The Glucose Intolerance Induced by Caffeinated Coffee Ingestion Is Less Pronounced than That Due to Alkaloid Caffeine in Men\(^1,2\)

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**ABSTRACT** Although acute alkaloid caffeine (CAF) ingestion results in an impaired glucose tolerance, chronic coffee (RCOF) ingestion decreases the risk of developing type 2 diabetes. This study examines the hypothesis that CAF ingestion impairs glucose tolerance to a greater extent than RCOF and that the ingestion of decaffeinated coffee (DECAF) results in a positive effect. Eleven healthy males underwent 4 double-blinded randomized trials. Each trial included the ingestion of either: 1) CAF in capsule form (4.45 mg/kg body weight), 2) RCOF (4.45 mg/kg body weight caffeine), 3) dextrose (placebo, PL) in capsule form, or 4) DECAF (equal in volume to the RCOF trial), followed 1-h later by a 2-h oral glucose tolerance test. Blood samples were collected at baseline (-30), 0 (time of treatment ingestion), 60 (initiation of oral glucose tolerance test), 75, 90, 120, 150, and 180 min. Area under the curve for glucose and insulin were higher \( (P \leq 0.05) \) following CAF than both PL and DECAF and, although a similar trend \( (P = 0.07) \) was observed following RCOF compared with DECAF, the effect was less pronounced. Interestingly, DECAF resulted in a 50% lower glucose response \( (P \leq 0.05) \) than PL, suggesting that the effects of PL and DECAF on glucose tolerance are not the same. These findings suggest that the effects of CAF and RCOF are not identical and may provide a partial explanation as to why acute CAF ingestion impairs glucose tolerance while chronic RCOF ingestion protects against type 2 diabetes. J. Nutr. 136: 1276–1280, 2006.

**KEY WORDS:** • chlorogenic acids • epinephrine • type 2 diabetes
acute glucose intolerance, other compounds present in coffee may attenuate this effect by altering other aspects of glucose metabolism.

On this basis, we resolved to investigate the impact of caffeinated (RCOF) and decaffeinated (DECAF) coffee consumption on the blood glucose and insulin responses during an OGTT and to compare these findings with those obtained from both alkaloid caffeine (CAF) and placebo (PL), ingestion. We hypothesized that CAF would result in an acute glucose intolerance identified by an elevated insulin response without a subsequent lowering in glucose response, whereas RCOF ingestion would result in a similar, yet blunted response. We further hypothesized that DECAF would result in a lower glucose and insulin response than that observed with PL due to the absence of caffeine and the presence of other biologically active ingredients.

MATERIALS AND METHODS
This study was approved by the University of Guelph’s Human Ethics Committee. All subjects (n = 11) were male, healthy, non-smokers, low to moderately active, and not taking any medications that alter glucose metabolism. Subject characteristics for age were 23.2 ± 0.6 y (mean ± SEM), for weight 76.4 ± 1.9 kg, for maximum oxygen consumption (VO_{max}) 51.8 ± 3.7 mL·kg⁻¹·min⁻¹ and for percent body fatness 15.6 ± 2.3%. All subjects but one were considered noncaffeine users as defined by the consumption of ≤2 caffeinated coffee or tea beverages and/or ≤5 caffeine-containing soft drinks per week. The one subject considered a caffeine user abstained from caffeine for 7 d prior to the first experiment and throughout the remainder of the study.

Prior to the first experiment, subjects underwent both a VO_{max} test and hydrostatic weighing, in addition to completing a 3-d food record. This diet was analyzed for macronutrient composition and consumed prior to each experiment. Also during this time subjects abstained from caffeine-containing beverages and/or substances and alcohol and maintained regular activity patterns. On the day prior to each experiment, subjects refrained from performing any strenuous activity to maintain muscle glycogen stores.

For a given subject, all experiments were conducted at the same time of day. Although most subjects (n = 9) entered the laboratory after a 10–12-h overnight fast, for those who began their experiments in the early afternoon (n = 2), a small breakfast was permitted. The composition of the breakfast was recorded and an identical breakfast was consumed prior to each experiment. All subjects completed 4 trials in a double-blinded, randomized fashion, separated by 1–2 wk. Upon entering the laboratory, a catheter was inserted into the antecubital vein for venous blood sampling. A baseline blood sample was collected (t = −30) and subjects remained rested for 30 min. Following a second blood sample (t = 0) subjects consumed either of the following 4 treatments: 1) 4.45 mg/kg dextrose in capsule form (placebo trial; PL) with 250 mL water, 2) 4.45 mg/kg alkaloid caffeine in capsule form (caffeine trial; CAF) with 250 mL water, 3) a volume of caffeinated coffee providing 4.45 mg/kg caffeine (regular coffee trial; RCOF), or 4) an equal volume of decaffeinated coffee (decaffeinated coffee trial; DECAF). The coffee used in this study was Maxwell House Dark Roast brand. The coffee preparation procedure is described in detail elsewhere (15). At 60 min post-treatment ingestion (t = 60), a blood sample was taken and a standard 2-h oral glucose tolerance test (OGTT) was initiated. Subjects remained rested over the next 2 h and blood samples were collected at regular intervals.

Blood samples collected in nonheparin-treated tubes were allowed to clot at room temperature. Samples were then centrifuged (1200 × g; 10 min) and the serum stored at −20°C for the later determination of insulin (Coat-a-Count RIA kit, Diagnostic Products Corporation), C-peptide (Human C-Peptide RIA kit, Linco Research), free fatty acids (NEFA kit, Wako Bioproducts), and glycerol (18). A 200 μL aliquot of heparinized blood was added to 1 mL of 0.6 mol/L perchloric acid (PCA) and, following centrifugation (1200 × g; 10 min), the supernatant (PCA extract) stored at −20°C and analyzed for whole blood glucose (19) and lactate (20). Another aliquot of heparinized blood was centrifuged and the plasma stored at −80°C for later determination of plasma methylxanthines by HPLC (21). For determining plasma epinephrine, 120 μL of 0.24 mol/L EGTA and reduced glutathione was added to the remaining blood in the heparin-treated tube and was centrifuged (1200 × g; 10 min). The supernatant portion was stored at −80°C until the analysis could be performed (Adrenaline RIA kit, Labor Diagnostika Nord GmbH).

All blood data were analyzed for time and treatment effects using a 2-way ANOVA for repeated measures and a Tukey test was used for multiple comparison analysis. Area under the curve (AUC) for glucose, insulin, and C peptide was calculated using the trapezoid method during the OGTT, with data at t = 0 min used as the baseline value. All AUC data were analyzed for treatment effects using a 1-way ANOVA for repeated measures, and, when differences were found, a Tukey test was used for multiple comparisons. For technical reasons, epinephrine concentrations were measured for a subset of subjects (n = 6). Due to differences in the initial starting concentrations among treatments, the change in epinephrine concentration between time 0 and 60 min was calculated (i.e., epinephrine concentration at time 60 – epinephrine concentration at time 0). A 1-way ANOVA was used to elucidate any treatment differences. To get an estimate of whole body insulin sensitivity, the insulin sensitivity index (ISI) was calculated using an equation described by Matsuda and DeFronzo (22). This equation uses both fasting and average OGTT values for plasma glucose and insulin. Although we do acknowledge that we have used whole blood glucose and serum insulin for the calculation, the use of this index was for comparison purposes among treatments only and not to comment on absolute values obtained. A 1-way ANOVA was used to elucidate any treatment effects. Linear regression analysis was performed to determine whether a relation existed between the fitness level or percentage of body fat and either AUC for glucose, insulin, or C peptide. Percentage of body fat was determined by hydrostatic weighing and was calculated using the Siri equation. Food records were analyzed using a computer program (ESHA Research, version 7.11).

All values are presented as mean ± SEM. Differences were significant at P ≤ 0.05 in 2-tailed testing.

RESULTS

Plasma methylxanthine concentrations. Plasma methylxanthine concentrations did not differ among the baseline periods and, in general, concentrations were undetectable, confirming compliance with pre-experimental dietary restrictions. During the PL (0.12 ± 0.04 μmol/L) and DECAF (0.21 ± 0.07 μmol/L) trials, plasma caffeine concentrations did not differ from baseline or from one another (P = 0.10). Within the CAF and RCOF trials, plasma caffeine concentrations increased, reaching peak concentrations of 33.1 ± 1.4 and 36.8 ± 1.5 μmol/L just prior to the initiation of the OGTT (t = 60 min), respectively. Thereafter, concentrations gradually declined but remained greater than baseline and greater than values obtained during both PL and DECAF trials. A treatment effect (P = 0.05) occurred between RCOF and CAF (RCOF > CAF) at all time points, despite the fact that the absolute difference in caffeine concentration was only 2.4 μmol/L. As expected, paraxanthine, theophylline, and theobromine concentrations closely paralleled those of caffeine concentrations (data not shown).

Serum insulin concentration. Serum insulin concentrations did not differ among trials at baseline (t = −30 or 0 min) and remained unchanged during the 60 min post-treatment ingestion (values ranging from 50 to 60 pmol/L) (Fig. 1A). As expected, in all trials, serum insulin concentrations increased rapidly within the first 30 min of the OGTT (P = 0.03), returning to baseline values by the end of the OGTT. CAF resulted in a greater serum insulin response (AUC, P = 0.05) than both PL and DECAF. The AUC tended to be greater (P = 0.09) after...
In all trials, C peptide concentrations did not differ between RCOF and DECAF, or between PL and DECAF, did not differ (P > 0.05).

**Blood glucose concentration and ISI.** Blood glucose concentrations were not different among treatments either at baseline (−30 and 0 min) or at 60-min post-treatment ingestion (mean 4.2 mmol/L) (Fig. 1B). Following the initiation of the OGTT, blood glucose concentrations increased rapidly in all trials, such that peak concentrations were achieved within the first 30 min of the OGTT (P ≤ 0.05). Thereafter, blood glucose concentrations steadily declined such that, by the end of the OGTT, glucose values had returned to baseline values. Interestingly, despite a higher AUC for insulin and C peptide (P ≤ 0.05) following CAF ingestion, the AUC for blood glucose (P ≤ 0.05) was also higher with CAF than with both PL and DECAF (Table 1). The difference between CAF and DECAF was further substantiated by a higher ISI with CAF (P ≤ 0.05) than with DECAF (Table 1); however ISI did not differ (P > 0.05) among any other treatments. In parallel to the differences reported between CAF and PL or DECAF, the 50% difference in glucose AUC between PL and DECAF (PL > DECAF) was also significant (P ≤ 0.05). Similar to the insulin response, RCOF resulted in a trend toward a higher glucose response (P = 0.06) than with DECAF. Finally, CAF resulted in a higher glucose AUC (P ≤ 0.05) than with RCOF, indicating that these treatments do not elicit a similar glucose response during an OGTT (Table 1).

**Serum FFA concentration.** Serum FFA concentrations were not different (P > 0.05) between trials at baseline (values ranging from 400 to 500 μmol/L). Following the ingestion the RCOF, PL, and DECAF treatments, the FFA concentrations remained unchanged from baseline values. In contrast, CAF resulted in a rapid increase in FFA concentrations of ~500 μmol/L during the first 60 min postcapsule ingestion (P ≤ 0.05). Despite this elevation in FFA concentration, upon initiation of the OGTT and subsequent increase in insulin (Fig. 1A), serum FFA concentrations declined in all trials such that, by 30 min into the OGTT, values were below (276 ± 41 μmol/L) those observed at baseline. By the end of the OGTT (t = 180 min), however, serum FFA concentrations returned to those at baseline in both the CAF and RCOF trials (P > 0.05). Overall, a higher FFA response (P ≤ 0.05) occurred following both CAF and PL than with DECAF. A trend (P = 0.07) for a higher FFA response in the RCOF trial than with the DECAF trial was observed (i.e., less decrease in FFA were observed within the first 60 min postigestion of the RCOF treatment than with the DECAF treatment).

**Serum glycerol concentrations.** At baseline, serum glycerol concentrations did not differ (P > 0.05) among trials. Within the RCOF, PL, and DECAF treatments, serum glycerol concentrations did not differ (P > 0.05) among treatments either at baseline or during the initial 60-min post-treatment ingestion (P > 0.05; values ranging from 0.36 to 0.39 nmol/L). Although C peptide concentrations increased in an expected parallel manner to insulin during the first 30 min of the OGTT (P ≤ 0.05), C peptide concentrations did not return to baseline values (mean 0.90 nmol/L) by the end of the OGTT, as observed with insulin. Similar to the serum insulin responses, AUC analysis revealed a higher C peptide response (P ≤ 0.05) with CAF than both PL and DECAF (Table 1). In addition, CAF resulted in a higher C peptide AUC (P ≤ 0.05) than RCOF. The C peptide responses between RCOF and DECAF, or between PL and DECAF, did not differ (P > 0.05).

**TABLE 1**

AUC for whole blood glucose, serum insulin, C peptide, and ISI in men during the 2-h OGTT following ingestion of PL, CAF, RCOF, and DECAF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PL</th>
<th>CAF</th>
<th>DECAF</th>
<th>RCOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>184 ± 40b</td>
<td>285 ± 40a</td>
<td>90 ± 29b</td>
<td>176 ± 46bc</td>
</tr>
<tr>
<td>2 h</td>
<td>22087 ± 3050b</td>
<td>32758 ± 4082a</td>
<td>20544 ± 2419b</td>
<td>26763 ± 2250ab</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>126 ± 14b</td>
<td>166 ± 15a</td>
<td>106 ± 12b</td>
<td>133 ± 15b</td>
</tr>
<tr>
<td>2 h</td>
<td>7.7 ± 0.8b</td>
<td>7.6 ± 0.6a</td>
<td>9.0 ± 0.5b</td>
<td>8.2 ± 0.7b</td>
</tr>
<tr>
<td>C peptide, nmol/L</td>
<td>126 ± 14b</td>
<td>166 ± 15a</td>
<td>106 ± 12b</td>
<td>133 ± 15b</td>
</tr>
<tr>
<td>2 h</td>
<td>7.7 ± 0.8b</td>
<td>7.6 ± 0.6a</td>
<td>9.0 ± 0.5b</td>
<td>8.2 ± 0.7b</td>
</tr>
<tr>
<td>ISI</td>
<td>8.7 ± 0.8b</td>
<td>7.6 ± 0.6a</td>
<td>9.0 ± 0.5b</td>
<td>8.2 ± 0.7b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; n = 11. Means at a time without a common letter differ, P ≤ 0.05.
concentrations remained unchanged from baseline throughout the experiments. Similar to the FFA response however, CAF resulted in an increased serum glycerol concentration (from 126 ± 8 to 154 ± 10 μmol/L) at 60 min post-treatment ingestion compared with all other trials (P ≤ 0.05). These concentrations returned to baseline values by 15 min into the OGTT and no longer differed from the other treatments. Overall, the serum glycerol response was greater (P ≤ 0.05) in the CAF trial than with all other trials.

**Blood lactate concentrations.** Blood lactate concentrations did not differ (P > 0.05) among trials at baseline. Following the ingestion of each treatment, blood lactate concentrations gradually increased, such that concentrations during the first 90 min of the OGTT were greater (P ≤ 0.05) than values obtained at baseline. The only treatment effect was a higher blood lactate concentration during the RCOF trial (1.5 ± 0.2 mmol/L) compared with the PL trial (1.1 ± 0.1 mmol/L) (P < 0.05), possibly due to what subjectively appears to be a modestly higher initial concentration of lactate (1.7 ± 0.3 for RCOF compared with 1.1 ± 0.1 mmol/L for PL).

**Plasma epinephrine concentrations.** Baseline epinephrine concentrations were similar (P > 0.05); values ranging from 0.36 to 0.45 nmol/L prior to the ingestion of all treatments; however, following the ingestion of CAF and RCOF, epinephrine concentrations increased significantly, such that values at 60, 120, and 180 min were higher than those observed at baseline. Although no overall treatment effect was obtained, analysis of epinephrine concentrations during the first hour following treatment ingestion (the delta between 0 and 60 min) revealed that both CAF (+ 0.15 nmol/L) and RCOF (+ 0.11 nmol/L) resulted in higher elevations in epinephrine concentrations than with both PL (−0.04 nmol/L) and DECAF (+ 0.02 nmol/L) (P ≤ 0.05).

**Linear regressions.** Body composition (% body fatness) or level of fitness (VO₂ max) were not correlated with the AUC for glucose, insulin, or C peptide during the OGTT for any of the treatments (data not shown).

**DISCUSSION**

This study is the first that we know of to directly compare the effects of CAF and RCOF ingestion on glucose metabolism within a single study. Although the present study confirms those previously reporting an impaired glucose tolerance following both CAF (5–8) and RCOF (16,23–25), our results demonstrate some notable differences. Whereas CAF resulted in higher glucose, insulin, and C peptide responses (P ≤ 0.05) compared with both PL and DECAF treatments, RCOF resulted in an attenuated response, such that trends were observed only for glucose (P = 0.06) and insulin (P = 0.09) when compared with DECAF, and no difference was observed when compared with PL. Furthermore, although the ISI was higher (P ≤ 0.05) following CAF than DECAF, no difference was observed between RCOF and DECAF. Interestingly, the dampened effect of RCOF occurred despite a small, albeit higher, caffeine concentration of 2–4 μmol/L (P ≤ 0.05) and suggests that the attenuated effect of RCOF ingestion on glucose tolerance cannot be attributed to a lower plasma caffeine concentration. Regardless, our results agree with those of Brown et al. (26), who demonstrate that the assumption that CAF consumption is equal to RCOF consumption leads to erroneous conclusions, at least with respect to the reduction of relative risk.

Interestingly, the effects observed between PL and DECAF closely parallel those observed between CAF and RCOF and may provide some additional explanation to the attenuated RCOF effect. Like RCOF, DECAF resulted in an attenuated glucose response, such that the AUC for glucose was 50% (P ≤ 0.05) of that observed with PL. Considering the fact that an identical amount of glucose was ingested in both the DECAF and PL trials, either DECAF resulted in a lower intestinal glucose absorption or a higher glucose clearance. Coffee is known to contain many biologically active compounds. Recent evidence demonstrates that the chlorogenic acids and quinide compounds found in DECAF may delay intestinal glucose absorption (16) and does enhance nonmuscle glucose uptake (17), respectively. Therefore, although it is not possible to ascertain from the present study which of these mechanisms is responsible for the attenuated glucose response, both would result in a lower glucose response. Furthermore, these compounds may also provide an explanation as to why RCOF had a blunted negative effect on glucose tolerance compared with CAF, in that the negative effect of CAF on glucose disposal may have been blunted by the other compounds in RCOF that either hinder glucose absorption (16) or enhance non skeletal muscle glucose disposal (17).

The exact mechanism by which caffeine exerts its negative effect on glucose metabolism remains controversial. Although CAF is a known nonselective adenosine receptor antagonist, adenosine’s role in skeletal muscle insulin-mediated glucose uptake also remains controversial (9,27–30). Until recently, we was believed that the small, albeit significant, elevation in epinephrine concentration observed following CAF ingestion was likely solely responsible for the impairment in glucose tolerance (6). However, although epinephrine is a potent antagonist of insulin-mediated glucose disposal (31–33), the infusio of epinephrine to concentrations similar to those observed following CAF ingestion did not significantly impede insulin-mediated glucose disposal (34), and therefore additional mechanisms are likely involved. Regardless, the present study did demonstrate significant elevations in epinephrine concentrations during both the CAF and RCOF trials; however, due to the fact that epinephrine concentrations were similar among these treatments, it is unlikely that this can explain the attenuated effect observed on glucose tolerance with RCOF.

Although CAF resulted in the expected increase in FFA and glycerol concentrations during the first hour after ingestion, RCOF ingestion did not. This is somewhat surprising inasmuch as the elevations in epinephrine concentrations were similar among these treatments. These findings not only provide further evidence that CAF and RCOF do not behave synonymously with respect to glucose metabolism but may also provide evidence that they have differential effects on other aspects of metabolism (i.e., lipolysis). In addition, the lack of increase in FFA/glycerol concentrations with similar epinephrine concentrations suggests that other components present in RCOF may blunt the effects of CAF not only on glucose disposal but on lipolysis as well.

Regardless of the exact mechanism involved, our findings may provide some insight into the conflicting observations among acute (5–8) and chronic (12–14,35) CAF ingestion studies. The present observations that DECAF decreased the glucose response by 50% compared with PL and that the effect of RCOF on the glucose response was attenuated by 40% compared with CAF are interesting. It appears that not only may some components of DECAF enhance glucose tolerance, but that it is likely that some components may attenuate or possibly antagonize the effects of CAF. This, along with the fact that the effects of CAF on epinephrine are blunted within 1–d (36) of ingestion, may also explain the beneficial aspects of chronic RCOF ingestion. A desensitization to the effects of epinephrine...
(i.e., downregulation to B-adrenergic receptors or a lack of increase in epinephrine) may lead to an attenuation of the mechanism by which CAF decreases glucose disposal. Taken together, if chronic RCOF ingestion results in the attenuation of the insulin-antagonistic effects of epinephrine while increasing exposure to insulin-sensitizing compounds, like chlorogenic acids and quinides, this may account for some, if not most, of the reduction in risk for the development of type 2 diabetes.

In conclusion, while this study confirms that CAF impairs glucose tolerance in men, RCOF results in only a trend toward a similar response and therefore the effects of CAF and RCOF cannot be considered to be the same. It is likely that although acute CAF results in an impaired glucose tolerance, other biologically active compounds in RCOF attenuate this effect, resulting in the observed blunted glucose intolerance. In addition, the different glucose response elicited by PL and DECAF further suggests that compounds present in coffee may enhance glucose tolerance and may provide a partial explanation for the reported decrease in incidence of type 2 diabetes in chronic coffee users. Finally, this study provides evidence that caution should be used when comparing the effects of CAF and RCOF and the reported decrease in incidence of type 2 diabetes in chronic coffee users. This study provides evidence that a similar response and therefore the effects of CAF and RCOF cannot be considered to be the same. It is likely that although acute CAF results in an impaired glucose tolerance, other biologically active compounds in RCOF attenuate this effect, resulting in the observed blunted glucose intolerance.

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