Caffeine-induced impairment of glucose tolerance is abolished by β-adrenergic receptor blockade in humans

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Thong, Farah S. L., and Terry E. Graham. Caffeine-induced impairment of glucose tolerance is abolished by β-adrenergic receptor blockade in humans. J Appl Physiol 92: 2347–2352, 2002.—The caffeine-induced impairment of insulin action is commonly attributed to adenosine receptor (AR) antagonism in skeletal muscle. However, epinephrine, a potent inhibitor of insulin actions, is increased after caffeine ingestion. We tested the hypothesis that the insulin antagonistic effects of caffeine are mediated by epinephrine, and not by AR antagonism, in seven healthy men. On four separate occasions, they received 1) dextrose (placebo, PL), 2) 5 mg/kg caffeine (CAF), 3) 80 mg of propranolol (PR), and 4) 5 mg/kg caffeine + 80 mg of propranolol (CAF + PR) before an oral glucose tolerance test (OGTT). Blood glucose was similar among trials before and during the OGTT. Plasma epinephrine was elevated (P < 0.05) in CAF and CAF + PR. Areas under the insulin and C-peptide curves were 42 and 39% greater (P < 0.05), respectively, in CAF than in PL, PR, and CAF + PR. In the presence of propranolol (CAF + PR), these responses were similar to PL and PR. These data suggest that the insulin antagonistic effects of caffeine in vivo are mediated by elevated epinephrine rather than by peripheral AR antagonism.

Adenosine receptor antagonists; caffeine; β-adrenergic receptor; euglycemic-hyperinsulinemic clamp; glucose uptake; insulin stimulation; propranolol; skeletal muscle.

METHODS

Subjects. Seven healthy, active men (24 ± 1 yr, 76 ± 4 kg body wt, 23 ± 1 kg/m² body mass index) were recruited to participate in the study, which was approved by the University of Guelph Human Ethics Committee. Informed, written consent was obtained from each subject before the experiment. The subjects were instructed to follow a mixed diet containing products and alcohol, and to avoid strenuous physical activity 2 days before the experiment. The subjects reported to the laboratory on four separate occasions after an overnight fast.

Experimental protocol. The subjects rested for 15 min, and a catheter was inserted into an antecubital vein for blood sampling and was kept patent with a normal saline drip. A resting blood sample, heart rate (HR), and blood pressure (BP) were obtained (−105 min), and another blood sample was obtained 15 min later (−90 min). Subsequently, the subjects received 5 mg/kg dextrose (placebo, PL), 5 mg/kg methylxanthines; epinephrine; insulin; adenosine; propranolol
caffeine (CAF), 80 mg of propranolol (PR), or 5 mg/kg caffeine and 80 mg propranolol (CAF + PR) in a randomized, double-blind fashion. The subjects remained at rest for 90 min. A blood sample was obtained at 0 min, and a 120-min OGTT was initiated by ingestion of 75 g of glucose (Trurol). The amount of dextrose administered in PL is a small percentage (<1%) of the glucose administered for the OGTT. Resting blood samples were obtained at 15, 30, 60, 90, and 120 min after the glucose drink. To provide measures of β-blockade, HR and BP were monitored throughout the OGTT, and the subjects then performed cycling exercise for 2 min at 100-, 150-, and 200-W workloads after the OGTT. HR and rating of perceived exertion were obtained at the end of each workload.

Analytic procedures. Blood (7–10 ml) was collected in a sodium heparin-containing tube. Whole blood (200 μl) was transferred to an Eppendorf tube and treated with 1 ml of 0.6 M perchloric acid for glucose, lactate, and glycerol analyses. The remainder of the blood was treated with 120 μl of 0.24 M EGTA and reduced glutathione (GSH) for determination of catecholamines. The catecholamine data are presented for five subjects because of technical difficulties. After centrifugation, aliquots of supernatant from the perchloric acid-treated samples and plasma from EGTA-GSH-treated samples were transferred to Eppendorf tubes and stored at −20°C, respectively, until the time of analysis. Another 7–10 ml of blood were collected in a nonheparinized tube for serum free fatty acid (FFA), insulin, and C-peptide analyses. Blood samples were allowed to clot at room temperature. Samples were separated by centrifugation, and serum was transferred to Eppendorf tubes and stored at −20°C until analysis.

All blood metabolites and hormone concentrations were determined as the average of duplicate determinations. To minimize the effects of assay variability, samples from each subject were analyzed in the same assay. Blood glucose and glycerol were analyzed according to the methods of Bergmeyer et al. (4) and Lowry and Passonneau (25), respectively. Plasma catecholamines were determined in EGTA-GSH-treated plasma by an HPLC method as described by Weiker et al. (36). Serum nonesterified FFA (NEFA) were measured by using a NEFA kit (Wako Chemicals). Radioimmunoassay kits were used to measure serum insulin (Coat-a-Count Insulin, Diagnostic Products) and serum C-peptide (Human C-Peptide RIA Kit, Linco Research). The minimum detectable limits were 7.2 pmol/l for insulin and 0.0331 nmol/l for C-peptide. The intra- and interassay coefficients of variation were ∼4 and 7%, respectively.

Calculations and statistical analyses. The areas under the curve for glucose, insulin, and C-peptide concentrations during the 120-min OGTT [AUC(0–120 min)] were calculated over 120 min by using the trapezoidal method. Indexes of whole body insulin sensitivity during the OGTT were calculated according to the following equation proposed by Matsuda and DeFronzo (27)

$$\text{BMI} \times \text{FPG} \times \text{FPI} \times (\text{G} \times \text{I})$$

where FPG is fasting plasma glucose, FPI is fasting plasma insulin, G is mean plasma glucose concentration during OGTT, and I is mean plasma insulin concentration during OGTT. The index of whole body insulin sensitivity during the OGTT calculated from this equation is highly correlated ($r = 0.73, P < 0.0001$) with the rate of whole body glucose disposal during the euglycemic insulin clamp (27).

Statistical analysis was performed with an SAS statistical package (Cary, NC). One- and two-way analyses of variance (ANOVA) with and without repeated measures were used to evaluate statistical differences as appropriate. Tukey’s post hoc comparison was made when statistical significance was found between observations. Statistical significance was accepted at $P < 0.05$. Values are means ± SE.

RESULTS

Basal HR and BP were similar among groups. HR was decreased ($P < 0.05$) in PR (45 ± 1 beats/min) and CAF + PR (43 ± 3 beats/min) compared with PL (50 ± 2 beats/min) and CAF (49 ± 3 beats/min) during the OGTT. CAF resulted in significantly higher systolic (122 ± 2 mmHg), but not diastolic, BP than PL (113 ± 2 mmHg), PR (108 ± 3 mmHg), and CAF + PR (111 ± 3 mmHg) during the OGTT. There were no differences in diastolic BP among groups (data not shown). During the exercise, the increase in HR was ∼50% lower in PR and CAF + PR than in PL and CAF and rating of perceived exertion was significantly greater in PR and CAF + PR than in PL and CAF (data not shown).

Blood glucose was similar among trials at −105 min. In response to an oral glucose load, blood glucose was significantly increased during the 120-min OGTT in all trials (Fig. 1). There were no differences in blood glucose before or during the entire OGTT between trials. Before the OGTT, insulin (Fig. 2) and C-peptide (Fig. 3) were not different between trials. In response to an oral glucose load, insulin (Fig. 2) and C-peptide (Fig. 3) concentrations were significantly higher at 15 min and remained higher ($P < 0.05$) for the remainder of the OGTT than basal levels. The insulin (Fig. 2) and C-peptide (Fig. 3) responses to CAF were greater from 15 to 60 min of OGTT than responses to PL, PR, and CAF + PR. Insulin and C-peptide were not different from PL, PR, or CAF + PR for the remainder of the OGTT. No differences were observed in insulin or C-peptide response to an oral glucose load in PR and

![Fig. 1. Blood glucose before and during a 120-min oral glucose tolerance test (OGTT) in subjects treated with dextrose at 5 mg/kg placebo (PL), caffeine at 5 mg/kg (CAF, ○), 80 mg of propranolol (PR, ▲), and CAF + PR (○). Values are means ± SE (n = 7). Blood glucose increased (P < 0.05) similarly in all 4 trials in response to a 75-g glucose load at 15 min and throughout the OGTT.](http://www.jap.org)
CAFEINE, EPINEPHRINE, AND GLUCOSE TOLERANCE

DISCUSSION

In the present study, caffeine administered in doses that would elicit plasma caffeine concentration of ~30–45 μM (13, 14) increased insulin response by 42% and reduced whole body insulin sensitivity index by 25%. These findings are in accordance with those previously reported (12, 14, 19, 33). On the basis of our previous finding that reduction in net glucose uptake in skeletal muscle was the major determinant of the caffeine-induced reduction in whole body glucose disposal (33), it is reasonable to speculate that the reduced glucose tolerance after caffeine ingestion in the present study resulted from decreased insulin-dependent glucose clearance in skeletal muscle.

It has been proposed that the mechanism by which caffeine impairs glucose uptake in vitro experimental models (31, 35) and insulin sensitivity and glucose tolerance in humans (12, 14) is adenosine receptor antagonism. However, caffeine also stimulates epinephrine release (13, 14, 33). Thus the caffeine-induced impairment of insulin actions could be secondary to epinephrine and not to adenosine receptor antagonism. Moreover, although a functional role for adenosine has been shown in isolated adipocytes (18, 21), cardiac muscle (23), and rodent skeletal muscle (15, 35), its role in insulin regulation of glucose transport in humans is unclear because whole body insulin sensitivity (19) and glucose uptake in the forearm (28) were unaffected by infusion of dipyridamole (an adenosine reuptake inhibitor) and adenosine, respectively. Moreover, the presence in human skeletal muscle of the adenosine A1 receptor, which is proposed to mediate adenosine interaction with insulin, is uncertain (26).

To test the hypothesis that reduction in insulin action in vivo after caffeine ingestion is mediated by elevated epinephrine levels, and not by adenosine receptor antagonism, we administered caffeine in the

![OGTT](http://jap.physiology.org/)

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**Table 1. Area under the insulin and C-peptide curves during 120-min OGTT**

<table>
<thead>
<tr>
<th></th>
<th>Insulin, pm/120 min</th>
<th>C-Peptide, nM/120 min</th>
</tr>
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<tbody>
<tr>
<td>PL</td>
<td>20.8 ± 2.0</td>
<td>124 ± 8.7</td>
</tr>
<tr>
<td>CAF</td>
<td>28.6 ± 2.6*</td>
<td>195 ± 13.8*</td>
</tr>
<tr>
<td>PR</td>
<td>17.9 ± 1.8</td>
<td>129 ± 15.2</td>
</tr>
<tr>
<td>CAF + PR</td>
<td>20.0 ± 1.5</td>
<td>146 ± 7.8</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7). PL, placebo; CAF, caffeine; PR, propranolol; CAF + PR, caffeine + propranolol; OGTT, oral glucose tolerance test. *P < 0.05 vs. PL, PR, and CAF + PR.

OGTT. No differences were observed in FFA and glycerol at any time point between PL and CAF + PR.

Plasma epinephrine concentration was significantly elevated after caffeine ingestion (CAF and CAF + PR) compared with PL and PR (Table 2). There were no differences in plasma epinephrine between PL and PR and between CAF and CAF + PR. Plasma norepinephrine concentration was similar between trials (Table 2). There was no effect of an oral glucose load on catecholamine concentrations.

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**Fig. 2.** Serum insulin before and during a 120-min OGTT in PL (●), CAF (○), PR (◆), and CAF + PR (●) groups. Values are means ± SE (n = 7). Insulin was increased (P < 0.05) in response to an oral glucose load in all trials. *P < 0.05 vs. PL, PR, and CAF + PR.

**Fig. 3.** Serum C-peptide before and during a 120-min OGTT in PL (●), CAF (○), PR (◆), and CAF + PR (●) groups. Values are means ± SE (n = 7). C-peptide was increased (P < 0.05) in response to an oral glucose load in all trials. *P < 0.05 vs. PL, PR, and CAF + PR.
presence and absence of a β-adrenergic receptor blocker, propranolol. Observation of marked reduction in HR during the OGTT and during exercise in the propranolol trials is consistent with reduction of sympathetic tone, suggesting that β-blockade was indeed achieved in this study. When caffeine was administered in combination with propranolol, despite similar levels of epinephrine and caffeine, insulin and C-peptide concentrations were comparable to those observed in placebo in response to an oral glucose load, suggesting that the greater insulin response to caffeine was indeed secondary to elevated epinephrine levels. A large body of evidence indicates that elevated epinephrine levels acting via β-adrenergic receptors selectively induce whole body insulin resistance by transiently increasing hepatic glucose production and impairing glucose clearance by skeletal muscle (2, 3, 8, 17, 22). It has also been found that epinephrine enhances β-cell responsiveness to glucose during an intravenous glucose tolerance test (2). The enhanced insulin response with caffeine ingestion in the present study is likely elicited to counter epinephrine’s opposing actions on insulin-stimulated glucose clearance by peripheral tissues and, possibly, an increase in hepatic glucose output, in an effort to maintain glucose homeostasis. Thus our data do not support the notion that the insulin antagonistic effects of caffeine in vivo are mediated by adenosine receptor antagonism in skeletal muscle. Instead, findings from the present and previous (33) studies suggest that the negative effects associated with caffeine ingestion on insulin action are coupled to increased epinephrine production and its subsequent inhibition of insulin-mediated glucose uptake in skeletal muscle. Although we did not assess the effects of epinephrine on insulin responses per se, the notion that endogenous catecholamines are implicated in the insulin antagonistic effects of caffeine is supported by findings that the methylxanthine-induced hyperglycemia (30, 32) and peripheral insulin resistance (30) were eliminated in adrenalectomized and control rats in the presence of propranolol (32). Furthermore, because of a lack of compensatory reactions by epinephrine to increased insulin secretion in these rodents, they developed lethal hypoglycemia in response to theophylline.

Table 2. Plasma epinephrine and norepinephrine before and during 120-min OGTT

<table>
<thead>
<tr>
<th></th>
<th>Time, min</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>-90</td>
<td>0</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Epi, nM</td>
<td>PL</td>
<td>0.22 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.23 ± 0.02</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CAF</td>
<td>0.25 ± 0.03</td>
<td>0.49 ± 0.07†</td>
<td>0.50 ± 0.09†</td>
<td>0.54 ± 0.08†</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>0.21 ± 0.03</td>
<td>0.32 ± 0.05</td>
<td>0.33 ± 0.07</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CAF + PR</td>
<td>0.25 ± 0.04</td>
<td>0.49 ± 0.09†</td>
<td>0.59 ± 0.09†</td>
<td>0.41 ± 0.07†</td>
</tr>
<tr>
<td>NE, pm</td>
<td>PL</td>
<td>1.85 ± 0.31</td>
<td>1.79 ± 0.17</td>
<td>2.20 ± 0.19</td>
<td>2.43 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>CAF</td>
<td>1.61 ± 0.09</td>
<td>2.21 ± 0.32</td>
<td>2.69 ± 0.42</td>
<td>2.67 ± 0.22</td>
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<tr>
<td></td>
<td>PR</td>
<td>1.71 ± 0.14</td>
<td>2.09 ± 0.14</td>
<td>2.36 ± 0.20</td>
<td>2.62 ± 0.20</td>
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<tr>
<td></td>
<td>CAF + PR</td>
<td>1.86 ± 0.37</td>
<td>2.67 ± 0.37</td>
<td>2.41 ± 0.27</td>
<td>2.30 ± 0.27</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5). Epi, epinephrine; NE, norepinephrine. *P < 0.05 vs. -90 min (within trial); †P < 0.05 vs. PL and PR.

Fig. 4. Free fatty acid concentration before and during 120-min OGTT in PL (●), CAF (○), PR (♦), and CAF + PR (▼) groups. Values are means ± SE (n = 7). Free fatty acid concentration was decreased (P < 0.05) in response to insulin in all trials. *P < 0.05 vs. PL, PR, and CAF + PR; †P < 0.05 vs. PL, CAF, and CAF + PR.

Fig. 5. Glycerol concentration before and during a 120-min OGTT in PL (●), CAF (○), PR (♦), and CAF + PR (▼) groups. Values are means ± SE (n = 7). Glycerol concentration was decreased (P < 0.05) in response to insulin in all trials. *P < 0.05 vs. PL, PR, and CAF + PR; †P < 0.05 vs. PL, CAF, and CAF + PR.
conclusion is supported by several inhibitory actions of caffeine on glucose tolerance in skeletal muscle during insulinoma and heparin infusion that high circulating FFA inhibits glucose uptake in skeletal muscle during insulinoma. Numerous studies have demonstrated by use of Inulin clearance that the caffeine-induced rise in FFA was abolished in the presence of propranolol could be secondary to inhibition of insulin secretion or stimulation of insulin clearance by some nonspecific effects of propranolol. However, we have found that propranolol, in the absence of caffeine, had no effect on insulin or C-peptide concentrations (Fig. 3), nor did it alter blood glucose levels (Fig. 1) compared with placebo in response to an oral glucose challenge. This would suggest that the effects observed when caffeine and propranolol were present concomitantly resulted from β-adrenergic receptor blockade on peripheral tissues, rather than from any nonspecific effects of propranolol. Similarly, C-peptide levels paralleled insulin levels in response to caffeine in the presence and absence of propranolol. On the basis of these observations, we can conclude that the greater insulin response to caffeine in the present study reflects altered insulin secretion, rather than altered clearance.

Caffeine ingestion resulted in higher circulating FFA (Fig. 5), likely secondary to epinephrine stimulation of lipolysis or via antagonism of the adenosine A₁ receptor. The finding that the caffeine-induced rise in circulating FFA was abolished in the presence of propranolol suggests that stimulation of lipolysis after caffeine ingestion resulted from elevated epinephrine levels (Fig. 5). Our data are in agreement with those reported by van Baak and Saris (34), who showed similar reductions in FFA when caffeine was administered in combination with propranolol during exercise. Numerous studies have demonstrated by use of Intralipid and heparin infusion that high circulating FFA inhibits glucose uptake in skeletal muscle during insulin stimulation (20, 29). However, it is unlikely that the inhibitory actions of caffeine on glucose tolerance in our study resulted from high circulating FFA. This conclusion is supported by several findings. Although circulating FFA was higher 90 min after caffeine ingestion and before the OGTT, the increase in insulin response was not observed until 15 min after the oral glucose load. Moreover, after caffeine ingestion, the increase in insulin concentration effectively decreased FFA to the level observed in placebo by 30 min of the OGTT, and yet insulin levels remained significantly higher until 90 min of the OGTT. Similarly, in our previous studies, insulin infusion prevented (14) or decreased (33) the caffeine-induced rise in plasma FFA to that observed in placebo, and yet, whole body insulin sensitivity (14, 33) and net glucose uptake in skeletal muscle (33) remained significantly lower than with placebo during the euglycemic-hyperinsulinemic clamps.

In summary, we have demonstrated that caffeine reduces glucose tolerance in healthy men in response to an oral glucose challenge. We have also provided evidence that caffeine’s negative impact on glucose tolerance was reversed in the presence of a β-adrenergic receptor blocker, propranolol. These data suggest that although caffeine can exert its inhibitory effects on glucose uptake in vitro by adenosine receptor antagonism, the insulin antagonistic effects of caffeine in vivo are mediated by elevated epinephrine levels. The findings from this study certainly cannot discount the potential role for adenosine receptor interaction with insulin actions in humans. Rather, our findings suggest that caffeine may not be an appropriate pharmacological tool to assess adenosine modulation of insulin actions on carbohydrate metabolism in vivo in light of the confounding effects elicited by the concomitant presence of epinephrine, which has profound counter-regulatory effects on insulin’s diverse actions in peripheral tissues.

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