Quinides of Roasted Coffee Enhance Insulin Action in Conscious Rats[1]

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ABSTRACT Consumption of large amounts of coffee has been shown to decrease the incidence of type 2 diabetes. However, the specific compounds and mechanisms responsible for this effect are not known. The aim of this study was to determine the effects of a decaffeinated coffee extract and a synthetic quinide, representative of those found in roasted coffee, 3,4-diferuloyl-1,5-quinolactone, on insulin-stimulated glucose disposal and muscle glucose uptake. Experiments were performed on conscious rats during hyperinsulinemic, euglycemic clamps receiving gastric infusions of saline, a decaffeinated coffee extract (DECAF) (220 mg/kg), or 3,4-diferuloyl-1,5-quinide (DIFEQ) (110 mg/kg). Following treatment, rats received an intravenous bolus of deoxy-[2-^{3}H] glucose to assess muscle glucose uptake (R_g, μmol · 100 g^{-1} · min^{-1}). Glucose infusions [mg/(kg · min)] required to maintain euglycemia during the tracer period were higher with DIFEQ (14.6 ± 0.7) than with saline (10.8 ± 0.7) and DECAF (11.5 ± 1.1). Despite increased glucose requirements, R_g in skeletal (soleus, gastrocnemius, superficial vastus lateralis) and cardiac muscle were unchanged. DECAF or DIFEQ did not affect heart rate, blood pressure, plasma nonesterified fatty acids or liver aminotransferase activity. These results demonstrate that DIFEQ increases whole-body glucose disposal independently of skeletal muscle R_g. J. Nutr. 133: 3529–3532, 2003.

KEY WORDS: • skeletal muscle glucose uptake • chlorogenic acid • diabetes • metabolism

Epidemiologic studies examining coffee consumption indicate that drinking large amounts of coffee drastically reduces the incidence of type 2 diabetes (1,2). These findings, verified by two independent investigations, show that the consumption of 1–3 cups of coffee per day in men resulted in a multivariate risk reduction of 0.93 for type 2 diabetes, whereas consumption of 4–5 and 6 or more cups of coffee resulted in risk reductions of 0.71 and 0.46, respectively (2). Paradoxically, the consumption of caffeine (5 mg/kg) in coffee results in impaired glucose tolerance in both healthy (3) and obese individuals (4). Further evidence suggests that when coffee and caffeine are consumed in combination, the resultant effect is different from that when each compound is consumed independently. Consumption of caffeine alone results in a greater glucose intolerance in response to an oral glucose tolerance test (OGTT) compared to an equivalent amount of caffeine consumed in coffee (5). In the same study, consuming decaffeinated coffee before an OGTT resulted in a blunted rise in blood glucose compared with subjects consuming water. Taken together, these results are dichotomous and suggest that the effects of coffee and caffeine are distinct. Although acute coffee consumption may be detrimental to glucose homeostasis, long-term coffee consumption may result in benefits that are protective to the development of type 2 diabetes. Alternatively, frequent coffee drinkers may become acclimated to the detrimental effects of caffeine, and thus benefit from any glucose-sensitizing effects of other components in coffee.

Coffee contains hundreds of biologically active compounds. The most abundant water-soluble constituents of coffee include phenolic polymers (8 g/100 g), polysaccharides (6 g/100 g), chlorogenic acids (4 g/100 g), minerals (3 g/100 g), organic acids (0.5 g/100 g), sugars (0.3 g/100 g) and lipids (0.2 g/100 g) (6). The most likely candidates in mediating the antidiabetic effects of coffee are chlorogenic acid–derived constituents. A typical cup of North American coffee contains ~200 g/250 mL chlorogenic acid (6). In addition, roasting coffee results in the transformation of some chlorogenic acid into new compounds that are unique to coffee. Loss of a water molecule and the formation of an internal ester bond from chlorogenic acids results in nonacidic quinolactones, or quinides. Studies examining chlorogenic acids and their derivatives showed that these compounds are absorbed and are biologically active (7,8). Therefore, the purpose of the present study was to determine the effects of a decaffeinated coffee extract (DECAF) and a synthetic quinide, 3,4-diferuloyl-1,5-quinolactone (DIFEQ) on insulin action in vivo. DIFEQ is a compound that is representative of the two most abundant quinides in coffee, 3- and 4-caffeoylquinide. We hypothesized that DECAF and DIFEQ would improve insulin action and that the primary site of action would be skeletal muscle, which comprises the bulk of insulin-sensitive tissue.

MATERIALS AND METHODS

Male Sprague Dawley rats (Harlan Industries, Indianapolis, IN) were housed individually and maintained at 23°C on a 0600–1800 h light cycle. Rats consumed a standard diet (Purina, Nestlé, St. Louis,

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[3] Abbreviations: ALT, plasma alanine aminotransferase; DECAF, instant decaffeinated coffee; DIFEQ, 3,4-diferuloyl-1,5-quinide; GIR, glucose infusion rate; [^{3}H]DG, deoxy-[2-^{3}H]glucose; [^{3}H]DGP, phosphorylated deoxy-[2-^{3}H]glucose; NEFA, nonesterified fatty acid; OGTT oral glucose tolerance test; R_g, glucose metabolic index.

MO) and water ad libitum. They were housed under these conditions for ~1 wk, by which time they weighed ~300 g. All procedures were approved by the Vanderbilt University Animal Care and Use Subcommittee and followed NIH guidelines for the care and use of laboratory animals.

Experimental compounds. *DIFEQ* The selection of DIFEQ as a suitable probe for investigating potential effects of quinides on glucose tolerance is based on the fact that the physicochemical properties of DIFEQ are representative of a number of compounds present in roasted coffee. DIFEQ was synthesized in five steps from *D*-quinic acid (Sigma-Aldrich, Milwaukee, WI) using the method of Wynne et al. (9) as described by Huynh-Ba (10). The resultant DIFEQ extract was diluted in saline to the appropriate concentration for administration to rats (110 mg/kg).

Decaffeinated coffee extract. To study the effects of coffee constituents, an extract of commercial decaffeinated instant coffee was prepared, in which water-soluble sugars, alkaloids and some of the quinic acids were removed. The extract used in the present study contained ~1.45 g/kg of total quinides with each rat receiving ~3.4 mg quinide/kg (11). To prepare the extract, 5 g of commercial instant coffee was dissolved in water (100 mL) and adjusted to pH 4–5 with 2 mmol/L hydrochloric acid. Lipophilic constituents were extracted with ethyl acetate (2 × 200 mL). Foaming was suppressed by the addition of 2 mL ethanol. The combined extract was washed with water (2 × 100 mL). Drying (Na_2SO_4) and evaporation of the solvent gave 0.50 ± 0.05 g residual oil (10% of the original weight). Coffee extract (0.5 g) was dissolved in 2 mL 50% Tween-80 and diluted with 8 mL water to give 50 g/L (10% Tween-80). The resultant decaffeinated coffee extract (DECAF) was diluted in saline to the appropriate concentration for administration to rats.

Surgical procedures. On the day of the surgery, rats were food deprived for 5 h to facilitate insertion of the gastric catheter. Surgical procedures were performed as previously described for the gastric (12), arterial and venous (13) catheters. To facilitate weight gain in the postsurgical period, rats were administered 14.23 kJ/100 g from glucose (0.5 kg/L) and 5.44 kJ/100 g from lipid (20%) via their gastric tube at a rate of 40 μL/min for the first 2 d after surgery. Animal weights and food intake were monitored daily, and only rats whose weights were restored to their presurgery level were used for experiments (≥6 d). On the day of the experiment, rats were food deprived for 5 h and catheters were flushed with heparinized saline (10^3 U/L) and connected to PE50 and silastic tubing for infusions and sampling.

Experimental procedures. Rats were conscious and unrestrained throughout the experiments. In total, experiments lasted for 120 min ($t = -60$ to 60 min). At $t = -60$ min, rats were administered a constant intravenous infusion of insulin at 14 pmol/(kg · min); glucose (0.5 g/mL) was infused to maintain glycemia at 7.0 mmol/L. At $t = 0$ min, a bolus infusion (5 μL/g body weight) of saline, DECAF (100 mg/kg) or DIFEQ (50 mg/kg) was administered over a 1-min interval through the gastric catheter. Immediately after the bolus, a constant infusion of saline, DECAF [2 mg/(kg · min)] or DIFEQ [1 mg/(kg · min)] was administered at a rate of 0.1 μL/(kg · min) for the remainder of the experiment. In total (bolus + constant infusion), this resulted in the administration of 11 μL/g body weight. At $t = 25$ min, an intravenous bolus of deoxy-[2-^{3}H]glucose ([^{3}H]DG) (50 μCi in 225 μL) was administered for determination of an index of muscle glucose uptake (R_g). The time period from $t = 0$ to 60 min is referred to as the "experimental period," whereas $t = 25$–60 min is the "tracer period."

Arterial blood samples (20 μL) were obtained every 10 min from $t = -60$ to $t = 0$ min for the measurement of plasma glucose to provide feedback used to adjust glucose infusion rates to maintain glycemia at 7.0 mmol/L. At times −60, 0, 20 and 60 min, large (150 μL) blood samples were withdrawn for the measurement of insulin, nonesterified fatty acids (NEFA) and [^{3}H]DG. In addition, small samples (50 μL) were obtained at 27, 30, 35, 40, 50 and 60 min for the measurement of [^{3}H]DG. To prevent declines in hematocrit, the erythrocytes taken between $t = -60$ and 0 min were washed in saline and infused after each sample. Arterial systolic, diastolic and mean blood pressures and heart rate were assessed by the arterial catheter using a blood pressure analyzer (Model 400, Digi-Med, Louisville, KY). Measurements were taken during 5-min intervals at −60 to −55, −5 to 0, 15 to 20, and 55 to 60 min. At $t = 60$ min, rats were anesthetized with pentobarbital sodium and soleus, gastrocnemius, superficial vastus lateralis and heart were excised and rapidly freeze-clamped in liquid nitrogen. Stomachs of the rats were visually inspected for retention of infused compounds and gastric catheters were tested by the infusion of dye into the stomachs. Rats with leaky ($n = 1$) or misplaced catheters ($n = 1$) were removed from the study.

Plasma analyses. *Metabolites.* Plasma glucose concentrations were measured by the glucose oxidase method using an automated glucose analyzer (Beckman Instruments, Fullerton, CA); immunoreactive insulin was measured using a double-antibody method (14). NEFA concentrations were determined spectrophotometrically using a kit obtained from Wako Chemicals (NEFA-C, Richmond, VA). Liver alanine aminotransferase activity (ALT) was assessed in plasma samples from $t = -60$ and $t = 60$ min using a kit from Stanbio (ALT/GPT 0930, Boerne, TX).

Tissue analyses. Tissues (skeletal and cardiac muscle) were analyzed for accumulation of free ([^{3}H]DG) and phosphorylated deoxy-[2-^{3}H]glucose ([^{3}H]DGP) as previously described (15).

Calculations. The R_g were calculated as previously described (16). A two-way repeated-measures ANOVA was performed to compare differences. To establish differences within the ANOVA, Tukey's post-hoc test was used. Significance levels of $P \leq 0.05$ were employed, and data are reported as means ± SEM.

RESULTS

Experimental rats in the saline, DECAF and DIFEQ groups weighed 300 ± 7, 302 ± 7, and 301 ± 7 g, respectively. Hematocrit did not differ between rats at the start (43 ± 2%, $t = -60$ min) or end of the experimental protocol (39 ± 1%, $t = 60$ min). Arterial plasma glucose levels were stable and did not differ between groups at any time point (**Fig 1***A*). In addition, no differences in plasma insulin or NEFA were apparent among the groups (Fig 1*B*, C). From baseline to t = 0 min, glucose infusions rates (GIR) were similar for all groups (**Fig 2**). After the saline infusion, DECAF, or DIFEQ at $t = 0$ min, there was no change in GIR for the saline and DECAF groups. In comparison, an increase in GIR occurred in the DIFEQ group from $t = 10$ to 30 min until a plateau was reached at $t = 30$ min; the mean GIR was 14.9 ± 0.5 mg/(kg · min). Results of the R_g measurements are depicted in **Table 1**. There were no differences within or between groups for either skeletal and cardiac muscle, indicating that neither DECAF nor DIFEQ administration affected insulin-stimulated glucose uptake in these tissues. In addition, no differences were apparent in heart rates (bpm), systolic, diastolic, and mean arterial blood pressures (mm Hg) with time or treatment (data not shown). Plasma ALT, an indicator of liver dysfunction was measured at the start ($t = -60$ min) and end ($t = 60$ min) of each experiment. Values were $< 1.0 \times 10^{-6}$ U/L for all samples and no differences occurred with time or treatment (data not shown).

DISCUSSION

The aim of the present study was to examine the effects of DECAF and DIFEQ on whole-body insulin action. Using chronically catheterized, conscious rats we demonstrated that ingestion of DIFEQ, but not DECAF enhanced GIR during the euglycemic-hyperinsulemic clamp compared with controls. To establish the contribution of skeletal muscle to the DIFEQ-induced increase in whole-body insulin action, [^{3}H]DG was administered to obtain an index of skeletal muscle uptake, R_g. Examination of soleus, gastrocnemius, superficial vastus lateralis and cardiac muscles showed no increase in R_g and thus no alteration in skeletal muscle glucose uptake with DIFEQ or DECAF treatment.

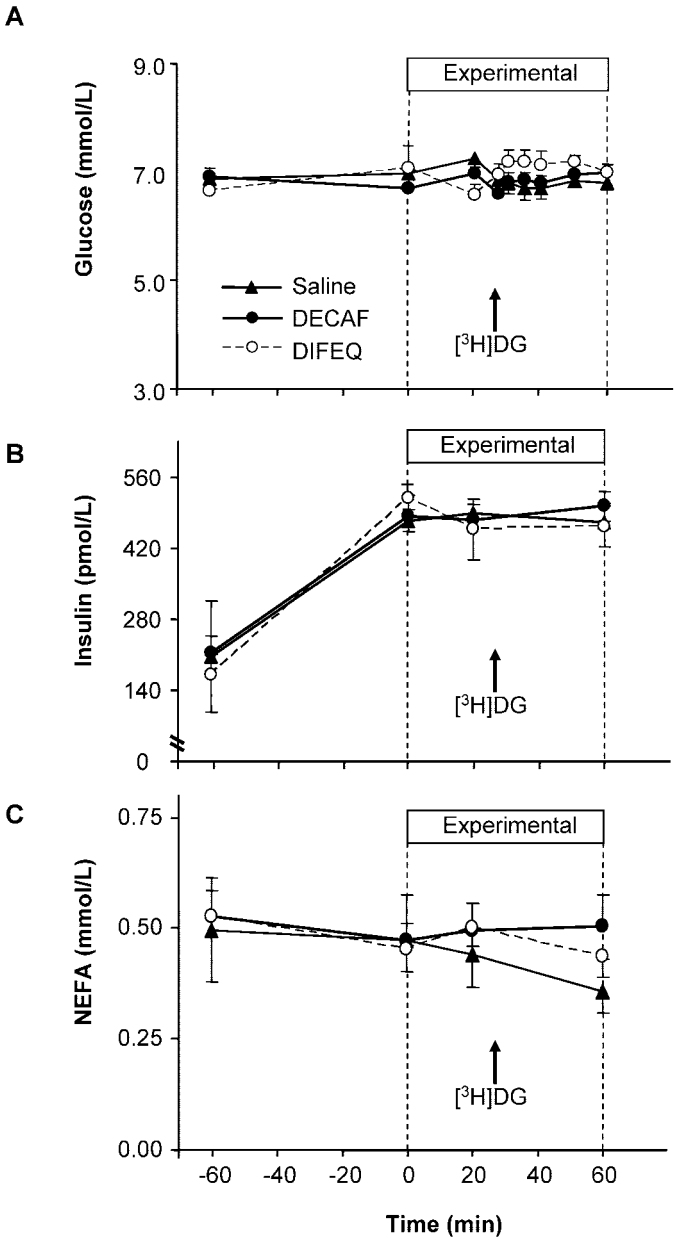

FIGURE 1 Arterial plasma measurements of (*A*) glucose (*B*) insulin and (*C*) nonesterified fatty acids (NEFA) during saline, decaffeinated coffee (DECAF) and 3,4-diferuloyl-1,5-quinide (DIFEQ) treatment in rats. Values are means ± SEM, $n = 7$.

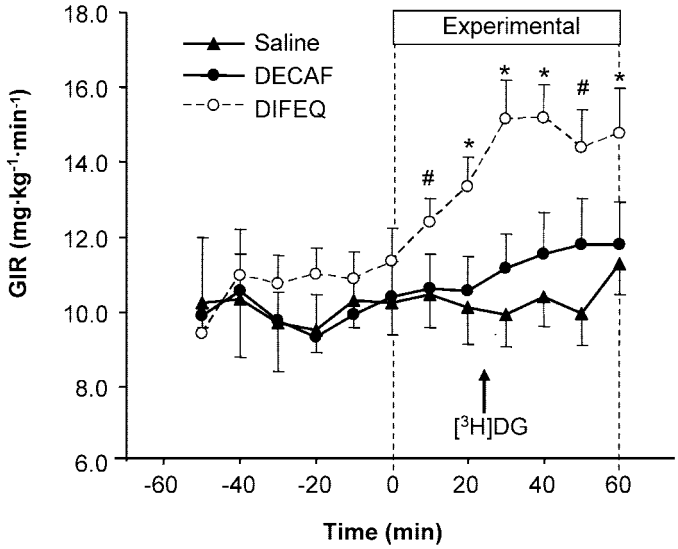

FIGURE 2 Mean intravenous glucose infusion rates (GIR) during saline, decaffeinated coffee (DECAF) and 3,4-diferuloyl-1,5-quinide (DIFEQ) treatment in rats. *Difference from both saline and DECAF; #different from saline alone. Values are means ± SEM, $n = 7$.

Because DIFEQ increased GIR without altering muscle R_g, it is reasonable to speculate that liver or adipose tissue was responsible for mediating its actions. Of these two tissues, the liver is the more likely candidate because the contribution of adipose tissue to whole-body glucose disposal is too small to account for the observed increase in GIR (17). If the liver is mediating the effects of DIFEQ or DIFEQ metabolites, then it would be expected to result in a decreased net hepatic glucose output. This effect of DIFEQ is consistent with previous studies examining chlorogenic acids showing that they inhibit liver glucose-6-phosphatase (G-6-Pase), the enzyme that catalyzes the terminal reaction of glycogenolysis and gluconeogenesis (18–21). Specifically, chlorogenic acid–derived compounds inhibit the transporting component T1 and result in concentration-dependent declines in net hepatic glucose output. Although inhibition of G-6-Pase by DIFEQ provides a plausible explanation for the present findings, further work is required to confirm the mechanism of action.

In addition to its effects on glucose homeostasis, an examination of the toxicity, absorption and timing of the actions of DIFEQ is also of interest. Because liver toxicity can result in decreased net hepatic glucose output, we assessed plasma ALT, an indicator of liver function. There was no increase in ALT in all rats, indicating that neither DECAF nor DIFEQ caused hepatotoxicity at the doses used. Additionally, DIFEQ did not adversely affect heart rate or blood pressure. Consideration of DIFEQ and DECAF absorption is also warranted. In the present study, DIFEQ administration increased GIR from both saline and DECAF after 20 min of administration. This time course was rapid and suggests that the experimental compound was quickly emptied from the stomach and absorbed in the rats. In contrast, the lack of an effect of DECAF on insulin action may be due to poor absorption of this solution. To resolve this issue, measurements of plasma metabolites of DECAF would have to be quantified after administration. Methods for measuring DIFEQ and DECAF metabolites on small volumes of plasma are not available but are currently being developed (11). Studies on chlorogenic acid indicate that it is absorbed in both rats and humans (7,22).

TABLE 1

Index of glucose uptake (R_g) in skeletal and cardiac muscle of rats treated with saline, DECAF, or DIFEQ[1,2]

Treatment	Soleus	Gastrocnemius	SVL	Heart
		μmol/(100 g · min)		
Saline	16.1 ± 3.0	1.9 ± 0.2	2.6 ± 0.3	58.6 ± 2.6
DECAF	17.8 ± 1.8	2.0 ± 0.2	2.9 ± 0.4	53.3 ± 5.0
DIFEQ	16.0 ± 2.6	1.9 ± 0.3	2.8 ± 0.5	50.4 ± 6.2

[1] Values are means ± SEM, $n = 7$.
[2] Abbreviations: DECAF, decaffeinated coffee extract; DIFEQ, 3,4-diferuloyl-1,5-quinide; SVL, superficial vastus lateralis.

Oral administration of 200 mL of coffee to humans results in rapid absorption and the appearance of the most abundant chlorogenic acid, 5-caffeoylquninic acid in plasma at >300% of baseline values 20 min postprandially. In addition, peak plasma values for the chlorogenic acid occurred at 60 min postprandially (7). This suggests that an individual consuming 6 or more cups of coffee per day would have a sustained elevation of chlorogenic acids and their derivatives. This may explain in part how heavy, long-term coffee consumption is preventative in the development of type 2 diabetes (2).

Although the present results do provide a potential mechanism by which coffee exerts its beneficial effects, further work is required to confirm this hypothesis. It must also be noted that the concentration of quinides in brewed coffee is relatively small (3 g/100 g) and varies depending on the type of coffee, roasting degree and method of preparation. Given this, it may be that the acute effects of quinides on insulin action are small and undetectable; however, when consumed at regular intervals (6 cups/d) over a long time period (20 y), the cumulative effect may be preventative in the development of glucose intolerance. In addition, other compounds in coffee, such as caffeine, lipids and minerals may be contributing to its long-term effects. Although coffee may be beneficial in preventing type 2 diabetes, there is no question that caffeine results in decreased glucose tolerance (3,5). Furthermore, coffee also possesses antioxidant properties (23) that may affect diabetes development as well as its progression (24).

If coffee, with or without caffeine, alters glucose homeostasis, then its consumption may be an important consideration in diabetes management. For example, the insulin dosage required for a given carbohydrate load may differ if an individual has consumed a large amount of coffee before the meal. Chronic coffee consumption and quinide administration may be of long-term benefit to type 2 diabetes if these compounds act to increase hepatic insulin sensitivity because studies have shown that this may be impaired in type 2 diabetes (25,26). In summary, the administration of a DIFEQ increased whole-body insulin action. This increase was not due to augmentation of skeletal muscle glucose uptake, and it is hypothesized that liver is the site at which DIFEQ acts to improve insulin action. These results support previous findings suggesting that coffee contains biologically active compounds other than caffeine that alter glucose metabolism.

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