Caffeine Ingestion Decreases Glucose Disposal During a Hyperinsulinemic-Euglycemic Clamp in Sedentary Humans

Felicia Greer,[1] Robert Hudson,[2] Robert Ross,[3] and Terry Graham[4]

The purpose of this investigation was to examine the effect of caffeine (an adenosine receptor antagonist) on whole-body insulin-mediated glucose disposal in resting humans. We hypothesized that glucose disposal would be lower after the administration of caffeine compared with placebo. Healthy, lean, sedentary (n = 9) men underwent two trial sessions, one after caffeine administration (5 mg/kg body wt) and one after placebo administration (dextrose) in a double-blind randomized design. Glucose disposal was assessed using a hyperinsulinemic-euglycemic clamp. Before the clamp, there were no differences in circulating levels of methylxanthines, catecholamines, or glucose. Euglycemia was maintained throughout the clamp with no difference in plasma glucose concentrations between trials. The insulin concentrations were also similar in the caffeine and placebo trials. After caffeine administration, glucose disposal was 6.38 ± 0.76 mg/kg body wt compared with 8.42 ± 0.63 mg/kg body wt after the placebo trial. This represents a significant ($P < 0.05$) decrease (24%) in glucose disposal after caffeine ingestion. In addition, carbohydrate storage was 35% lower ($P < 0.05$) in the caffeine trial than in the placebo trial. Furthermore, even when the difference in glucose disposal was normalized between the trials, there was a 23% difference in the amount of carbohydrate stored after caffeine administration compared with placebo administration. Caffeine ingestion also resulted in higher plasma epinephrine levels than placebo ingestion ($P < 0.05$). These data support our hypothesis that caffeine ingestion decreases glucose disposal and suggests that adenosine plays a role in regulating glucose disposal in resting humans. *Diabetes* 50:2349–2354, 2001

One role of insulin is the management of glucose uptake by a variety of tissues. Adenosine has been implicated as a local metabolite that can influence insulin binding and signaling and the translocation of glucose transporters. The many actions of adenosine are mediated by specific extracellular purinergic (P_1) receptors that are identified by their high affinity for adenosine compared with adenosine nucleotides (1) and by their antagonism by methylxanthines (2). Three types of adenosine receptors (A_1, A_2, and A_3) have been identified (3,4), with A_2 receptors subdivided into A_{2a} and A_{2b} receptors.

Glucose transport is a key element in insulin sensitivity, and skeletal muscle largely accounts for the decreased insulin sensitivity observed in obese individuals and those with type 2 diabetes (5). Few investigations have examined adenosine-insulin interaction in skeletal muscle, yet it is the major tissue responsible for insulin-mediated glucose uptake after carbohydrate ingestion. Thus, the effect of adenosine on insulin-mediated glucose uptake in this tissue is of particular significance (6,7).

Several studies investigating the role of adenosine on carbohydrate metabolism using rodent skeletal muscle have demonstrated a decrease in glucose uptake in the presence of an adenosine receptor antagonist (8–11). However, among these investigations, it is controversial as to whether this occurs in both resting and active muscle and whether all fiber types respond similarly. It has been reported that adenosine appears to have a glycogen-sparing effect in active oxidative rodent skeletal muscle (12), suggesting that adenosine's role in modulating glycogen metabolism may be linked to its role in regulating glucose uptake. However, few studies have used an intact animal, and no one has reported the effects of adenosine-insulin interaction on glucose disposal in humans.

Early studies investigating the effects of methylxanthines on glucose homeostasis in response to a glucose challenge in humans were descriptive in nature, reported inconsistent findings, and were fraught with methodological problems (13–17). More recent investigations have also produced conflicting results. Pizziol et al. (18) reported an increase in glucose concentration with no effect on insulin levels after the ingestion of caffeinated coffee during the administration of an oral glucose tolerance test. This contrasts a report by Battram et al. (19), who observed a significant increase in insulin and no difference in

From [1]Ohio University Eastern, St. Clairsville, Ohio; the [2]Department of Medicine, Division of Endocrinology, Queen's University, Kingston, Ontario, Canada; the [3]School of Physical and Health Education, Queen's University, Kingston, Ontario, Canada; and the [4]Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada.

Address correspondence and reprint requests to Felicia Greer, Ohio University Eastern, 45425 National Rd., St. Clairsville, Ohio 43950. E-mail: greerf@skynet.eastern.ohiou.edu.

Received for publication 25 September 2000 and accepted in revised form 16 July 2001.

FFA, free fatty acid; GS, glycogen synthase; HPLC, high-performance liquid chromatography; MRI, magnetic resonance imaging; RER, respiratory exchange ratio; RIA, radioimmunoassay.

blood glucose levels after the ingestion of an oral glucose load in the caffeine trial compared with the placebo trial. Once again, inadequate study design could be a reason for these conflicting results. Pizziol et al. (18) used coffee as their administration vehicle, the majority of subjects were women and were not monitored for their menstrual status, and the caffeine content of coffee was not indexed for body weight.

Thus, there are inconsistent results in rodent muscle and limited information on humans. Type 2 diabetes affects 5–20% of the population in Westernized societies (20) and is a disease characterized by insulin resistance. Caffeine, a trimethylxanthine, is the most common drug consumed in North America. Furthermore, it is a substance that has been implicated to decrease insulin sensitivity. If caffeine is a causative agent of skeletal muscle insensitivity at rest, recommendations concerning caffeine intake may be beneficial for certain individuals, such as those with type 2 diabetes. The effect of caffeine ingestion has never been investigated in humans using a hyperinsulinemic-euglycemic clamp technique, which is a sensitive method to measure whole-body glucose disposal. The purpose of this study was to investigate the influence of caffeine, a nonselective adenosine receptor antagonist, on whole-body glucose uptake in resting humans. We hypothesized that glucose disposal would be reduced after the administration of caffeine compared with that of placebo.

RESEARCH DESIGN AND METHODS

Subjects were recruited from the general public and from Queen's University. Inclusion criteria required that men were lean (BMI <25 kg/m^2), sedentary (i.e., did not participate in any regular physical activity), and were nonsmokers. Nine lean males (age 25 ± 0.5 years) served as subjects for this study. Subjects were screened for type 2 diabetes and medications that could possibly interfere with glucose disposal and were questioned regarding their daily physical activity habits. Furthermore, subjects were selected only if they were not regular caffeine users (<2 cups of coffee/week or <5 caffeine-containing soft drinks/week). The experimental procedures and possible risks of the study were explained, and all subjects gave written informed consent. The study was approved by Queen's University Ethics Committee.

Magnetic resonance images were obtained with a General Electric Signa Advantage 1.5-telsa scanner using software version 5.4.2 (Milwaukee, WI). The magnetic resonance imaging (MRI) protocol is described in detail elsewhere (21). Briefly, subjects laid in the magnet with arms placed overhead. Using the intervertebral space between the fourth and fifth lumbar vertebrae (L4-L5) as the point of origin, transverse images (10-mm slice thickness) were obtained every 40 mm from hand to foot, resulting in a total of ~46 images per subject. The total time required to obtain all MRI data for each subject was ~45 min. All MRI data were transferred to a computer workstation (Silicon Graphics, Mountain View, CA) for analysis using specially designed image analysis software (Tomovision, Montreal). These measurements were used to calculate skeletal muscle mass so glucose disposal could be expressed per kilogram of skeletal muscle.

Subjects arrived at the Special Investigations Unit of the Kingston General Hospital at 7:00 A.M. after an overnight fast on two occasions. Trials were separated by ~1 week. Subjects were asked to abstain from all methylxanthine-containing products for 48 h before each trial, and subjects recorded diet intake during this time. A catheter was placed in the medial anticubital vein for the infusion of a normal saline (0.9% NaCl) drip, as well as for the infusion of insulin, glucose, and potassium. A second catheter was placed in a heated hand vein, which was used to draw arterialized venous blood samples. Blood samples were taken at baseline and every 30 min for the duration of the clamp to measure insulin and C-peptide concentrations. At each hour, additional blood was drawn for the determination of catecholamines, methylxanthines, free fatty acids (FFAs), glycerol, and lactate. After the baseline blood sample (referred to as 0 min), subjects ingested a gelatin capsule containing either placebo (dextrose) or caffeine (5 mg/kg body wt), and the insulin clamp was initiated.

Glucose disposal was assessed using a hyperinsulinemic-euglycemic clamp procedure (22) that was performed by a physician who was present at all times. This technique assesses glucose disposal by determining the amount of glucose needed to maintain euglycemia during a constant insulin infusion. Insulin (Novolin, Toronto) was infused at a rate of 40 mU · m^{-2} · min^{-1} for 180 min. Twenty-percent glucose was infused at a rate to maintain a target plasma glucose concentration of 5.0–5.5 mmol/l. Glucose concentrations were monitored at 5-min intervals using an automated glucose analyzer (YSI 2,300 Glucose Analyzer; YSI, Yellow Springs, OH), and the glucose infusion was adjusted accordingly. Potassium chloride (42 mEq) was infused with an Imed Gemini PC-1 pump at a rate of 7.5 ml/min. The experiments were conducted in a random double-blind fashion. Indirect calorimetry (TEEM Aerosport) was conducted during the last 30 min of the clamp procedure to determine carbohydrate oxidation. Nonoxidative glucose disposal was calculated by subtracting the carbohydrate oxidation rate (obtained from V_{O_2} and respiratory exchange ratio [RER] measurements) from the total glucose uptake rate (based on the amount of glucose infused during the last 30 min of the clamp because the rate of glucose infusion is stable during this phase of the procedure). It was assumed that all carbohydrate oxidized was derived from the exogenous glucose, while the remaining glucose was stored.

Blood samples for the determination of catecholamines, methylxanthines, FFAs, glycerol, and lactate were immediately separated into two aliquots; 3 ml were transferred to a nontreated tube for the analysis of serum FFA, and 7 ml were transferred to a sodium heparinized tube for the analysis of other metabolites. A 100-μl aliquot of blood was added to 500 μl of 0.6 mol/l perchloric acid for the analysis of blood glycerol and lactate. A solution of 120 μl of 0.24 mol/l ethylene glycol-bis EGTA and reduced glutathione was then added to the remaining blood. The EGTA- and glutathione-treated plasma was analyzed in duplicate for epinephrine and norepinephrine concentrations using high performance liquid chromatography (HPLC) (Waters), as described by Weiker (23). Plasma caffeine, paraxanthine, theophylline, and theobromine were measured using fully automated HPLC (Waters). An aliquot of plasma (150 μl) was added to ~40 mg ammonium sulfate and 50 μl of 0.05% acetic acid. After adding 25 μl of an internal standard solution (7-*B*-hydroxypropyl theophylline) and 3 ml of chloroform/isopropyl alcohol (85:15 v/v) 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, resuspended in HPLC mobile-phase solvent (3% isopropanol, 0.05% Hac, and 0.5% methanol), and 100 μl was injected into a Beckman Ultrasphere IP C18 5-μl column. Methylxanthines were measured at 282 nm wavelength. Reagents for standards were obtained from Sigma Chemical. The blood perchloric acid extracts were analyzed enzymatically in duplicate for lactate and glycerol (24). Serum was analyzed enzymatically in duplicate for FFAs (25). Plasma insulin was measured using a radioimmunoassay (RIA) kit supplied by Diagnostic Products (Los Angeles), and C-peptide was measured using an RIA kit supplied by Linco Research (St. Louis, MO).

Statistics. Carbohydrate oxidation and storage data and glucose disposal were compared with respect to treatment using a one-way analysis of variance with repeated measures. The 95% CI is expressed for carbohydrate storage and glucose disposal data in addition to the P value. All blood data were analyzed using a two-way analysis of variance (time by treatment) for repeated measures. When significance was found ($P < 0.05$), Tukey's test was performed as the post hoc analysis. Data are reported as the mean ± SE.

RESULTS

There was a significant decrease ($P < 0.05$) in glucose disposal after caffeine ingestion compared with placebo ingestion. After caffeine administration, glucose disposal was 24% less (6.38 ± 0.76 mg · kg body wt^{-1} · min^{-1} vs. 8.42 ± 0.63 mg · kg body wt^{-1} · min^{-1}) than after placebo administration (Fig. 1). The confidence interval for the difference between these two means was −3.46 to −0.64. Average blood glucose concentrations during the last 30 min of the clamp were virtually identical in both trials (caffeine 5.2 ± 0.6 mmol/l, placebo 5.2 ± 0.4 mmol/l). When expressed per kilogram of skeletal muscle mass, glucose disposal in the caffeine trial was 15.5 ± 1.71 mg · kg^{-1} · min^{-1} compared with 20.44 ± 1.33 mg · kg^{-1} · min^{-1} in the placebo trial.

Calorimetry. V_{O_2} during the last 30 min of the clamp was not significantly different ($P > 0.05$) during the caffeine (3.8 ± 0.3 mg · kg^{-1} · min^{-1}) and placebo (3.1 ± 0.3 mg · kg^{-1} · min^{-1}) trials. Likewise, RER values were also similar (caffeine 0.83 ± 0.04, placebo 0.81 ± 0.02). There

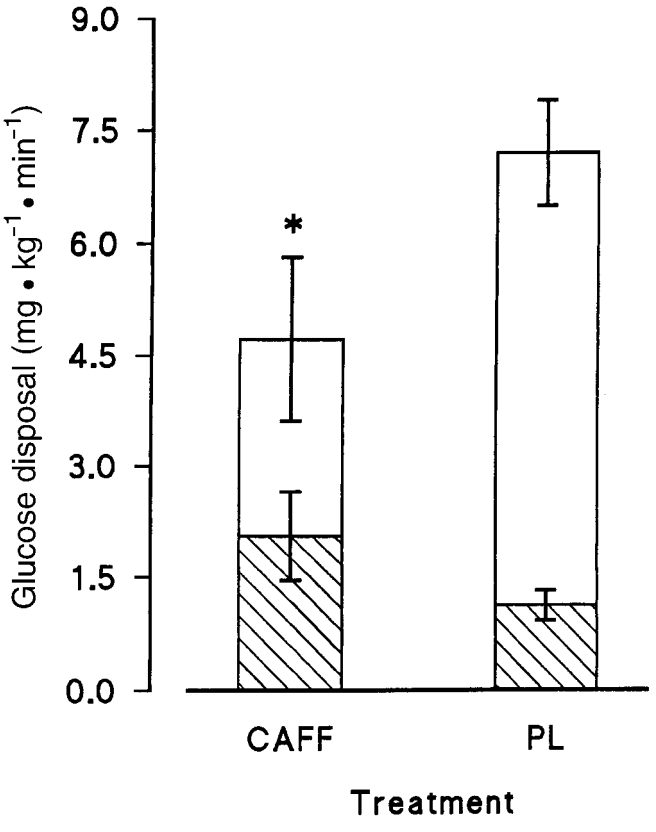

FIG. 1. The effect of caffeine ingestion on glucose disposal during the hyperinsulinemic-euglycemic clamp. Hatched bars indicate carbohydrate oxidation. Open bars indicate carbohydrate storage. *Statistically significant from placebo ($P < 0.05$). PL, placebo; CAFF, caffeine.

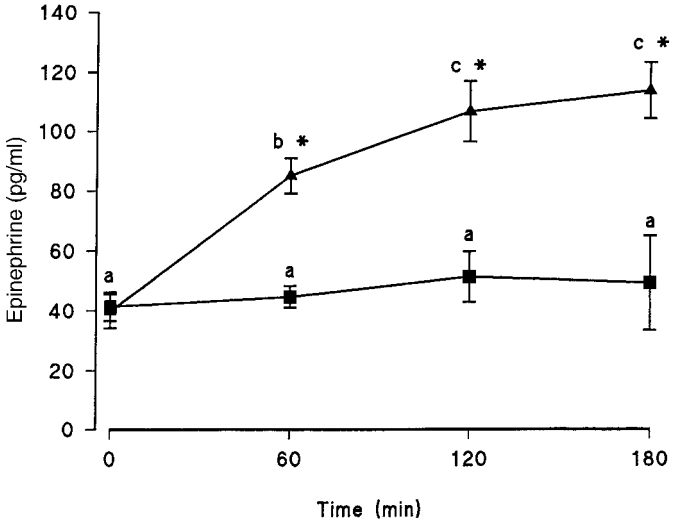

FIG. 2. Epinephrine responses to caffeine (CAFF, ▲) and placebo (PL, ■) ingestion during the hyperinsulinemic-euglycemic clamp. Values having the same letter within a given treatment are not significantly different from one another ($P < 0.05$). *Statistically significant from placebo ($P < 0.05$).

was a trend ($P = 0.07$) for carbohydrate oxidation to be higher in the caffeine trial (2.06 ± 0.6 mg · kg^{-1} · min^{-1}) than in the placebo trial (1.12 ± 0.2 mg · kg^{-1} · min^{-1}). When expressed per kilogram of skeletal muscle, carbohydrate oxidation in the placebo trial was 0.04 ± 0.01 mg · kg^{-1} · min^{-1} compared with 0.07 ± 0.02 mg · kg^{-1} · min^{-1} in the caffeine trial. Consequently, carbohydrate storage was significantly lower ($P < 0.05$) in the caffeine trial (4.72 ± 1.1 mg · kg^{-1} · min^{-1}) than in the placebo trial (7.20 ± 0.7 mg · kg^{-1} · min^{-1}) (Fig. 1). The confidence interval for the difference between these two means was −5.90 to −3.01. This 35% decrease in carbohydrate storage after caffeine ingestion is consistent with the decreased glucose uptake in the caffeine trial. The distribution of glucose was calculated by setting the glucose disposal at 100% for each treatment (to account for the lower glucose uptake in the caffeine trial). Subsequently, the percent of glucose either oxidized or stored was determined. In the caffeine trial, 62.4 ± 12.4% of the glucose was stored, and in the placebo trial, 85.5 ± 2.8% was stored. This represents a 23% ($P = 0.06$) decrease in the amount of carbohydrate stored in the caffeine trial, even after accounting for the lower glucose uptake after methylxanthine administration.

Methylxanthines. Plasma caffeine concentration before capsule ingestion was negligible in both trials, confirming 48 h abstinence from methylxanthine-containing products. Within 60 min after the ingestion of caffeine, plasma caffeine concentration appeared to reach steady state. At 1 and 3 h, mean plasma caffeine concentrations in the caffeine trial were 45.0 ± 10.3 and 45.1 ± 6.5 μmol/l, respectively. Other methylxanthine concentrations were similar to previous reports (26,27), when a similar caffeine dose was administered.

Catecholamines. One hour after caffeine ingestion, the plasma epinephrine level was significantly increased compared with that of placebo ($P < 0.05$) (Fig. 2). Epinephrine remained significantly elevated throughout the clamp in the caffeine compared with the placebo trials. Norepinephrine concentration was not affected by either caffeine or placebo ingestion. Norepinephrine concentrations remained constant throughout the duration of the clamp and were not different from baseline ($P > 0.05$). (Table 2).

Insulin. Baseline plasma insulin were not obtained because of technical problems encountered when obtaining blood samples at this time period. Resting insulin concentrations reported in the literature are ~14 μU/ml in healthy young subjects (7,22). Because our subjects were screened for type 2 diabetes and were otherwise healthy, it was assumed that their baseline insulin concentrations would have been similar. Furthermore, resting C-peptide concentrations were very similar at the start of both trials, indicating low basal insulin concentrations. Based on this assumption, plasma insulin concentrations were dramatically increased by 30 min after the beginning of the clamp compared with baseline. Insulin concentrations were not different in the caffeine trial compared with the placebo trial throughout the 180-min clamp (Table 1).

C-peptide. Plasma C-peptide concentrations were similar throughout the 180-min hyperinsulinemic-euglycemic clamp in both trials ($P > 0.05$). There was a decrease in C-peptide over time, so that at 60 min, C-peptide concentration was significantly lower ($P < 0.05$) than at 0 and 30 min (Table 1). This is consistent with a decrease in insulin secretion that would be expected because of the infusion of high levels of exogenous insulin.

Blood lactate. Blood lactate concentration was not affected by the ingestion of caffeine or placebo ($P > 0.05$). Furthermore, lactate levels were similar throughout the

TABLE 1
Summary of plasma insulin and C-peptide concentration during the 180-min hyperinsulinemic-euglycemic clamp

Treatment	Time (min)						
	0	30	60	90	120	150	180
Insulin (μU/ml)							
PL	N/A	57.7 ± 5.4	59.9 ± 6.8	66.2 ± 6.3	64.0 ± 6.9	66.0 ± 8.7	62.5 ± 5.5
CAFF	N/A	49.7 ± 4.3	57.5 ± 4.3	54.8 ± 4.3	59.3 ± 5.7	62.5 ± 6.5	57.2 ± 6.6
C-peptide (ng/ml)							
PL	1.4 ± 0.2	1.3 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	1.0 ± 0.2
CAFF	1.5 ± 0.2	1.4 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2

Data are means ± SE. Treatment is indicated as placebo (PL) or caffeine (CAFF). N/A, not available. Available data are not statistically different from one another ($P > 0.05$).

hyperinsulinemic-euglycemic clamp procedure compared with baseline ($P > 0.05$) (Table 2).

Blood glycerol. Caffeine ingestion had no significant effect on blood glycerol concentrations compared with placebo ingestion ($P > 0.05$). Glycerol concentration decreased significantly ($P < 0.05$) when exogenous insulin was infused compared with basal concentrations. (Table 2).

Serum FFAs. Serum FFA concentrations were not significantly different in the caffeine trial compared with those of the placebo trial ($P > 0.05$). However, 1 h into the clamp session, FFA concentrations were significantly decreased ($P < 0.05$) compared with initial levels and remained suppressed throughout the remainder of the clamp (Table 2).

DISCUSSION

This study examined the effect of caffeine, a nonselective adenosine receptor antagonist on whole-body glucose disposal in humans. The most significant finding was a 24% decrease in glucose uptake and a 35% decrease in carbohydrate storage after caffeine ingestion compared with placebo ingestion. This is the first study to investigate the effects of caffeine on whole-body glucose uptake in humans using a hyperinsulinemic-euglycemic clamp technique.

Because of various gender differences that exist regarding carbohydrate management, insulin sensitivity, and caffeine metabolism, we felt that a homogenous subject pool would provide the most consistent results. Menstrual position, oral contraceptive use, pregnancy, and menopause are all conditions that can affect caffeine metabolism (28,29), carbohydrate management, and insulin sensitivity (30). Furthermore, estrogen has been reported to inhibit caffeine metabolism and the pharmacokinetics of caffeine. Thus, depending on a woman's reproductive status, for a given caffeine dose, caffeine levels could vary (29). Whereas women should also be investigated, we felt that because this was the first study to examine glucose disposal in human subjects using a hyperinsulinemic-euglycemic clamp, a homogenous population of men was the most appropriate.

Skeletal muscle is a very important tissue in whole-body glucose management, accounting for 50–80% of glucose clearance after the ingestion of a carbohydrate load (6,31). Thus, any alterations in the insulin sensitivity of skeletal muscle could have severe implications for whole-body glucose uptake. Crist et al. (8) investigated the effects of an A_1 adenosine receptor antagonist on glucose uptake in various tissues of lean rodents using a hyperinsulinemic-euglycemic clamp. This is the only study, to our knowledge, that has examined the effects of an adenosine receptor antagonist on glucose uptake in intact animals. They reported no change in glucose uptake by the liver, a 49% decrease in glucose uptake by adipose tissue, and a 12–16% decrease in glucose uptake by skeletal muscle in the presence of an adenosine receptor antagonist. Because skeletal muscle represents a much larger mass in the body and because the amount of glucose taken up by this tissue was 10-fold greater compared with adipose, the majority of the decrease in glucose clearance after adenosine receptor antagonism was caused by skeletal muscle. In the present study, the results reflecting a 24% decrease in glucose uptake after caffeine ingestion in resting humans

TABLE 2
Summary of blood-borne metabolites during the 180-min hyperinsulinemic-euglycemic clamp

Treatment	Time (min)			
	0	60	120	180
NE (pg/ml)				
PL	202.3 ± 29.1*	197.7 ± 21.5*	232.7 ± 24.1*	218.9 ± 17.7*
CAFF	220.7 ± 30.2*	249.3 ± 32.3*	257.4 ± 30.6*	225.9 ± 28.9*
Lactate (mmol/l)				
PL	1.7 ± 0.1*	1.7 ± 0.1*	1.6 ± 0.1*	1.6 ± 0.2*
CAFF	1.5 ± 0.1*	1.6 ± 0.1*	1.7 ± 0.1*	1.5 ± 0.1*
Glycerol (μmol/l)				
PL	54.6 ± 14.1*	35.9 ± 11.4†	37.8 ± 13.2†	33.9 ± 12.6†
CAFF	66.5 ± 14.4*	39.8 ± 14.0†	35.9 ± 10.9†	34.9 ± 9.5†
FFA (mmol/l)				
PL	0.6 ± 0.1*	0.3 ± 0.1†	0.2 ± 0.01†	0.2 ± 0.02†
CAFF	0.6 ± 0.1*	0.3 ± 0.02†	0.2 ± 0.02†	0.2 ± 0.02†

*Data are means ± SE. Treatment is indicated as placebo (PL) or caffeine (CAFF). NE, norepinephrine. Symbols represent significance between time points. Data with the same symbol are not statistically different from one another ($P > 0.05$).

are slightly higher, yet consistent, with a previous report (8). Because skeletal muscle is primarily responsible for glucose disposal under hyperinsulinemic-euglycemic conditions (7), it is highly probable that the decrease in glucose uptake in the present study was caused by alterations in this tissue's sensitivity to insulin.

Glucose taken up by the muscle could have one of three primary fates: oxidation; storage as glycogen; or conversion to three carbon compounds (predominately lactate), which could subsequently be transported to the liver (32). In the present study, blood lactate concentrations were stable throughout the hyperinsulinemic-euglycemic clamp procedure and were not different in the caffeine trial compared with the placebo trial. However, in both trials, carbohydrate oxidation was significantly lower compared with carbohydrate storage. These data are compatible with previous studies (7,33) estimating that the majority of an infused or oral glucose load is stored in muscle. Possible factors influencing glucose storage include membrane transport (34), glycogen synthase (GS) (which has been demonstrated to be activated by glucose and insulin infusions in proportion to glucose uptake [35] and skeletal muscle blood flow), or fiber type (36). It is unlikely that fiber type played a role in the reduction of carbohydrate storage after caffeine ingestion, because conditions for both trials were similar and because subjects served as their own control. Furthermore, caffeine has been demonstrated to increase total peripheral resistance with no difference in blood flow (37). Another possible site of action could be the modulation of hexokinase II. The inhibition of hexokinase would interfere with the turnover rate of the receptor and hence depress glucose transport or uptake. Although Han et al. (10) found no effect of adenosine on this enzyme, more studies are needed to confirm their results.

An alteration in GS, the enzyme responsible for glycogen synthesis in the muscle, could be another explanation for the observed decrease in glucose uptake in the present study. It has been suggested that the failure to activate glycogen storage could lead to a reduced uptake of glucose by the muscle (32). Vergauwen et al. (12) reported a significant increase in glycogen breakdown in contracting oxidative muscle fibers after the administration of adenosine receptor antagonists in the presence of high insulin levels (100 μU/ml). Furthermore, GS fractional activity was depressed twofold in the soleus muscle, without any effect on glycogen phosphorylase during exercise. This is consistent with insulin's action in modulating GS (31). Thus, adenosine appears to exert a "glycogen sparing" effect in oxidative muscle by facilitating insulin-stimulated glycogen synthesis. Even after accounting for lower glucose uptake in the caffeine trial compared with the placebo trial, there was a strong trend for less of the glucose to be taken up than to be stored after caffeine (62.3%) rather than placebo (85.5%) administration. Although muscle GS activity was not measured, the lower carbohydrate storage in the caffeine trial was not only caused by less glucose being taken up by the muscle. Thus, it is possible that adenosine may have altered glucose uptake by modulating intracellular glucose transporter trafficking. This possible mechanism of adenosine needs to be investigated.

Insulin is a well-documented inhibitor of net hepatic glucose output (38). Steady-state insulin concentrations in the present study were within levels reported to significantly decrease hepatic glucose production (22) and very similar between trials. Thus, we are confident that the insulin levels infused were sufficient to suppress hepatic glucose output in the present study. Because it is likely that hepatic glucose output was suppressed and because the amount of insulin infused for a given subject was similar during both trials, an effect of caffeine on hepatic glucose output would not be expected. Consequently, this implies that the impaired glucose uptake observed in the caffeine trial was caused by alterations in the function of skeletal muscle rather than that of liver.

It is well established that caffeine administration results in an increase in plasma epinephrine levels in humans (39,26,27). However, an increase in plasma epinephrine does not always result in detectable effects on metabolism. Conversely, caffeine-induced metabolic effects have been reported without an increase in catecholamine levels (40,41). Epinephrine has been reported to oppose the action of insulin by acting on the mitogen-activated protein kinase signaling cascade (42). Although this may be a possible mechanism to explain the effect of caffeine in the present study, the epinephrine levels induced by Napoli et al. (42) were considerably higher than those in the present study. Furthermore, it would not explain the findings of other investigations where epinephrine was not present in the muscle preparation used (9–11).

There was no effect of treatment on blood glycerol concentration (an indicator of lipolysis) or on serum FFA levels. Insulin is a potent inhibitor of lipolysis (43). One hour into the clamp session, glycerol and FFA concentrations were significantly lower compared with baseline levels. This would be expected because exogenous glucose and high amounts of insulin were being infused throughout the duration of the clamp.

In conclusion, the results of this study show that the administration of caffeine results in a significant decrease in insulin-mediated glucose uptake in resting humans. Carbohydrate storage is also significantly reduced after caffeine ingestion compared with placebo ingestion. Furthermore, even when the difference in glucose disposal between the caffeine and placebo trials was accounted for, there was a 23% difference in the amount of carbohydrate stored after caffeine administration compared with placebo administration. Because caffeine is a nonselective adenosine receptor antagonist, these findings support the theory that adenosine is an important regulator of insulin-mediated glucose uptake and possibly glycogen synthesis. Furthermore, because skeletal muscle is the primary tissue responsible for insulin-mediated glucose uptake at rest, we propose that this tissue is critical in this metabolic alteration. Although highly speculative, these findings raise the possibility that caffeine ingestion (possibly in the form of coffee) could play a role in the development of insulin resistance. Because caffeine is a commonly consumed substance and one that may be a causative agent of skeletal muscle insulin insensitivity, recommendations concerning caffeine intake may be beneficial for certain individuals, such as those at risk for the development of type 2 diabetes. Caffeine has been proven an effective tool with which to study skeletal muscle metabolism and is

safe for human subjects. Thus, it may be a useful agent when studying insulin-adenosine interaction on carbohydrate metabolism and in expanding our knowledge of metabolic diseases.

ACKNOWLEDGMENTS

This study was supported in part by the Natural Sciences and Engineering Research Council of Canada.

The authors would like to acknowledge the excellent technical assistance of Premila Sathasivam, Susan Rhymer, and Diana Hall.

REFERENCES

1. Ramkumar V, Pierson G, Stiles G: Adenosine receptors: clinical implications and biochemical mechanisms. *Prog Drug Res* 32: 195–210, 1988
2. Biaggioni I, Paul S, Puckett A, Arzubiaga C: Caffeine and theophylline as adenosine receptor antagonists in humans. *J Pharmacol Exp Therap* 258:588–593, 1991
3. Daly J, Butts-Lamb P, Padgett W: Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. *Cell Mol Neurobiol* 3:69–80, 1983
4. Sajjadi F, Firestein G: cDNA cloning and sequence analysis of the human A_3 adenosine receptor. *Biochim Biophys Acta* 1179:105–107, 1993
5. Bonadonna RC, Saccomani MP, Seely L, Zych KS, Ferrannini E, Cobelli C, DeFronzo RA: Glucose transport in human skeletal muscle: the in vivo response to insulin. *Diabetes* 42:191–198, 1993
6. Baron A, Brechtel G, Wallace P, Edelman S: Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol* 255: E769–E774, 1988
7. DeFronzo RA, Jacot E, Jequier E, Maider E, Wahren J, Felber JP: The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30:1000–1007, 1981
8. Crist G, Xu B, LaNoue K, Lang C: Tissue-specific effects of *in vivo* adenosine receptor blockade on glucose uptake in Zucker rats. *FASEB J* 12:1301–1308, 1998
9. Derave W, Hespel P: Role of adenosine in regulating glucose uptake during contractions and hypoxia in rat skeletal muscle. *J Physiol* 515:255–263, 1999
10. Han D-H, Hansen PA, Nolte LA, Hollowszy JO: Removal of adenosine decreases the responsiveness of muscle glucose transport to insulin and contractions. *Diabetes* 47:1671–1675, 1998
11. Vergauwen L, Hespel P, Richter EA: Adenosine receptors mediate synergistic stimulation of glucose uptake and transport by insulin and contractions in rat skeletal muscle. *J Clin Invest* 93:974–981, 1994
12. Vergauwen L, Richter EA, Hespel P: Adenosine exerts a glycogen sparing action in contracting rat skeletal muscle. *Am J Physiol* 272:E762–E768, 1997
13. Deakins M: Effects of caffeine on human sugar-tolerance curves. *Proc Soc Exp Biol Med* 40:588–589, 1939
14. Feinberg L, Sandberg H, De Castro O, Bellet S: Effects of coffee ingestion on oral glucose tolerance curves in normal human subjects. *Metab* 17:916–922, 1968
15. Goldman J, Ovadia J: The effect of coffee on glucose tolerance in normal and prediabetic pregnant women. *Obstet Gynecol* 33:214–218, 1969
16. Jankelson O, Beaser S, Howard F, Mayer J: Effect of coffee on glucose tolerance and circulating insulin in men with maturity-onset diabetes. *Lancet* 1:527–529, 1967
17. Wachman A, Hattner R, George B, Bernstein D: Effects of decaffeinated and nondecaffeinated coffee ingestion on blood glucose and plasma radioimmunoreactive insulin responses to rapid intravenous infusion of glucose in normal man. *Metab* 9:539–546, 1970
18. Pizziol A, Tikhonoff V, Palcari C, Russo E, Mazza A, Ginocchio G, Onesto C, Pavan L, Casiglia E, Pessina A: Effects of caffeine on glucose tolerance: a placebo-controlled study. *Eur J Clin Nutr* 52:846–849, 1998
19. Battram D, Rowland M, Marko N, Graham T: Caffeine ingestion and response to an oral glucose tolerance test (Abstract). *Can J Appl Physiol* 24:425, 1999
20. Harris MI: Impaired glucose tolerance in the U.S. population. *Diabetes Care* 12:464–474, 1989
21. Ross R, Rissanen J, Pedwell H, Clifford J, Schragge P: Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. *J Appl Physiol* 81:2445–2455, 1996
22. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
23. Weiker H: Determination of free and sulfoconjugated catecholamines in plasma and urine by high performance liquid chromatography. *Int J Sports Med* 9 (Suppl. 2):S68–S74, 1988
24. Bergmeyer H: *Methods of Enzymatic Analysis*. New York, Academic Press, 1974
25. Miles J, Glasscock R, Aitkins J, Gerich J, Haymond M: A micro-fluorometric method for the determination of FFA in plasma. *J Lipid Res* 24:96–99, 1983
26. Graham T, Spriet L: Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *J Appl Physiol* 78:2867–2874, 1995
27. Greer F, Friars D, GrahamT: Comparison of caffeine and theophylline ingestion: exercise, metabolism and endurance. *J Appl Physiol* 89:1837–1844, 2000
28. Abernethy DR, Todd EL: Impairment of caffeine clearance by chronic use of low-dose oestrogen-containing oral contraceptives. *Eur J Clin Pharmacol* 28:425–428, 1985
29. Lane LD, Steege JF, Rupp SL, Kuhn CM: Menstrual cycle effects on caffeine elimination in the human female. *Eur J Clin Pharmacol* 43:543–546, 1992
30. Nuutila P, Knuuti MJ, Mäki M, Laine H, Ruotsalainen U, Teräs M, Haaparanta M, Solin O, Yki-Järvinen H: Gender and insulin sensitivity in the heart and skeletal muscles: studies using positron emission tomography. *Diabetes* 44:31–36, 1995
31. Dent P, Lavoinne A, Nakielny S, Caudwell B, Watt P, Cohen P: The molecular mechanism by which insulin stimulates glycogen synthesis in mammalian skeletal muscle. *Nature* 348:302–308, 1990
32. Lillioja S, Mott D, Zawadski J, Young A, Abbott W, Bogardus C: Glucose storage is a major determinant of *in vivo* insulin resistance in subjects with normal glucose tolerance. *J Endocrinol Metab* 62:922–927, 1986
33. Taylor R, Price T, Katz L, Shulman R, Shulman G: Direct measurement of change in muscle glycogen concentration after a mixed meal in normal subjects. *Am J Physiol* 265: E224–E229, 1993
34. Elbrink J, Bihler I: Membrane transport: its relation to cellular metabolism rates. *Science* 188:1177–1180, 1975
35. Bogardus C, Lillioja S, Stone K, Mott D: Correlation between muscle glycogen synthase activity and *in vivo* insulin action in man. *J Clin Invest* 73:1185–1190, 1984
36. James D, Jenkins A, Kraegen E: Heterogeneity of insulin action in individual muscles *in vivo*: euglycemic clamp studies in rats. *Am J Physiol* 248:E567–E574, 1985
37. Graham T, Helge J, MacLean D, Kiens B, Richter E: Caffeine ingestion does not alter carbohydrate or fat metabolism in skeletal muscle during exercise. *J Physiol* 529:837–847, 2000
38. Steele R, Bishop J, Dunn A, Altszuler N, Rathgeb I, DeBodo R: Inhibition by insulin of hepatic glucose production in the normal dog. *Am J Physiol* 208:301–306, 1965
39. Graham T, Spriet L: Performance and metabolic responses to a high caffeine dose during prolonged exercise. *J Appl Physiol* 71:2292–2298, 1991
40. Mohr T, van Soeren M, Graham T, Kjaer M: Caffeine ingestion and metabolic responses of tetraplegic humans during electrical cycling. *J Appl Physiol* 85:979–985, 1998
41. van Soeren M, Mohr T, Kjaer M, Graham T: Acute effects of caffeine ingestion at rest in humans with impaired epinephrine responses. *J Appl Physiol* 80:999–1005, 1996
42. Napoli R, Gibson L, Hirshman MF, Boppart MD, Dufresne SD, Horton ES, Goodyear LJ: Epinephrine and insulin stimulate different mitogen-activated protein kinase signaling pathways in rat skeletal muscle. *Diabetes* 47:1549–1554, 1998
43. Arner P: Differences in lipolysis between human subcutaneous and omental adipose tissues. *Ann Med* 27:435–438, 1995