Caffeine-Induced Impairment of Insulin Action but Not Insulin Signaling in Human Skeletal Muscle Is Reduced by Exercise

Farah S.L. Thong,[1] Wim Derave,[2] Bente Kiens,[3] Terry E. Graham,[1] Birgitte Ursø,[4]
Jørgen F.P. Wojtaszewski,[3] Bo F. Hansen,[4] and Erik A. Richter[3]

We investigated the effects of caffeine ingestion on skeletal muscle glucose uptake, glycogen synthase (GS) activity, and insulin signaling intermediates during a 100-min euglycemic-hyperinsulinemic (100 μU/ml) clamp. On two occasions, seven men performed 1-h one-legged knee extensor exercise at 3 h before the clamp. Caffeine (5 mg/kg) or placebo was administered in a randomized, double-blind fashion 1 h before the clamp. During the clamp, whole-body glucose disposal was reduced ($P < 0.05$) in caffeine (37.5 ± 3.1 μmol · min^{-1} · kg^{-1}) vs. placebo (54.1 ± 2.9 μmol · min^{-1} · kg^{-1}). In accordance, the total area under the curve over 100 min ($AUC_{0-100 \, min}$) for insulin-stimulated glucose uptake in caffeine was reduced ($P < 0.05$) by ~50% in rested and exercised muscle. Caffeine also reduced ($P < 0.05$) GS activity before and during insulin infusion in both legs. Exercise increased insulin sensitivity of leg glucose uptake in both caffeine and placebo. Insulin increased insulin receptor tyrosine kinase (IRTK), insulin receptor substrate 1–associated phosphatidylinositol (PI) 3-kinase activities, and Ser^{473} phosphorylation of protein kinase B (PKB)/Akt significantly but similarly in rested and exercised legs. Furthermore, insulin significantly decreased glycogen synthase kinase-3α (GSK-3α) activity equally in both legs. Caffeine did not alter insulin signaling in either leg. Plasma epinephrine and muscle cAMP concentrations were increased in caffeine. We conclude that 1) caffeine impairs insulin-stimulated glucose uptake and GS activity in rested and exercised human skeletal muscle; 2) caffeine-induced impairment of insulin-stimulated muscle glucose uptake and downregulation of GS activity are not accompanied by alterations in IRTK, PI 3-kinase, PKB/Akt, or GSK-3α but may be associated with increases in epinephrine and intramuscular cAMP concentrations; and 3) exercise reduces the detrimental effects of caffeine on insulin action in muscle. *Diabetes* 51:583–590, 2002

Skeletal muscle is the major site of glucose disposal and thus is critical in maintaining glucose homeostasis. Insulin and exercise are potent stimulators of skeletal muscle glucose transport and glycogen metabolism. Diminished response to insulin, but not exercise/contraction signals leading to glucose transport in skeletal muscle, is a major factor responsible for insulin resistance associated with type 2 diabetes. Consequently, interest in elucidating the molecular and regulatory mechanisms involved in insulin- and exercise-mediated glucose transport and glycogen metabolism has intensified. The prevailing theory is that insulin stimulates translocation of GLUT4 (1,2) and activation of glycogen synthase (GS) (3) by activating the classical insulin receptor substrate (IRS)/phosphatidylinositol (PI) 3-kinase signal transduction pathway.

The signaling intermediaries downstream from PI 3-kinase remain to be delineated. Several recent studies have suggested the serine/threonine kinase protein kinase B (PKB) (also known as Akt) as a potential link between insulin-stimulated PI 3-kinase and the glucose transport process (4,5). Activation of PKB/Akt, which in turn phosphorylates and inactivates glycogen synthase kinase-3 (GSK-3), has been proposed to facilitate insulin's activation of GS (6,7). Although earlier reports of PKB and GSK-3 as potential downstream signaling intermediates that mediate insulin's actions on glucose transport and GS activity have been incongruous (8,9), recent evidence from knockout mice indicates that Akt2 participates in insulin signaling in muscle (10). After a single bout of exercise, whole-body glucose disposal is markedly increased, and this is due predominantly to an enhanced response to insulin on glucose uptake and GS activity in previously exercised skeletal muscle (11–15). Several studies that have examined changes in insulin signaling after a single bout of exercise have found that neither insulin binding to its receptor (16,17) nor insulin receptor–mediated signaling to the level of insulin receptor tyrosine kinase (IRTK), IRS-1 associated PI 3-kinase, and GSK-3α (11,12) are mechanisms by which exercise increases insulin sensitivity in skeletal muscle. Whether enhanced PKB activity

links insulin stimulation to glucose transport in the post-exercise period is still unclear (11,18).

Methylxanthines such as caffeine and theophylline are known adenosine receptor antagonists (19). In rat adipocytes (20) and in the perfused contracting rat hindlimb (21), methylxanthines inhibited insulin-stimulated glucose uptake, and this inhibitory action was attributed to the antagonistic effects of methylxanthine on the A_1 adenosine receptor. We have previously shown that caffeine administration at doses eliciting plasma concentrations equivalent to concentrations obtainable after drinking 3–4 cups of coffee (19) decreased whole-body insulin sensitivity in sedentary males (22). However, it is not known whether the reduction in whole-body glucose disposal after caffeine ingestion resulted from diminished glucose uptake in skeletal muscle. The signal transduction pathway that may couple methylxanthines to insulin stimulation of glucose transport and glycogen metabolism in skeletal muscle has not been investigated. Finally, caffeine is a widely consumed drug, in beverages such as coffee, tea, and caffeinated soft drinks. Its impact on insulin-stimulated glucose metabolism is therefore of interest to the population as a whole and especially for patients with insulin resistance or diabetes. Therefore, the purpose of the present study was to examine the effects of caffeine ingestion on glucose uptake and GS activity in rested and previously exercised human skeletal muscle during a euglycemic-hyperinsulinemic clamp.

RESEARCH DESIGN AND METHODS

Subjects. Seven healthy, moderately active men were recruited to participate in this study. The subjects' age, weight, height, and BMI were 26 ± 2 years, 78 ± 4 kg, 180 ± 3 cm, and 24 ± 1 kg/m^2, respectively. This study was approved by the Ethics Committee of Copenhagen and Frederiksberg County, and informed consent was obtained from each subject. On three separate occasions, the subjects became accustomed to the one-legged knee extensor apparatus before determination of peak work capacity ($V_{O_{2peak}}$) via an incremental knee extensor test. They were instructed to follow a mixed diet, abstain from caffeine-containing products and alcohol, and avoid strenuous physical activity 2 days before the experiment. On the day of the experiment, the subjects ate a light breakfast 2 h before arriving at the laboratory at 7:00 A.M.
Experimental protocol. On two separate occasions, separated by 10–14 days, the subjects performed 60 min of repeated (1 kick/s) one-legged knee extensor exercise alternating every 5 min at a workload eliciting 75 and 100% of the knee extensor $V_{O_{2peak}}$. The selection of the exercising leg was randomized. Three subjects exercised the left leg, whereas four exercised the right leg. After the exercise, subjects rested in a supine position. Teflon catheters were then inserted below the inguinal ligament in the femoral artery and bilateral femoral veins. A thermistor (Baxter, CA) was inserted through each femoral venous catheter and was advanced 6–8 cm proximal to the catheter tip. The thermistors were connected to an Edslab cardiac output computer (Baxter, CA), and a Gould TA 2000 recorder (Cleveland, OH) was used to record venous temperature for blood flow measurements. For glucose and insulin infusions, catheters were inserted into an antecubital and a forearm vein, respectively.

At 2 h after exercise, 5 mg/kg body wt of either caffeine or placebo (dextrose) in gelatin capsules was administered to the subject in a randomized, double-blind fashion. A one-step euglycemic-hyperinsulinemic clamp was initiated 3 h postexercise by an intravenous bolus injection of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) over 1 min (9 mU/kg). This was followed by 100-min constant infusion of insulin at 1.5 mU $\cdot$ min^{-1} $\cdot$ kg^{-1}. To maintain plasma K$^+$ concentration during the insulin clamp, an oral dose of 30 mmol KCl (Kaleorid, Leo, Denmark) was administered to each subject 15 min before initiation of the insulin clamp. Blood samples were drawn simultaneously from the femoral arterial and both femoral venous catheters at -60 and at 0 (1 h postpill ingestion), 10, 20, 30, 50, 75, and 100 min after the initiation of the insulin clamp. Blood flow was measured in both thighs, using a modified thermodilution method as previously described (14). Pneumatic cuffs were placed below the knees and were inflated to 230 mmHg during blood sampling and blood flow measurements. Bilateral muscle biopsies were
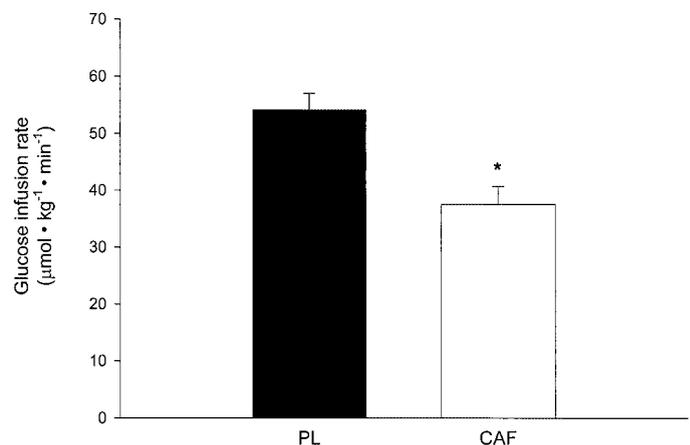

FIG. 1. Glucose infusion rates (μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}) in placebo and caffeine during the euglycemic-hyperinsulinemic clamp. Values are means $\pm$ SE ($n = 7$). PL, placebo; CAF, caffeine. *$P < 0.05$ vs. placebo.

taken under local anesthesia from the vastus lateralis at 0, 30, and 100 min of insulin infusion and were quickly frozen in liquid nitrogen. The two muscle biopsies were obtained within 30 s at each time point.
Analytical procedures. Plasma and blood glucose were determined in duplicate using a dual-channel glucose analyzer (YSI-2700Select; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a radioimmunoassay method (insulin radioimmunoassay kit; Amersham Pharmacia Biotech, Uppsala, Sweden). Plasma free fatty acid (FFA) was determined by a noesterified fatty acid kit (Wako Bioproducts, Richmond, VA). Plasma glycerol was measured by an enzymatic method (23). Plasma catecholamines were determined in 120 μl of 0.24 mol/l EGTA/glutathione-treated plasma by a high-pressure liquid chromatography method as described by Weiker et al. (24).

Muscle biopsies were freeze-dried and dissected free of blood, fat, and connective tissue for measuring muscle glycogen, GS activity, and cAMP, but not for determining the insulin signaling intermediates. Muscle glycogen concentration was measured by a modified acid hydrolysis method as previously described by Adamo and Graham (25). Muscle GS activity was determined by a modified method as previously described (14). GS activity was determined in the presence of 0, 0.17, and 8 mmol/l glucose-6-phosphate (G-6-P). GS activities are expressed as either the percent fractional velocity (calculated as 100 times the activity in the presence of 0.17 mmol/l G-6-P divided by the activity at 8 mmol/l G-6-P) or as the percent G-6-P–independent form of GS (I-form), with the percent I-form calculated as 100 times the activity in the absence of G-6-P divided by the activity at 8 mmol/l G-6-P [saturated]). GS activity at 100 min was determined in six subjects only because of analytical difficulty with this sample in one subject. Muscle cAMP was measured using a radioimmunoassay kit (Amersham Pharmacia Biotech, Uppsala, Sweden).

For determination of IRTK activity and PKB Ser^{473} phosphorylation, ~10 mg frozen muscle was processed as previously described (12). Solubilized protein concentrations were determined using a bicinchoninic acid Protein Reagent kit (Pierce Chemical) using a microtiter plate protocol at 37°C for 30 min. The chemicals used were of analytical grade from Sigma Chemical, unless otherwise stated.

IRTK activity was measured as previously described (12). For determination of IRS-1–associated PI 3-kinase and GSK-3α, muscle biopsies weighing ~20–30 mg were processed and quantified according to previously described methods (12,26). Phosphorylation of PKB on Ser^{473} was measured as an indication of in vivo PKB activity because these have been shown to be highly correlated (J.F.P.W., unpublished observations). Briefly, for immunoblotting, aliquots of muscle lysates containing 50 mg protein were separated by SDS-PAGE and transferred to polyvinylidine fluoride (PVDF) membranes. The PVDF membranes were blocked with 1% BSA in Tris-buffered saline containing 10 mmol/l Tris, 50 mmol/l NaCl, and 0.05% Tween-20 and were incubated with phospho-specific serine (S^{473}) PKB antibody (New England Biolabs, Beverly, MA). Detection was made with an alkaline phosphatase–conjugated anti-rabbit IgG (Zymed). Specific bands for PI 3-kinase and PKB were quantified using a phosphorimager (Molecular Dynamics), and GSK-3α was quantified using a Packard scintillation counter.
Calculations. The net exchanges of glucose and insulin were calculated using the direct Fick method by multiplying the arteriovenous differences in concentration by blood flow. Total stimulated glucose uptake was calculated
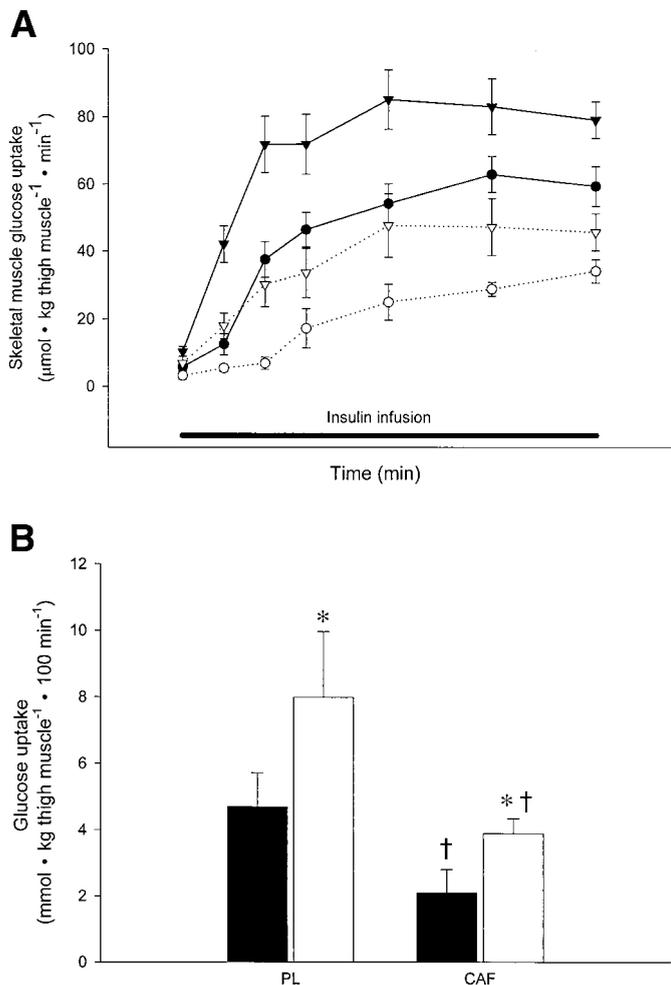
FIG. 2. *A*: Insulin-stimulated glucose uptake (μmol $\cdot$ kg thigh muscle^{-1} $\cdot$ min^{-1}) in placebo rested leg (●), placebo exercised leg (▲), caffeine rested leg (○), and caffeine exercised leg (△) during the euglycemic-hyperinsulinemic clamp. Solid and dotted lines represent placebo and caffeine trials, respectively. Values are means ± SE (n = 6). Glucose uptake was higher (P < 0.05) in the exercised versus rested legs in both placebo and caffeine. Caffeine decreased (P < 0.05) glucose uptake in both rested and exercised legs from 10 to 100 min of insulin infusion vs. placebo. *B*: Total (AUC$_{0-100 \text{ min}}$) glucose uptake in rested (■) and exercised (□) thighs (mmol $\cdot$ kg thigh muscle^{-1} $\cdot$ 100 min^{-1}) in placebo and caffeine during the euglycemic-hyperinsulinemic clamp. Values are means ± SE (n = 6). CAF, caffeine; PL, placebo. *P < 0.05 vs. rested thigh; †P < 0.05 vs. placebo.

as the area under the curve (AUC) over 100 min using the trapezoidal method. Leg exchange, glucose AUC, and blood flow data are expressed per kilogram of thigh muscle mass. Lean thigh volume was estimated using the method described by Jones and Pearson (27), where skin and subcutaneous adipose tissue volumes were subtracted from the total thigh volume. Quadriceps

muscle mass was estimated from the lean thigh volume according to Andersen and Saltin (28). Total thigh muscle mass was calculated from the volume ratio (40:60) between the quadriceps femoris and the hamstring and adductor muscles (12). Activities of the insulin signaling intermediates are expressed in arbitrary units relative to an insulin-stimulated standard.

Statistical analysis. Statistical analysis was performed using an SAS statistical package (Cary, NC). One-, two-, or three-way ANOVA with or without repeated measures were used to evaluate statistical differences as appropriate. A Tukey post hoc comparison was made when statistical significance was found between observations. Statistical significance was accepted at P < 0.05. All data presented are the means ± SE.

RESULTS

Arterial plasma insulin increased (P < 0.05) immediately after the bolus injection of insulin and reached a plateau by 20 min in both the placebo and caffeine trials. Plasma insulin concentrations were similar between placebo (556 ± 40 pmol/l) and caffeine (510 ± 45 pmol/l) during the insulin clamp. Plasma glucose concentration during the insulin infusion was maintained at 5.2 ± 0.1 and 5.3 ± 0.1 mmol/l in the placebo and caffeine trials, respectively. After caffeine ingestion, whole-body glucose disposal was decreased (P < 0.05) compared with placebo, as indicated by a 30% reduction in glucose infusion rates needed to maintain euglycemia (Fig. 1).

The arteriovenous differences for glucose are from six subjects because of technical difficulty with femoral artery catheterization in one subject. Thigh insulin clearance was similar in rested and exercised legs and was not affected by caffeine ingestion (data not shown). In both placebo and caffeine trials, insulin-stimulated glucose uptake by the previously exercised leg was significantly higher compared with the rested leg (Fig. 2*A*). Glucose uptake in caffeine was decreased (P < 0.05) compared with placebo in both rested and exercised legs during the 100-min insulin infusion (Fig. 2*A*). Total insulin-stimulated leg glucose uptake was decreased (P < 0.05) by 2.59 ± 1.36 mmol $\cdot$ kg^{-1} $\cdot$ 100 min^{-1} in the rested leg (55%) and by 4.10 ± 1.98 mmol $\cdot$ kg^{-1} $\cdot$ 100 min^{-1} in the previously exercised leg (51%) after caffeine ingestion (Fig. 2*B*). Thigh blood flow increased with insulin infusion and was similar between the rested and exercised leg and between placebo and caffeine trials throughout the entire duration of the insulin clamp (Table 1). Thus, the significantly higher glucose uptake by the exercised leg was caused by higher glucose extraction by the previously exercised muscle. Similarly, the significantly lower glucose uptake after caffeine ingestion was caused by lower glucose extraction by both the rested and previously exercised leg.

Before caffeine ingestion, arterial plasma FFA and glycerol were similar to the placebo trial. However, at 60 min

TABLE 1
Blood flow in rested and exercised legs in placebo and caffeine trials during 100 min of the euglycemic-hyperinsulinemic clamp

	Time (min)	PL rested	PL exercised	CAF resting	CAF exercised
Blood flow* (ml $\cdot$ kg^{-1} $\cdot$ min^{-1})	0	29 ± 4	29 ± 3	33 ± 5	38 ± 4
	10	38 ± 7†	49 ± 12†	51 ± 6†	51 ± 6†
	20	44 ± 7†	50 ± 13†	56 ± 6†	54 ± 6†
	30	47 ± 7†	51 ± 12†	56 ± 6†	55 ± 7†
	50	49 ± 8†	54 ± 11†	60 ± 8†	64 ± 9†
	75	53 ± 10†	54 ± 10†	61 ± 9†	66 ± 10†
	100	53 ± 10†	53 ± 8†	62 ± 9†	64 ± 10†

Values are means ± SEM (n = 6). CAF, caffeine; PL, placebo. *Expressed per kilogram thigh muscle mass; †P < 0.05 vs. time 0 min (within leg).

TABLE 2
Arterial FFA, glycerol, epinephrine, and norepinephrine concentrations in placebo and caffeine during 100 min of the euglycemic-hyperinsulinemic clamp

		Time (min)							
		−60	0	10	20	30	50	75	100
FFA (μmol/l)	PL	490 ± 112	524 ± 75	421 ± 72*	238 ± 36*	171 ± 22*	128 ± 9*	122 ± 11*	117 ± 10*
	CAF	484 ± 64	839 ± 132*†	584 ± 47*†	315 ± 24*†	218 ± 14*†	175 ± 16*†	145 ± 13*	143 ± 16*
Glycerol (μmol/l)	PL	51.9 ± 4.2	54.6 ± 7.8	42.3 ± 5.1*	33.0 ± 3.4*	34.0 ± 3.8*	31.8 ± 5.3*	33.7 ± 6.3*	32.4 ± 5.5*
	CAF	51.7 ± 6.3	74.8 ± 8.2*†	59.3 ± 6.8*†	53.6 ± 8.9*†	44.3 ± 3.8*†	42.7 ± 2.6*†	37.9 ± 2.9*	40.5 ± 2.4*
Epinephrine (nmol/l)	PL	0.19 ± 0.02	0.19 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.23 ± 0.01	0.25 ± 0.02	0.21 ± 0.02	0.26 ± 0.05
	CAF	0.19 ± 0.02	0.39 ± 0.03*†	0.44 ± 0.02*†	0.49 ± 0.04*†	0.60 ± 0.07*†	0.49 ± 0.06*†	0.43 ± 0.04*†	0.40 ± 0.04*†
Norepinephrine (pmol/l)	PL	0.55 ± 0.06	0.55 ± 0.06	0.52 ± 0.04	0.54 ± 0.04	0.55 ± 0.05	0.53 ± 0.03	0.51 ± 0.04	0.54 ± 0.05
	CAF	0.55 ± 0.06	0.65 ± 0.04*	0.65 ± 0.04*†	0.69 ± 0.06*†	0.71 ± 0.06*†	0.70 ± 0.06*†	0.68 ± 0.07*†	0.68 ± 0.06*†

Data are means ± SE ($n = 7$). *$P < 0.05$ vs. −60 min (within trial); †$P < 0.05$ vs. placebo. CAF, caffeine; PL, placebo.

after caffeine ingestion, FFA and glycerol were significantly higher compared with placebo (Table 2). Insulin infusion suppressed FFA and glycerol ($P < 0.05$) in both placebo and caffeine, but FFA and glycerol remained significantly higher in caffeine than placebo until 50 and 30 min of insulin infusion, respectively (Table 2). Plasma epinephrine and norepinephrine were significantly increased after caffeine ingestion (Table 2). Insulin infusion did not affect catecholamine concentrations.

At 3 h after exercise, muscle glycogen concentration was significantly lower in the exercised compared with the rested leg in both the placebo (45% lower) and caffeine (44% lower) trials (Table 3). Insulin significantly, but modestly, increased muscle glycogen at 100 min in the exercised but not in the rested leg in placebo. Glycogen concentration in the exercised leg remained lower ($P < 0.05$) compared with the rested leg in both placebo and caffeine trials at all time points. Caffeine ingestion did not alter glycogen concentration.

GS activity, expressed as either the percent fractional velocity (Fig. 3A) or percent I-form (Fig. 3B) was significantly higher in the exercised compared with the rested leg in both placebo and caffeine trials before and during the insulin clamp. In placebo, insulin infusion increased GS activity at 30 min in rested and exercised legs compared with basal, and GS activity remained higher ($P < 0.05$) at 100 min compared with basal in both legs. In caffeine trials, insulin increased GS activity at 30 min, with no further detectable increase observed after 30 min of insulin infusion in both rested and exercised legs. GS activity remained higher ($P < 0.05$) in the exercised compared with the rested legs in caffeine trials (Fig. 3A and B). Caffeine ingestion resulted in significant reduction

in GS activity in the rested and exercised legs at all time points (Fig. 3A and B). The caffeine-induced reduction in GS activity was observed before and throughout the insulin infusion compared with placebo. Neither insulin nor prior exercise affected muscle cAMP concentrations, whereas caffeine ingestion resulted in significantly higher, but similar, muscle cAMP levels in both the rested and exercised legs (Table 3).

Basal IRTK, IRS-1–associated PI 3-kinase, and GSK-3α activities and PKB phosphorylation on Ser[473] were similar between the rested and exercised legs and between placebo and caffeine trials. Insulin induced a significant, but similar, increase in IRTK activity (approximately threefold) in both the rested and exercised legs in placebo and caffeine trials (Fig. 4). Similarly, insulin resulted in a comparable threefold ($P < 0.05$) increase in PI 3-kinase activity at 30 min in both rested and exercised legs and in placebo and caffeine trials (Fig. 5). At 100 min of insulin infusion in placebo trials, but not caffeine trials, insulin-stimulated PI 3-kinase activity decreased ($P < 0.05$) in the exercised leg and was significantly lower compared with the rested leg (Fig. 5). Insulin infusion resulted in a significant, but similar, 2.5-fold increase ($P < 0.05$) in PKB phosphorylation at 30 min in rested and exercised legs in placebo and caffeine trials (Fig. 6). No further increase in PKB activity was observed after 30 min of insulin infusion. Insulin decreased ($P < 0.05$) GSK-3α activity similarly (~50%) in both the rested and exercised legs in placebo and caffeine trials at 30 min of insulin infusion, and no further decreases were observed thereafter (Fig. 7). Caffeine ingestion did not alter insulin's effects on IRTK, PI 3-kinase and GSK-3α activities, and PKB phosphorylation in either the rested or the exercised legs.

TABLE 3
Skeletal muscle glycogen and cAMP concentrations in rested and exercised legs in placebo and caffeine during 100 min of the euglycemic-hyperinsulinemic clamp

	Time (min)	Placebo rested	Placebo exercised	Caffeine resting	Caffeine exercised
Glycogen (mmol glucosyl units · kg^{-1} dry wt)	0	496 ± 35	277 ± 39*	509 ± 27	286 ± 40*
	30	507 ± 33	286 ± 30*	492 ± 36	285 ± 27*
	100	487 ± 36	310 ± 30*†	496 ± 40	307 ± 29*
cAMP (μmol · kg^{-1} dry wt)	0	2.8 ± 0.5	2.2 ± 0.7	3.3 ± 0.6‡	3.7 ± 0.4‡
	30	2.3 ± 0.4	2.5 ± 0.7	3.4 ± 0.7‡	3.8 ± 0.3‡
	100	2.9 ± 0.6	3.1 ± 0.5	4.1 ± 0.3‡	3.4 ± 0.5‡

Values are means ± SE ($n = 7$). *$P < 0.05$ vs. rested leg (within trial); †$P < 0.05$ vs. time 0; ‡$P < 0.05$ vs. placebo.
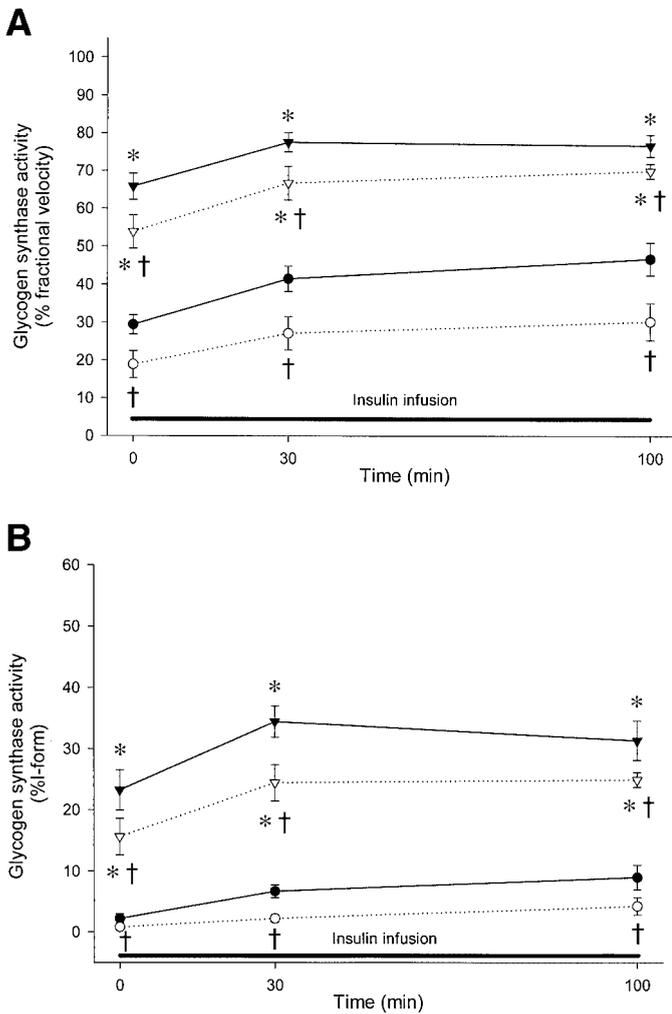
FIG. 3. Skeletal muscle glycogen synthase activity expressed as percent fractional velocity (*A*) and percent I-form (*B*) during the euglycemic-hyperinsulinemic clamp in placebo rested leg (●), placebo exercised leg (▲), caffeine rested leg (○), and caffeine exercised leg (△). Solid and dotted lines represent placebo and caffeine trials, respectively. Values are means ± SE ($n = 7$). *$P < 0.05$ vs. rested leg; †$P < 0.05$ vs. placebo.

DISCUSSION

Ingestion of caffeine at a dose equivalent to drinking 3–4 cups of coffee decreased whole-body glucose disposal by 30%, in agreement with our previous report (22) (Fig. 1). Moreover, we have demonstrated for the first time that the caffeine-induced reduction in insulin-stimulated glucose uptake in human skeletal muscle is a major contributor to diminished whole-body glucose disposal (regression analysis of absolute change in insulin-stimulated leg glucose uptake versus glucose infusion rates: $r^2 = 0.93$, $P < 0.05$). In the present study, caffeine decreased glucose uptake by ~55% in the rested leg and 51% in the exercised leg. Yet, after caffeine ingestion, glucose uptake by exercised muscle remained 40% higher ($P < 0.05$) compared with the rested leg, suggesting that the enhanced insulin sensitivity to glucose uptake in the postexercise period was not abolished by caffeine, but merely that the general level of glucose uptake was decreased by caffeine ingestion. Stated differently, our results also show that exercise reduces the deleterious effects of caffeine and that caffeine reduces the beneficial effects of exercise on muscle insulin
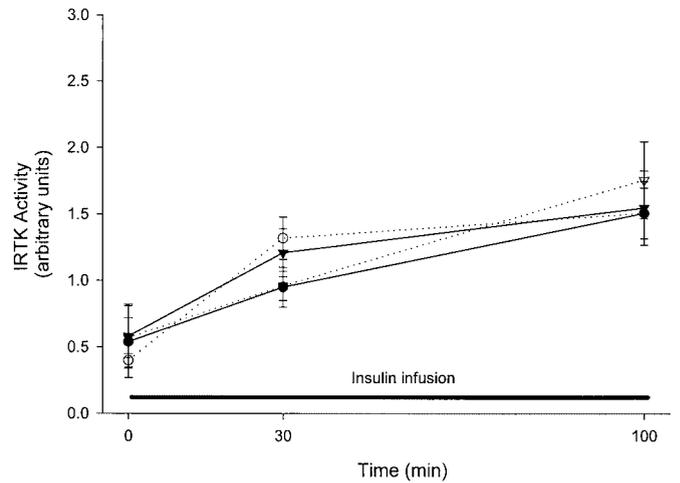

FIG. 4. Skeletal muscle IRTK activity during the euglycemic-hyperinsulinemic clamp in placebo rested leg (●), placebo exercised leg (▲), caffeine rested leg (○), and caffeine exercised leg (△). Solid and dotted lines represent placebo and caffeine trials, respectively. Values are means ± SE ($n = 7$) and are expressed in arbitrary units normalized to the number of insulin receptors.

action. Furthermore, after caffeine ingestion, the reduction in GS activity in the exercised leg was not different compared with the rested leg. Because caffeine resulted in similar relative magnitudes of inhibition in glucose uptake and GS activity in both the rested and previously exercised legs, caffeine likely inhibited glucose uptake and GS activity by a common mechanism in both rested and exercised muscle.

It is interesting to note that in the present study, the inhibitory effects of caffeine on glucose uptake occur only in the presence of high insulin levels (since glucose uptake was not different at 0 min compared with placebo). This is
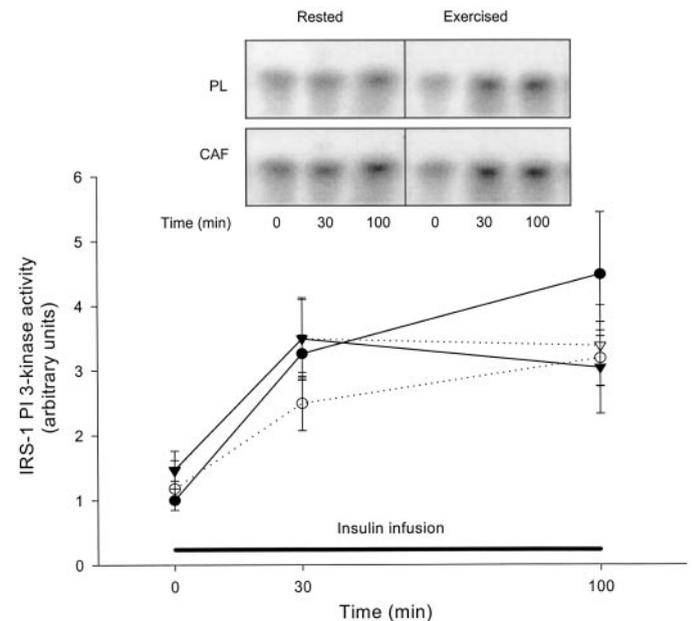

FIG. 5. Skeletal muscle IRS-1–associated PI 3-kinase activity during the euglycemic-hyperinsulinemic clamp in placebo rested leg (●), placebo exercised leg (▲), caffeine rested leg (○), and caffeine exercised leg (△). Solid and dotted lines represent placebo and caffeine trials, respectively. Values are means ± SE ($n = 7$). CAF, caffeine; PL, placebo. *$P < 0.05$ vs. rested leg (within placebo and caffeine).
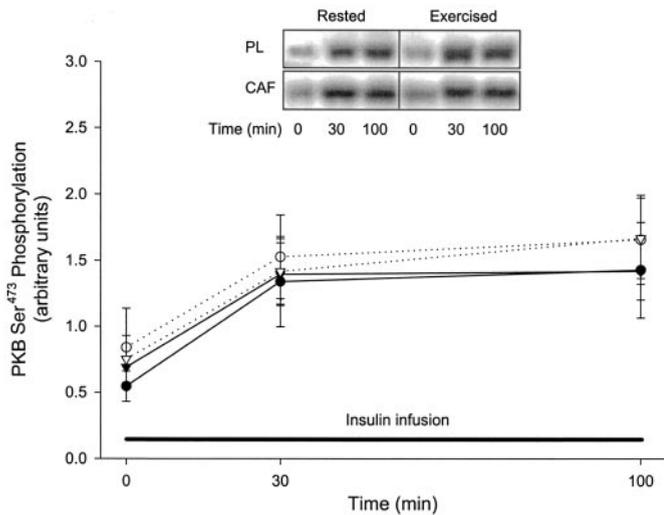
FIG. 6. Skeletal muscle PKB Ser[473] phosphorylation during the euglycemic-hyperinsulinemic clamp in placebo rested leg (●), placebo exercised leg (▲), caffeine rested leg (○), and caffeine exercised leg (△). Solid and dotted lines represent placebo and caffeine trials, respectively. Values are means ± SE ($n = 7$). CAF, caffeine; PL, placebo.

in accordance with our previous observations that caffeine reduced glucose disposal during a euglycemic clamp in humans (22) and during electrical stimulation of perfused muscle in the presence of 100 μU/ml of insulin (21), but not during exercise in humans, when plasma insulin is low (29). Thus, it appears that caffeine exerts its inhibitory effects on glucose uptake only when insulin is above normal resting concentrations. In contrast, the effect of caffeine on GS activity was present both at low preinfusion insulin concentrations and during hyperinsulinemia.

A potential mechanism by which caffeine exerted its inhibitory effects on glucose uptake and GS activity in skeletal muscle is by competitively blocking adenosine binding to its receptors. In the perfused contracting rat hindlimb in the presence of insulin, caffeine at 77 μmol/l reduced glucose uptake comparably to that observed with a selective A_1 adenosine receptor antagonist, CPDPX (21), suggesting caffeine exerted its inhibitory effect by selec-
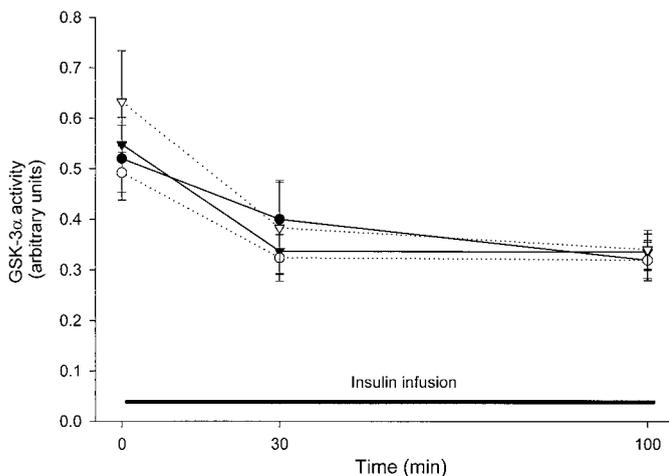

FIG. 7. Skeletal muscle GSK-3α during the euglycemic-hyperinsulinemic clamp in placebo rested leg (●), placebo exercised leg (▲), caffeine rested leg (○), and caffeine exercised leg (△). Solid and dotted lines represent placebo and caffeine trials, respectively. Values are means ± SE ($n = 7$).

tively blocking the A_1 adenosine receptor. A dose of 5 mg/kg caffeine in the present study would elicit a plasma concentration of ~45 μmol/l (30), which approaches the K_i of 40–44 μmol/l for A_1 and A_2 adenosine receptors, suggesting that caffeine is a competitor for binding to adenosine receptors (19). However, although it is possible that caffeine in our study mediated its inhibitory effects on glucose uptake by blocking adenosine binding to the A_1 adenosine receptor, the presence of the A_1 adenosine receptor in this tissue remains uncertain (31).

After caffeine ingestion, plasma FFA was significantly higher compared with placebo (see Table 2). Numerous studies have demonstrated by use of intralipid and heparin infusion that high circulating FFA inhibits glucose uptake in skeletal muscle during insulin stimulation (32,33). However, in the present study, although plasma FFA was higher 1 h after caffeine ingestion compared with placebo, insulin infusion effectively decreased plasma FFA to that observed in placebo after 50 min, and yet glucose uptake remained significantly lower during 100 min of insulin infusion. Similarly, intramuscular G-6-P and glucose concentrations were not different between placebo and caffeine trials and between the rested and exercised legs during the insulin clamp (data not shown). Therefore, it is unlikely that in the present study, the inhibitory action of caffeine on skeletal muscle glucose uptake resulted from either high circulating plasma FFA or from G-6-P inhibition of hexokinase.

In the present study, caffeine ingestion resulted in significantly higher plasma epinephrine and intramuscular cAMP concentrations. Epinephrine has been shown to inhibit insulin-stimulated glucose transport and/or glycogen synthesis in human (34,35) and rodent (36–38) skeletal muscle. The recent finding that insulin sensitivity to glucose uptake is increased in stimulatory guanine nucleotide-binding protein (G_s) α–knockout mice provide further evidence that G_sα-coupled pathways, possibly including epinephrine stimulation of G_s protein, negatively regulate insulin signaling (39). Similarly, epinephrine is known to potently counteract insulin activation of GS (40,41). Thus, it is likely that the inhibitory actions of caffeine on glucose uptake and GS activity in the present study were secondary to epinephrine. However, the inhibitory effects of caffeine in this study were not mediated by alterations in the known components of the insulin signaling cascade because after caffeine ingestion, the responses of IRTK, PI 3-kinase, PKB, and GSK-3α were comparable to that observed in placebo trials. Similarly, the caffeine-induced reduction in GS activity was not accompanied by alterations in insulin-stimulated GSK-3α activity. Thus, our results suggest that caffeine inhibited these metabolic processes by altering signaling intermediates that are either further downstream or are independent of this signaling cascade. The observation that downregulation of GS activity after caffeine ingestion occurred before insulin infusion would further imply that the mechanism involved in caffeine-induced inhibition of skeletal muscle GS activity must be distinct from those activated by insulin. Furthermore, our data also suggest that the insulin antagonistic effects of epinephrine are not mediated by downregulating the insulin signaling intermediates measured in this study. The molecular mechanism involved in the

epinephrine effect is not well characterized. One possibility is that cAMP activation of protein kinase A leads to serine/threonine phosphorylation of different isoforms of the signaling intermediates measured in this study or in separate pathways, which might decrease the ability of these molecules to be activated by insulin. Furthermore, downregulation of GS activity could be mediated by an epinephrine-induced decreased association of GS with the glycogen targeting subunits of protein phosphatase-1 (PP1), leading to GS inactivation (42).

After an acute bout of exercise, whole-body glucose disposal is enhanced, and this is attributed mainly to enhanced insulin sensitivity to glucose transport and glycogen metabolism in skeletal muscle (13,43). In accordance with a number of studies (11,12,14), we have found that 3 h after one-legged knee extensor exercise, net glucose uptake was ~45% higher in the previously exercised leg than in the rested leg during physiological hyperinsulinemia. This was observed regardless of whether the subjects received placebo or caffeine, and regardless of the fact that insulin induced similar increases in IRTK, IRS-1–associated PI 3-kinase, and PKB in the rested and exercised legs. We actually observed a decrease in insulin-stimulated IRS-1–associated PI 3-kinase activity in the exercised leg by 100 min of insulin infusion, resulting in significantly lower activity compared with the rested leg, in accordance with our previous observations (12). Similarly, GSK-3α activity was reduced by insulin to a similar extent in rested legs compared with exercised legs, despite a significantly higher GS activity in the previously exercised leg, consistent with previous reports (12). Nonetheless, the findings of the present study suggest that increased insulin sensitivity of glucose transport and GS activity in the postexercise period results from activation of signaling intermediates that are either more distal of IRTK, IRS-1 PI 3-kinase, PKB, and GSK-3α or are independent of the PI 3-kinase pathway (11,12). It is possible that the enhanced insulin sensitivity to glucose transport and GS activity is merely a general response to glycogen depletion after exercise (44).

In summary, we have shown that caffeine ingestion reduced whole-body insulin sensitivity, a finding that is attributed to a ~50% reduction in glucose uptake by both rested and exercised skeletal muscle during physiological hyperinsulinemia. We have also shown that caffeine reduced GS activity in both rested and exercised muscle. These inhibitory effects of caffeine were not accompanied by alterations in IRTK, IRS-1–associated PI 3-kinase, or GSK-3α activities or changes in PKB Ser^{473} phosphorylation. Caffeine's inhibitory effects may be mediated by signaling pathways that are further downstream from these insulin signaling intermediates or that are independent of this insulin signaling cascade. The detrimental effects of caffeine on insulin action in muscle were reduced by exercise. Caffeine increased circulating levels of epinephrine and muscle concentrations of cAMP, possibly indicating that this might contribute to the insulin-antagonistic effects of caffeine. Finally, caffeine is a ubiquitous substance in the human diet, and the dose of caffeine administered to the subjects in this study was equivalent to ~3–4 cups of coffee, which is typical of human consumption. Caffeine ingestion may be particularly deleterious for maintaining metabolic homeostasis, especially for individuals with impaired glucose tolerance and insulin resistance or frank diabetes.

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