate after giving written, informed consent of all experimental procedures and on the completion of a Health Screening questionnaire. All subjects were healthy, nonsmokers, and recreationally active. Subject characteristics for age ranged from 21 to 27 yr [$23.7 \pm$ (SE) 0.6 yr], for body weight from 65 to 98 kg (75.8 ± 2.8 kg), for height from 167 to 206 cm (179.5 ± 3.5 cm), and for maximal oxygen consumption ($\dot{V}O_{2\max}$) from 47–69 ml·kg⁻¹·min⁻¹ (60.9 ± 2.4 ml·kg⁻¹·min⁻¹). Seven of the 10 subjects were considered caffeine users as defined as the consumption of ≥ 2 caffeinated coffee or tea beverages and/or ≥ 5 caffeine-containing soft drinks per week.

Preexperimental Procedures

An incremental $\dot{V}O_{2\max}$ test was performed on an electronically braked cycle ergometer (Quinton Excaliber) to establish power outputs that were theoretically equal to both 75 and 130% $\dot{V}O_{2\max}$. One week before the first experimental trial, subjects completed a 45-min practice ride at 75% $\dot{V}O_{2\max}$ to familiarize themselves with the workload and experimental procedures and to verify the work intensities. Three days before the first trial, subjects completed a 3-day diet record, consuming no less than 150 g of CHO per day. Subjects were then required to follow this diet before the second trial. During the 3-day period before each trial, subjects were asked to maintain regular activity habits and to refrain from consuming any caffeinated substances and/or beverages and alcohol. On the day before the experiments, subjects were asked to refrain from performing any strenuous activity. The goals of both the dietary and activity controls were to achieve a similar level of glycogen before each trial.

Experimental Design and Procedures

The following protocol was employed to achieve a plasma concentration of caffeine that would remain high throughout recovery, to deplete muscle glycogen stores, and to maximize muscle glycogen resynthesis. Subjects completed two trials, one ingesting caffeine (6 mg/kg of body weight) and the other placebo capsules. Trials were randomized for order, double-blinded, and separated by ~ 1 wk. Subjects exercised to voluntary exhaustion on a cycle ergometer at 75% $\dot{V}O_{2\max}$ followed by a series of 30-s sprints at 130% $\dot{V}O_{2\max}$. Subjects then rested over the next 5 h, during which blood and muscle

samples were taken and a commercial CHO-electrolyte beverage (Gatorlode) was consumed.

Approximately 30 min before reporting to the laboratory, the subjects consumed a high-CHO breakfast, which was recorded and consumed before the second trial. When they arrived, a catheter was inserted into the antecubital vein for venous blood sampling and kept patent with a normal saline infusion. A baseline blood sample was taken and measured for methylxanthine concentrations to confirm that subjects refrained from consuming caffeinated products before testing. Subjects then ingested either 3 mg/kg of alkaloid caffeine or dextrose (placebo) in capsule form. Subjects rested for 30 min and then consumed 1 mg/kg of caffeine or dextrose. Immediately after ingestion, the subjects began exercising on the cycle ergometer at 75% $\dot{V}O_{2\max}$ to voluntary exhaustion, which was determined at the point at which revolutions per minute fell below 60. At 30 and 60 min during exercise, subjects again consumed 1 mg/kg of caffeine or dextrose, which resulted in a total caffeine/dextrose dose of 6 mg/kg of body weight. All capsules were ingested with 250 ml of water, and water was consumed ad libitum throughout the exercise. Blood samples were taken every 30 min, and oxygen consumption was determined at 10 and 45 min to ensure that subjects remained at a work intensity of 75% $\dot{V}O_{2\max}$. Because the goal of the exercise protocol was to deplete glycogen levels and not to monitor performance, the power output was adjusted accordingly to maintain 75% of $\dot{V}O_{2\max}$. After the exhaustive exercise, the subject's leg was prepared for a muscle biopsy. The initial leg used was randomly selected for the first trial, and then the order was reversed for the second trial. Once the preparation was completed (~ 5 –10 min), the subjects then performed five, 30-s sprints at 130% $\dot{V}O_{2\max}$, with a 2-min rest period in between each sprint.

Immediately after the last sprint (*time 0*), a blood sample was taken, followed by a muscle biopsy by the use of the percutaneous needle biopsy technique using suction. Immediately after the biopsy, subjects consumed 374 ml of a maltodextrin-dextrose solution, providing 75 g of CHO (Gatorlode). Throughout the remaining 5 h of recovery, muscle biopsies were taken at 30, 120, and 300 min. The biopsy at 30 min was taken from the same incision as the biopsy at *time 0*, and the biopsies at 120 and 300 min were taken from one incision on the opposite leg. Blood samples were collected every 30 min during the first 2 h of recovery and every 1 h throughout the last 3 h of recovery. Additional 374 ml of CHO beverage was consumed at 1, 2, 3, and 4 h of recovery, which in total provided 375 g of CHO or 5.0 ± 0.2 g·kg⁻¹·h⁻¹ of CHO during recovery.

Analyses

Blood samples were collected in both nontreated and heparinized tubes. In the nontreated tube, 7 ml of blood were allowed to clot at room temperature. After centrifugation, the serum was stored at -20°C for the later determination of serum insulin (Coat-a-Count RIA kit, Diagnostic Products, Los Angeles, CA), C-peptide (Human C-peptide RIA kit, Linco Research, St. Charles, MO), free fatty acids (FFA) (NEFA kit, Wako Bioproducts, Richmond, VA), and glycerol (28). In the heparinized tube, 10 ml of blood were collected. Two hundred microliters of heparinized blood were added to 1 ml of 0.6 M perchloric acid, and, after centrifugation, the supernatant (perchloric acid extract) was stored at -20°C and used for the determination of whole blood glucose (5) and lactate (16). Another 200- μl aliquot of heparinized blood was centrifuged, and the plasma was used for the determination of methylxanthines by high-performance liquid chromatography as previously described (3). For technical reasons, data for all blood metabolites are available for nine subjects.

Muscle biopsies were immediately immersed in liquid nitrogen and stored at -80°C . A portion was then cut, dissected free of blood and connective tissue and freeze-dried. Approximately 2 mg were used for the determination of pro- (PG) and macroglycogen (MG) by a method previously described by Adamo and Graham (1). Where sample size permitted ($\sim 75\%$ of samples), two independent pieces of muscle were

analyzed and the values averaged. Muscle glycogen synthase (GS) activity was determined by a modified method as previously described (34). For technical reasons, PG, MG, and GS data are available for nine subjects in the placebo trial and for 10 subjects for the caffeine trial.

Calculations and Statistics

Blood metabolites were analyzed for both time and treatment effects by using a two-way ANOVA for repeated measures, and when differences were found, a Tukey's test was used for post hoc analysis. Area under the curve (AUC) was calculated by using the trapezoid method during the 5 h of recovery for blood glucose and serum insulin. Data at exhaustion (*time 0*) were taken as the baseline values. All AUC data were analyzed for treatment effects by using a paired *t*-test.

The net synthesis rates for total (PG+MG) glycogen, PG, and MG were determined by taking the difference between two data points and dividing by the time interval in hours. The percentage of PG and MG of total glycogen was determined by dividing the PG or MG concentration by the total glycogen (PG+MG) concentration and multiplying by 100 for each time point. GS activities are expressed as either the percent glucose-6-phosphate (G-6-P)-independent form of GS (%I form) or as the percent fractional velocity. The %I form was calculated as the activity in the absence of G-6-P divided by the activity at 8 mM G-6-P multiplied by 100%, and the percent fractional velocity was calculated as the activity in the presence of 0.17 mM G-6-P divided by the activity at 8 mM G-6-P multiplied by 100%. PG+MG, PG, MG, net synthesis rates and GS were analyzed for time and treatment effects by using a two-way ANOVA, and when differences were found, a Tukey's test was used for post hoc analysis. Differences were accepted as significant if $P \leq 0.05$. All data are presented as means \pm SE.

RESULTS

Exercise and Diet Characteristics

The mean times to exhaustion for both the placebo and caffeine trials were 91.3 ± 9.2 and 95.1 ± 6.1 min, respectively ($P > 0.05$). Analysis of 3-day diet records confirmed that the subjects consumed a diet consisting of 60% CHO, with an average of 550 g of CHO consumed daily (data not shown). The breakfast meal consumed the day of the exercise trials (0.5 h before entering the laboratory) was 70% CHO and provided on average 103 g of CHO (data not shown).

Blood Glucose Concentration

At exhaustion (*time 0*), no difference in blood glucose concentration was observed between treatments ($P > 0.05$). After the ingestion of the CHO beverage, blood glucose levels increased rapidly, reaching a peak concentration by 60 min postexercise. Blood glucose levels then steadily declined over the next 4 h of recovery and approached baseline values by 5 h postexercise despite repeated ingestions of the CHO beverage. Blood glucose concentration at all time points between 30 and 240 min were greater than that at exhaustion, and the blood glucose concentration at 60 min was greater than that at all other time points except at 90 min (Table 1). There was a significantly higher blood glucose concentration in the caffeine trial ($P = 0.05$), and although a 44% increase in AUC was observed in the caffeine trial compared with the placebo trial, a paired *t*-test indicated that the difference was not significant ($P = 0.06$) (data not shown).

Table 1. Blood metabolite data during 5 h of recovery

	Recovery Time, min							
	0*	30	60*	90	120*	180*	240*	300
	<i>Glucose, mM</i>							
Placebo	3.4 \pm 0.2 ^a	5.2 \pm 0.3 ^b	6.0 \pm 0.3 ^c	5.6 \pm 0.2 ^{b,c,d}	5.0 \pm 0.2 ^{b,d,e}	4.6 \pm 0.3 ^{e,f}	4.0 \pm 0.2 ^{f,g}	3.6 \pm 0.2 ^{a,g}
Caffeine†	3.5 \pm 0.1 ^a	5.8 \pm 0.4 ^b	6.8 \pm 0.3 ^c	6.3 \pm 0.4 ^{b,c,d}	5.7 \pm 0.3 ^{b,d,e}	4.9 \pm 0.2 ^{e,f}	4.8 \pm 0.2 ^{f,g}	3.9 \pm 0.2 ^{a,g}
	<i>Insulin, pM</i>							
Placebo	18 \pm 5 ^a	94 \pm 22 ^a	200 \pm 42 ^b	313 \pm 44 ^c	238 \pm 42 ^{c,d}	303 \pm 36 ^c	250 \pm 41 ^{c,d}	211 \pm 31 ^{b,d}
Caffeine	18 \pm 3 ^a	104 \pm 14 ^a	185 \pm 29 ^b	286 \pm 22 ^c	294 \pm 46 ^{c,d}	304 \pm 38 ^c	320 \pm 38 ^{c,d}	197 \pm 23 ^{b,d}
	<i>C-peptide, nM</i>							
Placebo	0.2 \pm 0.0 ^a	0.6 \pm 0.1 ^b	1.2 \pm 0.2 ^c	1.8 \pm 0.2 ^d	1.6 \pm 0.2 ^d	1.8 \pm 0.1 ^d	1.7 \pm 0.1 ^d	1.6 \pm 0.1 ^d
Caffeine	0.2 \pm 0.0 ^a	0.6 \pm 0.1 ^b	1.1 \pm 0.1 ^c	1.7 \pm 0.1 ^d	1.8 \pm 0.1 ^d	2.0 \pm 0.1 ^d	2.0 \pm 0.1 ^d	1.6 \pm 0.1 ^d
	<i>Lactate, mM</i>							
Placebo	8.4 \pm 1.3 ^a	3.4 \pm 0.5 ^b	2.1 \pm 0.2 ^{b,c}	1.8 \pm 0.2 ^c	1.7 \pm 0.1 ^c	1.6 \pm 0.2 ^c	1.5 \pm 0.1 ^c	1.7 \pm 0.2 ^c
Caffeine	9.2 \pm 1.3 ^a	4.2 \pm 0.5 ^b	2.5 \pm 0.2 ^{b,c}	2.2 \pm 0.1 ^c	2.0 \pm 0.3 ^c	1.8 \pm 0.2 ^c	1.6 \pm 0.2 ^c	1.8 \pm 0.2 ^c
	<i>FFA, μM</i>							
Placebo	902 \pm 132 ^a	876 \pm 138 ^a	536 \pm 98 ^b	362 \pm 74 ^{b,c}	276 \pm 44 ^c	181 \pm 12 ^c	157 \pm 9 ^c	149 \pm 9 ^c
Caffeine†	1,137 \pm 130 ^a	1,318 \pm 35 ^a	704 \pm 108 ^b	433 \pm 84 ^b	331 \pm 53 ^{b,c}	203 \pm 26 ^c	164 \pm 15 ^c	160 \pm 24 ^c
	<i>Glycerol, μM</i>							
Placebo	315 \pm 27 ^a	237 \pm 20 ^b	188 \pm 16 ^c	159 \pm 11 ^{c,d}	142 \pm 10 ^{c,d}	120 \pm 7 ^d	120 \pm 7 ^d	118 \pm 10 ^d
Caffeine†	403 \pm 34 ^a	306 \pm 35 ^b	206 \pm 20 ^{b,c}	150 \pm 17 ^{c,d}	155 \pm 12 ^{c,d}	128 \pm 8 ^d	112 \pm 8 ^d	111 \pm 8 ^d

Values are means \pm SE. FFA, free fatty acids. *Time points at which the carbohydrate beverage was ingested. †Significant overall treatment effect ($P \leq 0.05$). For each metabolite, time points sharing a lowercase letter are not significantly different ($P > 0.05$) from each other within a trial.

Serum Insulin Concentration

Serum insulin concentrations were not different between treatments at exhaustion. Throughout the 5 h of recovery, serum insulin concentrations increased such that all time points between 60 and 240 min were significantly greater than at exhaustion. Compared with blood glucose concentration, which rose rapidly to a peak at 60 min, serum insulin concentration increased more slowly and did not reach a peak value until 90 min into recovery. As well, unlike blood glucose levels, which declined during the final 4 h of recovery, the serum insulin concentration remained elevated and constant. At no point in time were any differences observed between treatments ($P > 0.05$; Table 1). AUC for serum insulin was also similar between treatments (data not shown).

Other Blood Metabolites

Table 1 summarizes all blood metabolite data. Both C-peptide and lactate levels were not significantly different at exhaustion between treatments. After ingestion of CHO, C-peptide levels increased to a peak concentration at 90 min and remained elevated throughout the rest of recovery in a similar fashion to insulin. The high level of lactate observed in both trials at exhaustion declined by 60% ($P < 0.05$) during the first 30 min of recovery and then continued to decline steadily thereafter. As expected with caffeine ingestion, both serum glycerol and FFA were elevated at exhaustion in the caffeine trial ($P \leq 0.05$). After the ingestion of CHO and the subsequent rise in serum insulin, both glycerol and FFA concentrations declined, reaching resting values by 2 h into recovery. The baseline methylxanthine data confirmed that subjects refrained from consuming caffeinated products before each experiment and that the caffeine ingestion pattern achieved a moderate dose of caffeine, such that an average concentration of 30 μM was observed throughout 5 h of recovery (data not shown).

Glycogen Concentrations

Total glycogen (PG+MG) concentration. At exhaustion, no differences in PG+MG glycogen concentrations were observed between the placebo and caffeine treatments (Fig. 1). Throughout the 5 h of recovery, PG+MG concentration increased significantly ($P < 0.05$), such that by 300 min, PG+MG concentrations approximated normal resting levels (i.e., 300–350 mmol/kg dw) in both placebo and caffeine groups. At no time were any differences observed between treatments ($P > 0.05$). As well, a strong correlation ($r = 0.92$) ($y = 0.89x - 0.77$) was observed between the two independent muscle samples (from 1 biopsy) analyzed for PG+MG concentration.

PG. At exhaustion, PG concentration represented 76–77% of total glycogen in both trials. During the first 30 min of recovery, PG concentration doubled ($P \leq 0.05$) and during the next 90 min of recovery, PG levels continued to increase ($P < 0.05$). By 300 min PG concentrations increased ($P < 0.05$) to 203 ± 11 and 187 ± 9 mmol/kg dw in the placebo and caffeine trials, respectively, and represented 68% of PG+MG concentration. At no point in time were any differences observed between treatments ($P > 0.05$; Fig. 1).

MG. Unlike PG, MG concentration remained unchanged until 2 h into recovery, and even then the concentration was not

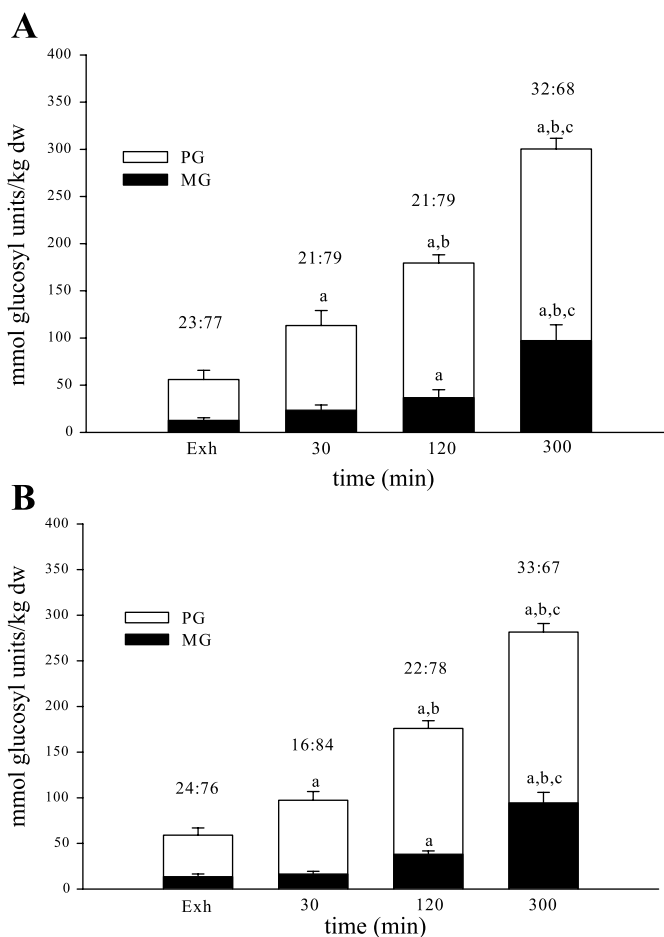


Fig. 1. Total glycogen [proglycogen (PG) + macroglycogen (MG)] glycogen concentrations during 5 h of recovery in the placebo trial (A) and caffeine trial (B). Histogram represents PG+MG concentration: open bar, PG; solid bar, MG. Values are means \pm SE. Ratios indicate the percent contribution of PG and MG to PG+MG for each time point. dw, Dry weight. Lowercase letters indicate significant differences ($P < 0.05$) vs. exhaustion (Exh; a), 30 min (b), and 120 min (c) within a treatment. No differences were observed between treatments.

different from that at 30 min ($P > 0.05$). From 120 to 300 min, MG levels increased ($P \leq 0.05$) further to 97 ± 17 and 94 ± 12 mmol/kg dw in the placebo and caffeine trials, respectively. At no time during recovery were any differences in MG concentrations observed between treatments ($P > 0.05$; Fig. 1).

Net Glycogen Resynthesis Rates

PG+MG. The net PG+MG resynthesis rates during the first 30 min of recovery were 114 ± 15 and 72 ± 14 mmol \cdot kg dw $^{-1}\cdot$ h $^{-1}$ for the placebo and caffeine trials, respectively (Fig. 2). These rates significantly decreased ($P \leq 0.05$) during the following 90 min of recovery and remained constant throughout the rest of the recovery period. The net rate of PG+MG resynthesis during the entire 300 min of recovery was 49 mmol \cdot kg dw $^{-1}\cdot$ h $^{-1}$. At no time were differences observed between the placebo and caffeine groups ($P > 0.05$).

PG. During the first 30 min of recovery when insulin levels were low, the net rates of resynthesis for PG within the placebo and caffeine trials were 93 ± 23 and 71 ± 13 mmol \cdot kg dw $^{-1}\cdot$ h $^{-1}$, respectively (Fig. 2). During the next 90 min of

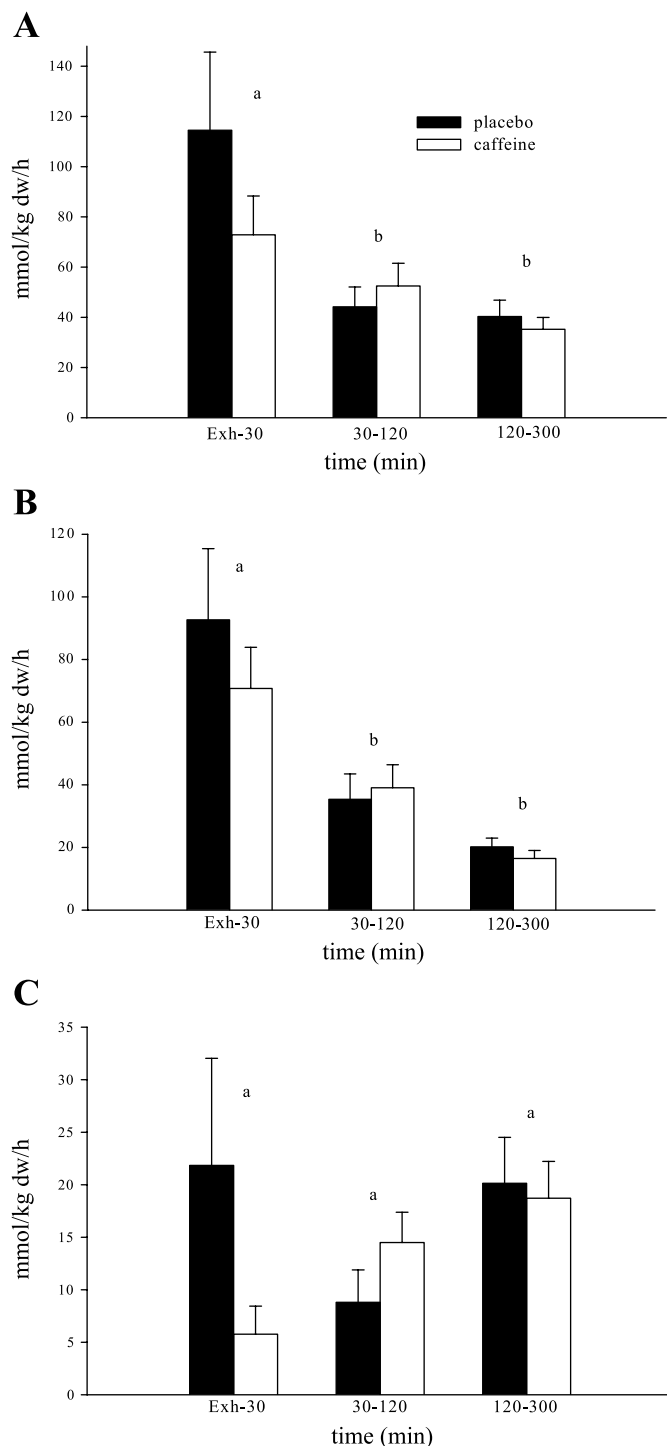


Fig. 2. Net PG+MG (A), PG (B), and MG (C) resynthesis rates during 5 h of recovery. Values are means \pm SE. Solid bars, placebo trial; open bars, caffeine trial. Bars sharing a lowercase letter are not significantly different ($P > 0.05$) from each other. No differences were observed between treatments.

recovery and as insulin levels increased, the net PG resynthesis rate decreased significantly ($P < 0.05$) and remained constant throughout the remaining 3 h of recovery. At no point in time were any treatment effects observed ($P > 0.05$). The mean rate of PG resynthesis from exhaustion to 5 h postexercise was 32 ± 11 mmol \cdot kg dw $^{-1}\cdot$ h $^{-1}$. It is interesting to note that the net

PG resynthesis rates throughout the 5 h of recovery paralleled the net PG+MG resynthesis rates.

MG. No significant treatment or time effects were observed in the net rates of MG resynthesis ($P < 0.05$) (Fig. 2). The rate of MG resynthesis was 76 and 74% lower than the rates of PG resynthesis during the first 30 min and subsequent 90 min of recovery. However, during the last 3 h of recovery when insulin levels were high the rates of resynthesis for both PG (20 ± 3 mmol \cdot kg dw $^{-1}\cdot$ h $^{-1}$) and MG (20 ± 4 mmol \cdot kg dw $^{-1}\cdot$ h $^{-1}$) were similar. The mean rate of MG resynthesis during the entire 5 h of recovery was 17 ± 4 mmol \cdot kg dw $^{-1}\cdot$ h $^{-1}$ and was 50% lower than the PG resynthesis rate observed during recovery ($P \leq 0.05$).

GS

%I form. At exhaustion, there were no differences observed in GS activity between treatments, as measured by the %I form. It is interesting to note that the %I form of GS remained unchanged between exhaustion and 30 and 120 min into recovery ($P > 0.05$) despite significant increases in insulin concentration and PG+MG concentration. By 300 min, %I form of GS declined significantly as PG+MG concentration approached resting values ($P \leq 0.05$). At no time were differences observed between treatments ($P > 0.05$) (Fig. 3).

Percent fractional velocity. At exhaustion, no differences in percent fractional velocity of GS between treatments were observed ($P > 0.05$). Fractional velocity of GS remained unchanged between exhaustion and 30 and 120 min of recovery and then declined such that the fractional velocity at 300 min (66.8%) was significantly different from that observed at 30 and 120 min of recovery ($P \leq 0.05$). Similar to the %I form, these changes in fractional velocity of GS occurred independent of increases in insulin and PG+MG glycogen concentration. Although no treatment effects were observed in glycogen concentration, glycogen resynthesis rate, or %I form of GS, caffeine ingestion was associated with a significant decrease in fractional velocity of GS ($P = 0.05$; Fig. 3).

DISCUSSION

This study examined 1) the effect of caffeine ingestion during exercise on the net resynthesis of PG and MG during early recovery and 2) the pattern of PG and MG resynthesis after exhaustive exercise in humans. The present findings demonstrate that caffeine ingestion did not impede either the amount or rate of PG or MG resynthesis, despite the fact that caffeine resulted in higher blood glucose and FFA and glycerol responses and lower percent fractional velocity of GS. The present results also confirm previous findings from our laboratory that PG resynthesis predominates during early recovery (2); however, early and repeated sampling times and an aggressive CHO ingestion pattern now provide evidence that the rate of PG resynthesis was underestimated and may be regulated by different factors than previously reported.

Caffeine's lack of effect on PG and MG resynthesis in the present study is surprising because previous studies have consistently reported significant negative effects of caffeine ingestion on insulin-mediated glucose disposal in the resting state (12, 13, 25, 36). The lack of a caffeine effect on postexercise glycogen synthesis could suggest that caffeine affects CHO metabolism in resting, but not in active or postexercised,

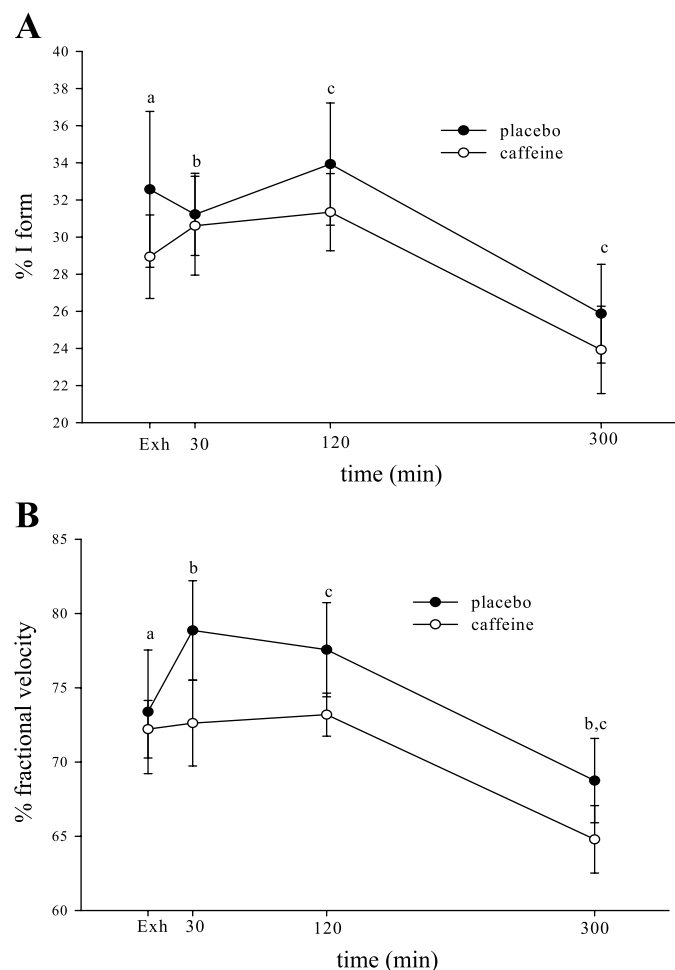


Fig. 3. Glycogen synthase activity during 5 h of recovery expressed as percent glucose-6-phosphate-independent (% I) form (A) and % fractional velocity (B). Values are means \pm SE. \bullet , Placebo trial; \circ , caffeine trial. Time points sharing a lowercase letter are significantly different ($P \leq 0.05$) from each other within a trial. Caffeine resulted in a significantly lower % glycogen synthase fractional velocity compared with placebo.

muscle. Graham et al. (11) demonstrated no change in glucose uptake and net glycogen breakdown during exercise, suggesting that caffeine affects insulin-dependent mechanisms and not insulin-independent mechanisms. In contrast to these findings, Thong et al. (36) reported that caffeine ingestion followed by a hyperinsulinemic-euglycemic clamp resulted in significant decreases in both leg glucose uptake ($\sim 50\%$) and GS activity ($\sim 17\%$ fractional velocity of GS and $\sim 35\%$ I form) after one-legged knee extensor exercise in humans. Despite these decreases, no differences were found in glycogen concentration between the caffeine and placebo treatments. We suspect that the lack of caffeine effect on glycogen metabolism in their study was due to 1) a short period of observation (100 min), and 2) glycogen resynthesis conditions that resulted in a low rate of glycogen resynthesis, such that a minimal increase in glycogen concentration was observed in each trial. The present study achieved a much higher rate of glycogen resynthesis, which was probably due to both a lower level of glycogen at exhaustion and a large, frequent, and immediate CHO provision. Nevertheless, our results confirm the findings of Thong et al. that caffeine ingestion does not impede glycogen resynthe-

sis during recovery in humans despite a significant decrease in GS activity (36). The findings of the present study suggest that the factors driving glycogen synthesis postexercise, such as the insulin-independent mechanisms, low level of glycogen at exhaustion, and high insulin and glucose levels, may override caffeine's potential negative effects on glucose metabolism previously seen during resting conditions (12, 13, 25, 36).

Another possible explanation for a lack of caffeine effect on PG and MG resynthesis could be because of the high degree of intersubject variability of both PG and MG concentrations at exhaustion and 30 min postexercise. It is possible that this variability masked caffeine's negative effects on glycogen recovery during the initial 30-min period. This variability could be attributed to a variety of factors, including the training status of subjects. Hickner et al. (19) reported that trained individuals have an increased ability to resynthesize glycogen after glycogen-depleting exercise than sedentary individuals. However, within our study, statistical analysis revealed no differences in the rates of glycogen resynthesis between those subjects who were trained ($n = 7$; $\dot{V}O_{2\max} = 60$ ml \cdot kg $^{-1}\cdot$ min $^{-1}$) and those who were sedentary ($n = 3$; 38 ml \cdot kg $^{-1}\cdot$ min $^{-1}$). This suggests that training status did not contribute significantly to the variability in PG and MG concentrations.

Caffeine ingestion also resulted in both higher blood glucose and FFA and glycerol responses. Although the present model does not allow for a direct conclusion to be made, a higher blood glucose response after caffeine ingestion has been reported under resting and postexercise conditions and may suggest an impairment in glucose disposal (11, 12, 13, 25, 36). It remains unclear, however, why no corresponding increase in the insulin response was observed. Regardless, the increased glucose response did not result in differences in glycogen resynthesis between the placebo and caffeine treatments. With respect to the FFA and glycerol responses, our findings confirm those seen in resting studies that the caffeine-induced increase in the concentration of FFAs declines as insulin levels increase (11, 12, 13, 36).

The present net rates of glycogen resynthesis are as high as any previously reported in the literature during 2 and 5 h of recovery (2, 6, 7, 23, 32, 33, 37, 38). However, our early, repeated sampling and separation of glycogen into two fractions allows for a more detailed look at the glycogen resynthesis process. Our study is the first that we know of to include a 30-min postexercise sample while utilizing a protocol involving both biopsies and a CHO-feeding regime. Most studies utilizing biopsies and exogenous CHO take their first sample at ≥ 2 h postexercise and therefore underestimate the magnitude of the initial rate of total glycogen resynthesis (7, 23, 32). As well, studies involving NMR spectroscopy that have included a 30-min sample typically do not include a CHO-feeding regime (33, 37). Only one recent study by Ivy et al. (22) has combined NMR spectroscopy and CHO supplementation. Although their rates of glycogen resynthesis are comparable with the present study's reported rates of total glycogen resynthesis, their study did not separate muscle glycogen into fractions. Our separation of glycogen into two fractions allows us to estimate into which fraction the incoming glucose molecules are entering. Incoming glucose molecules can either be incorporated into 1) new glycogen particles formed from glycogenin, 2) existing PG particles, or 3) existing MG particles. Although

our data are only semiquantitative because we report net changes in glycogen concentrations, they are nevertheless quite informative. For example, if MG were the dominant fraction being resynthesized, either by existing PG growing into MG or existing MG particles growing larger, we would expect either a decrease or no change in PG concentration, respectively. In the present study, however, we report that the initial and largest changes in glycogen concentration occur in the PG fraction, suggesting that either new granules are being formed or incoming glucose molecules are being added to already existing PG particles. This finding is supported by work from Marchand et al. (29), who, using electron microscopy, reported that there is an increase in glycogen particle number rather than an increase in glycogen particle diameter during the first 4 h of recovery from exhaustive exercise in humans. Similarly, Elsner et al. (9) reported that cultured myotubes synthesize glycogen predominantly by forming new granules rather than by increasing granule size.

Although this overall pattern of PG and MG resynthesis is similar between our present and previous work (2), differences were observed with respect to the magnitude and timing of changes in glycogen concentrations. For example, the net rates of PG and MG resynthesis were five- and twofold greater in the present study, respectively, which resulted in a MG concentration of ~ 100 mmol/kg dw and a PG+MG concentration of 300–350 mmol/kg dw being obtained by 5 h of recovery compared with 24 h in the previous study. These higher rates of resynthesis in the present study may be attributed to both a lower PG+MG concentration at exhaustion and a more aggressive CHO feeding schedule, both of which enhance the rate of glycogen resynthesis (2). Despite this acceleration, the basic pattern remained: 1) PG is the predominate form resynthesized early in recovery, 2) there is little net resynthesis of MG until a PG+MG concentration of ~ 250 mmol/kg dw is obtained, and 3) the increase in MG concentration occurs without a corresponding decrease in PG concentration, suggesting that when MG resynthesis does occur that PG resynthesis continues.

The magnitudes of change in glycogen concentrations are not the only differences observed between our present and previous work (2). The present results also suggest that the net rate of PG resynthesis may not be regulated as previously suggested. Adamo et al. (2) observed the highest rate of PG resynthesis during the first 4 h of recovery when insulin levels (339 pM) were high and speculated that the process responsible for PG resynthesis was insulin sensitive. In contrast, the present study observed the highest net rate of PG resynthesis during the first 30 min of recovery when insulin levels (101 pM) were near resting values. As well, by the time insulin levels peaked (303 pM), the net rate of PG resynthesis had declined 46%. Together, these findings suggest that factors other than insulin are responsible for determining the rate of PG resynthesis. Although regression analysis revealed no correlation between the rate of PG, MG, or PG+MG resynthesis and either PG, MG, or PG+MG concentration (respectively) or GS activity (data not shown), it is interesting that the rate of PG and PG+MG resynthesis did parallel changes in blood glucose concentration. Glucose has been shown to stimulate glycogen resynthesis in cultured human myoblasts after glycogen depletion (17).

Studies of both rodent and human muscle have demonstrated a strong relationship between glycogen concentration and the rate of glycogen resynthesis and GS activity (27, 30, 31). The reason for the lack of correlation between these factors within the present study is unknown. It is possible that with our low level of glycogen at exhaustion, early sampling time, and the large, immediate, and frequent provision of CHO, that the potential for glycogen to regulate the rate of its resynthesis is overridden and that GS activity was controlled by different factors throughout early recovery. For example, during the initial 120 min postexercise, GS activity remained constant despite a 55% increase in PG+MG concentration and a 61% decrease in the rate of PG+MG resynthesis. It is possible that the elevated blood glucose and insulin concentrations during this time may have stimulated GS activity. Our findings are supported by Greiwe et al. (14) and Hickner et al. (19), who reported sixfold increases in glycogen concentration with no corresponding decreases in GS activity during the initial 6 h of recovery from exhaustive exercise in humans and with the provision of exogenous CHO. As well, studies in cultured human myoblasts have demonstrated that the effects of glucose and insulin on the rate of glycogen resynthesis are additive and together may override glycogen's ability to control the rate of its resynthesis (17). In contrast to what occurs during the initial 2 h postexercise, during the final 3 h of recovery when insulin levels were at their highest and PG+MG concentration increased by 40%, both the %I form and percent fractional velocity of GS decreased significantly by 24 and 12%, respectively. Although no significant correlation was found between glycogen concentration and GS activity (data not shown), these data do support previous reports that glycogen concentration is a stronger predictor of GS activity than insulin during this period of recovery (27, 30, 31). These data therefore suggest that the relationship between glycogen concentration and GS activity and the factors regulating the rate of glycogen resynthesis may vary during recovery from exhaustive exercise in combination with an aggressive CHO-feeding protocol.

It is noteworthy that during the first 30 min of recovery when the net PG+MG and PG rates of resynthesis were greatest that the blood glucose concentration continued to increase. Also, despite repeated ingestions of CHO throughout recovery and a decline in the rate of PG+MG resynthesis, blood glucose levels declined between 60 and 300 min. These changes in blood glucose concentration may be explained by a change in hepatic glycogen synthesis (15, 20). Studies have reported that immediately after glycogen-depleting exercise, the majority of glycogen resynthesis occurs in the muscle and that only when glycogen levels are partially repleted will the incoming glucose molecules be redirected for hepatic glycogen resynthesis (15, 20).

In conclusion, our observations demonstrate that the ingestion of caffeine during exercise does not impede PG and MG resynthesis after glycogen-depleting exercise when there was a large, immediate, and frequent ingestion of a CHO beverage in humans. Our data suggest that the postexercise factors driving glycogen resynthesis are stronger than caffeine's potential negative effects on glucose metabolism. Our results also confirm the results of our previous work that PG is the predominant glycogen pool resynthesized in early recovery, that MG is not resynthesized until a PG+MG concentration of ~ 250 mmol/kg dw is obtained, and that the increase in MG concen-

tration occurs without a corresponding decrease in PG concentration (2). Our data further suggest that the process controlling the rate of PG resynthesis is insulin insensitive and that the immediate and frequent supply of CHO can enhance the magnitude of PG and MG resynthesis but does not influence the overall pattern of their resynthesis.

ACKNOWLEDGMENTS

The authors thank 1) Premila Sathasivam for technical assistance, 2) the volunteers who helped on trial days, and 3) the subjects for time and dedication to this project.

GRANTS

This study was supported by the National Science and Engineering Research Council (NSERC) and the Gatorade Sports Science Institute. D. S. Battram currently holds a NSERC studentship and at the time of this project was supported by a Gatorade Sports Science Institute student research award. J. Shearer currently holds a NSERC fellowship and at the time of this project was supported by an Industrial NSERC Award sponsored by the Gatorade Sports Science Institute.

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