Chlorogenic acid differentially affects postprandial glucose and glucose-dependent insulinotrophic polypeptide response in rats

Jasmine M. Tunnicliffe, Lindsay K. Eller, Raylene A. Reimer, Dustin S. Hittel, and Jane Shearer

Abstract: Regular coffee consumption significantly lowers the risk of type 2 diabetes (T2D). Coffee contains thousands of compounds; however, the specific component(s) responsible for this reduced risk is unknown. Chlorogenic acids (CGA) found in brewed coffee inhibit intestinal glucose uptake in vitro. The objective of this study was to elucidate the mechanisms by which CGA acts to mediate blood glucose response in vivo. Conscious, unrestrained, male Sprague–Dawley rats were chronically catheterized and gavage-fed a standardized meal (59% carbohydrate, 25% fat, 12% protein), administered with or without CGA (120 mg·kg\(^{-1}\)), in a randomized crossover design separated by a 3-day washout period. Acetaminophen was co-administered to assess the effects of CGA on gastric emptying. The incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) were measured. GLP-1 response in the presence of glucose and CGA was further examined, using the human colon cell line NCI-H716. Total area under the curve (AUC) for blood glucose was significantly attenuated in rats fed CGA (\(p < 0.05\)). Despite this, no differences in plasma insulin or nonesterified fatty acids were observed, and gastric emptying was not altered. Plasma GIP response was blunted in rats fed CGA, with a lower peak concentration and AUC up to 180 min postprandially (\(p < 0.05\)). There were no changes in GLP-1 secretion in either the in vivo or in vitro study. In conclusion, CGA treatment resulted in beneficial effects on blood glucose response, with alterations seen in GIP concentrations. Given the widespread consumption and availability of coffee, CGA may be a viable prevention tool for T2D.

Key words: Coffee, chlorogenic acid, blood glucose, type 2 diabetes, GIP, GLP-1, incretins.

Résumé : La consommation régulière de café diminue significativement le risque de diabète de type 2 (T2D). On ne connaît pas le constituant spécifique responsable de la diminution du risque parmi les milliers de constituants contenus dans le café. L’acide chlorogénique (CGA) présent dans le café infusé inhibe la captation intestinale du glucose in vitro. Cette étude se propose d’élucider le mécanisme par lequel le CGA intervient dans la réponse du glucose sanguin in vivo. On insère de façon chronique un cathéter à des rats Sprague–Dawley conscients et sans restriction puis on leur donne par gavage un repas standard (59 % de sucre, 25 % de gras, 12 % de protéines), avec ou sans CGA (120 mg·kg\(^{-1}\)), en se conformant à un devis aléatoire contrebalancé : entre les traitements, on compte 3 jours d’élimination. En même temps, on administre de l’acétaminophène afin d’évaluer les effets du CGA sur la vidange gastrique. On évalue les concentrations des incrétines GLP-1 (peptide-1 semblable au glucagon) et GIP (peptide insulinotrope dépendant du glucose). On étudie plus à fond la réponse du GLP-1 en présence du glucose et de CGA par l’analyse de la lignée cellulaire NCI-H716. Chez les rats ayant reçu du CGA, on observe une diminution significative de la surface totale sous la courbe (AUC) de glucose (\(p < 0.05\)). Toutefois, on n’observe aucune différence des concentrations plasmatiques d’insuline et d’acides gras libres; la vidange gastrique n’est pas modifiée. La réponse plasmatique du GIP est émoussée chez les rats ayant reçu du CGA; jusqu’à 180 min après le repas, on observe une concentration de pointe plus faible et une plus petite AUC (\(p < 0.05\)). On n’observe aucune variation de la sécrétion de GLP-1 dans les conditions in vivo et in vitro de l’étude. En conclusion, le traitement au CGA procure des effets bénéfiques sur la réponse sanguine du glucose et sur les modifications des concentrations de GIP. Du fait de la consommation généralisée et de la disponibilité du café, le CGA apparaît comme un outil de prévention viable du T2D.

Mots-clés : café, acide chlorogénique, glucose sanguin, diabète de type 2, GIP, GLP-1, incrétines.

[Traduit par la Rédaction]
Introduction

Type 2 diabetes (T2D) is rapidly rising in prevalence. Characterized by either inadequate insulin production or the inability to utilize insulin produced, T2D results in elevated blood glucose levels (Reaven 1993). Epidemiological studies show decaffeinated and caffeinated coffee consumption to be correlated to reduced risk of T2D in a dose-dependent manner (Higdon and Frei 2006; Salazar-Martinez et al. 2004; Soriguer et al. 2004; Yamaji et al. 2004). A systematic review by van Dam and Hu (2005) found relative risk reductions of 0.65 ± 0.12 (95% confidence interval) with ≥6 cups per day. Despite convincing epidemiological evidence, the specific coffee component(s) and physiological mechanisms responsible for these anti-diabetic effects are unclear. Because caffeine, ingested either alone or as a part of coffee, acutely impairs insulin sensitivity, a component of coffee other than caffeine is likely responsible (Battram et al. 2006; Graham et al. 2001; Greer et al. 2001; van Dijk et al. 2009).

Chlorogenic acids (CGA) are the main antioxidants in brewed coffee, and are found in concentrations similar to caffeine (Clifford 1999). Upon ingestion, small amounts of CGA are absorbed intact from the stomach and small intestine, with the remainder undergoing hydrolysis and subsequent absorption in the colon (Lafay et al. 2006a, 2006b; Olthof et al. 2003; Renouf et al. 2010). CGA have a prolonged presence in the intestine, with only 43% of orally administered CGA being recovered intact from the small intestine of rats 1 h after ingestion (Azuma et al. 2000). Consumption of glucose together with coffee (Battram et al. 2006; Johnston et al. 2003; Shearer et al. 2007), CGA (Bassoli et al. 2008), or CGA-enriched coffee (Thom 2007) reduces the rise in blood glucose concentration, potentially explaining the anti-diabetic effects of coffee. Of interest in this study, the timing and macronutrient composition of a meal may also play a role in the protective effects of coffee and CGA. Sartorelli and colleagues (2010) recently found the greatest benefits of coffee on T2D when individuals consumed coffee at lunch, often the largest meal of the day.

Although the exact physiological mechanisms by which coffee and CGA lower blood glucose are not known, studies have shown that these compounds reduce intestinal glucose absorption and inhibit hepatic glucose output in vitro (Rodriguez de Rotillo and Hadley 2002; Welsch et al. 1989). There is also evidence to suggest that CGA may alter the release of gastrointestinal hormones that enhance insulin secretion (Baggio and Drucker 2007; Elrick et al. 1964). Glucose-dependent insulinoëtropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) 1, known as incretins, are responsible for these anti-diabetic effects. Because of incretin, as shown in the intestine, but proximally concentrated in the jejunum and released in response to the absorption of fat, glucose, and protein (Cataland et al. 1974; Deacon 2005; Elliott et al. 1993). GIP is secreted by K-cells dispersed throughout the intestine, but proximally concentrated in the jejunum and released in response to the absorption of fat, glucose, and protein (Cataland et al. 1974; Deacon 2005; Elliott et al. 1993; Falko et al. 1975). GLP-1 is secreted from distally concentrated intestinal L-cells in response to the presence of carbohydrates, fatty acids, essential amino acids, or fiber (Baggio and Drucker 2007; Holst 2007). It would be expected that the inhibition of glucose absorption in the intestine would lower the rate of GIP secretion while increasing GLP-1, owing to sustained glucose presence in the intestine. Johnston and colleagues (2003) have shown both decaffeinated and non-decaffeinated coffee ingestion to reduce GIP, and decaffeinated coffee to enhance GLP-1 response following a 25 g oral glucose tolerance test in human subjects. Greenberg et al. (2009) also found GIP to be lower after decaffeinated coffee consumption. Coffee consumed prior to nutrient intake may not produce the same effect, as neither decaffeinated coffee nor CGA consumed 30 min before an oral glucose tolerance test altered incretin response (Olthof et al. 2011). As such, differential incretin response has been proposed as a mechanism by which coffee consumption lowers glucose response, and thus T2D risk (McCarty 2005).

To gain insight into the protective effects of CGA on glucose control, the objective of this study was to determine the effects of CGA consumed with a mixed meal. Novel findings show CGA to have no effect on the rate of gastric emptying, but to profoundly influence GIP release. These results are independent from those of plasma insulin and fatty acid levels, which did not change with CGA administration. Additionally, the ability of CGA to inhibit glucose absorption, and thereby alter GLP-1 response in vitro, was further explored using the human colon cell line NCI-H716. GLP-1 was not found to be affected by CGA, either in vivo or in vitro.

Materials and methods

Animals

Procedures were approved by the University of Calgary Animal Care and Use Committee (protocol No. BI 2008-42), and abide by the Canadian Association for Laboratory Animal Science guidelines for animal experimentation. Male Sprague–Dawley rats, weighing 260–300 g, were housed individually in a temperature-controlled room with a 12 h light / 12 h dark cycle. Rats were given ad libitum access to drinking water, and were maintained on a standard diet (5001 Laboratory Rodent Diet) 234.0 g·kg

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Table 1. Oral gavage composition.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/100 g⁻¹</th>
<th>% kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>32.41</td>
<td>29.40</td>
</tr>
<tr>
<td>Sucrose</td>
<td>29.79</td>
<td>30.03</td>
</tr>
<tr>
<td>Protein (casein)</td>
<td>13.58</td>
<td>12.32</td>
</tr>
<tr>
<td>Lard</td>
<td>4.85</td>
<td>11.00</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>6.79</td>
<td>15.40</td>
</tr>
<tr>
<td>Dyetrose</td>
<td>4.85</td>
<td>0.00</td>
</tr>
<tr>
<td>AIN-93M Mineral Mix</td>
<td>3.39</td>
<td>0.72</td>
</tr>
<tr>
<td>AIN-93-VX Vitamin Mix</td>
<td>0.97</td>
<td>0.95</td>
</tr>
<tr>
<td>L-cystine</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.19</td>
<td>0.00</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>3.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Note: On each experiment day, oral gavage dose was calculated (4 g·kg⁻¹ BW) for the meal tolerance test. On the randomly assigned treatment day, 120 mg·kg⁻¹ BW chlorogenic acid (CGA) was added to the gavage mixture.

Experimental protocol

Experiments were performed in a crossover design, with animals randomly assigned to initial placebo or CGA treatment. Experiments were separated by a 3-day washout period. Rats were fasted overnight (12 h) with ad libitum access to water. The arterial catheter was connected to additional PE50 tubing to allow for blood collection, and was flushed with heparinized saline (10 U heparin·mL⁻¹) between blood draws to prevent clotting. Baseline blood was collected ~5 min before oral gavage. On each experiment day, rats were weighed and a meal dose (4 g·kg⁻¹ body weight (BW)) was calculated for the meal tolerance test. The oral gavage consisted of 59% carbohydrate, 25% fat, and 12% protein (Table 1), with a dry food (1 g)/water ratio of 1.5 mL. Acetaminophen (124 mg·kg⁻¹ BW) was added to the mixture to track the rate of gastric emptying, as it is minimally absorbed in the stomach and rapidly absorbed in the small intestine, and does not require the removal of the stomach for analysis (Hatanaka et al. 1994; Porsgaard et al. 2003). Blood was collected at 15, 30, 45, 60, 90, 120, and 180 min after meal ingestion. For animals receiving CGA, the meal contained 120 mg·kg⁻¹ BW CGA (Sigma–Aldrich Canada, Oakville, Ont., Canada).

Plasma measurements

At each time point, blood glucose was measured with a whole blood glucose monitor (OneTouch Ultra 2, Milpitas, Calif., USA). Additional samples were collected in a chilled EDTA-coated tube, containing 1 µL Diprotin A, a dipeptidyl peptidase-IV inhibitor to prevent GLP-1 and GIP degradation (Balkan et al. 1999). Immediately after collection, plasma was separated by centrifugation and stored at −80 °C until analysis. Analysis of insulin and total GIP was carried out using the Rat Gut Hormone Panel LINCOples Kit (Millipore Corporation, Billerica, Mass., USA). Samples of plasma (12.5 µL) were analyzed using antibody-immobilized beads specific for these 2 hormones. Analysis of hormone concentration was determined using Luminex100 (Luminex Corporation Inc., Austin, Tex.). Active GLP-1 was quantified using the Glucagon-Like Peptide-1 (Active) ELISA kit (Millipore Corporation). Nonesterified free fatty acids (NEFA) were measured using HR Series NEFA-HR (2) kit (Wako, Chuo-Ku, Osaka, Japan). Acetaminophen levels were determined using Acetaminophen-SL Assay (Diagnostic Chemicals Limited, Charlottetown, P.E.I., Canada).

Cell model

The NCI-H716 cell line is an enteroendocrine L-cell line derived from a poorly differentiated human colon adenocarcinoma; it has been shown to secrete GLP-1 in a regulated manner (Purk et al. 1987). Methods were adapted from Reimer and colleagues (2001). Briefly, identical cell culture conditions and GLP-1 secretion study techniques were utilized.

Cell culture

Cells were grown in suspension in RPMI 1640 medium (Life Technologies, Carlsbad, Calif., USA), supplemented with 10% fetal bovine serum (Life Technologies), 2 mmol·L⁻¹ L-glutamine (Life Technologies), 100 IU·mL⁻¹ penicillin (Life Technologies), and 100 µg·mL⁻¹ streptomycin (Life Technologies). Media mixture was added every 3–4 days until the desired cell density was reached, then every 7 days for proliferation maintenance.

Secretion study and analysis

Two days prior to the experiments, 1 × 10⁶ cells were seeded into 12-well plates coated with Matrigel (Becton Dickinson, Franklin Lakes, N.J., USA) to induce endocrine differentiation for GLP-1 secretion (de Bruïne et al. 1993). Media was simultaneously changed to low-glucose Dulbecco’s modified Eagle’s medium (Life Technologies), supplemented with 10% fetal bovine serum, 2 mmol·L⁻¹ L-glutamine, 100 IU·mL⁻¹ penicillin, and 100 µg·mL⁻¹ streptomycin, as described above. Cells were washed, and the differentiation media was replaced with Krebs–Ringer bicarbonate buffer, containing 0.2% bovine serum albumin. CGA was added to the Krebs–Ringer bicarbonate buffer solution, and the pH was adjusted to 7.2 with NaOH. Cells were incubated in six 12-well plates, with treatments in duplicate, of control (no test agents, no glucose), positive control (2% meat hydrolysate), and either 100 µmol·L⁻¹ 5-CGA with various concentrations of glucose or 5% glucose with various concentrations of 5-CGA. After the 2 h incubation period, supernatants and cells were collected separately. Supernatants were removed, and 50 µg·mL⁻¹ anti-protease phenylmethylsulfonyl fluoride (Sigma), with 2 µL·mL⁻¹ DPP-IV inhibitor Diprotin A (Calbiochem, Gibbstown, N.J.), was added to the collection to prevent GLP-1 degradation. Cells were scraped with lysis buffer, containing 50 µg·mL⁻¹ phenylmethylsulfonyl fluoride and 2 µL·mL⁻¹ Diprotin A. Both cells and supernatants were stored at −80 °C until analysis. GLP-1 was measured using the GLP-1 (7–36) Active RIA kit (Linco Research, St. Charles, Mo., USA) assay after 10-fold dilution of both cells and supernatants.

Statistical analysis

In each of the CGA and no-CGA treatments, differences between baseline body mass and blood glucose, GIP, GLP-1, insulin, NEFA, and acetaminophen were calculated by subtracting baseline values from the intervention values (SigmaStat for Windows, version 3.5, San Jose, Calif., USA). Total area under the curve (AUC) was calculated using the trape-
Table 2. Baseline characteristics of rats fed placebo or CGA.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo</th>
<th>CGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>266.80±4.90</td>
<td>264.90±4.70</td>
</tr>
<tr>
<td>Fasting NEFA, mmol·L⁻¹</td>
<td>0.65±0.04</td>
<td>0.59±0.04</td>
</tr>
<tr>
<td>Fasting glucose, mmol·L⁻¹</td>
<td>5.46±0.24</td>
<td>5.18±0.19</td>
</tr>
<tr>
<td>Fasting insulin, pg·mL⁻¹</td>
<td>347.50±47.30</td>
<td>364.40±73.40</td>
</tr>
<tr>
<td>Fasting GIP, pg·mL⁻¹</td>
<td>51.70±8.30</td>
<td>60.00±4.10</td>
</tr>
<tr>
<td>Fasting GLP-1, pmol·L⁻¹</td>
<td>6.06±1.02</td>
<td>5.64±0.93</td>
</tr>
</tbody>
</table>

Note: Measurements were made following a 12 h overnight fast. All blood samples were obtained from the arterial blood catheter. Data represent means ± SE, n = 12. There were no significant differences. GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; NEFA, nonesterified free fatty acids.

zoidal method. Comparison of results for individual time points and AUC were done using paired t tests. Differences between treatments in the NCI-H716 cell line were determined with 1-way analysis of variance. Differences with p < 0.05 were considered significant. Data are expressed as means ± SE.

Results

In vivo rat feeding study

Baseline measurements

There were no significant differences in baseline blood glucose, plasma insulin, GIP, GLP-1, or NEFA between the CGA and placebo groups, or between the values for each of the experiment days (Table 2).

Blood glucose

Blood glucose rapidly increased from fasting levels with both placebo and CGA treatments following gavage, and concentrations were significantly higher at 15 min in both groups (p < 0.001) (Fig. 1). However, blood glucose concentration in rats was significantly lower at 60 min (p = 0.024) in the CGA group than in the placebo group. Blood glucose levels appeared to be attenuated over the course of the experiment for rats given CGA treatment. AUC was significantly lower in the CGA group than in the placebo group (p = 0.021) (Table 3). Blood glucose did not return to baseline in either CGA- or placebo-treated rats 180 min after meal gavage (p < 0.001).

Incretins

Plasma GIP increased above baseline following gavage in both groups, with a maximum response occurring at 60 min in placebo-treated rats, compared with 45 min in CGA-treated rats (Fig. 2A). Ingesting CGA in a mixed-meal gavage resulted in significantly reduced GIP levels at 30 min (p = 0.036) and 60 min (p = 0.006). There was a significantly lower GIP AUC (p = 0.029) in the CGA group over 180 min than in the placebo group (Table 3). Plasma GIP concentrations returned to baseline after 180 min in both groups. GLP-1 concentrations decreased shortly after meal ingestion in both groups, then increased again between 45 and 90 min (Fig. 2C). Despite attenuated secretion of GIP with CGA, there were no statistically significant differences in GLP-1 between groups at any time point, or in the AUC over 180 min (Table 3).

Insulin and NEFA

There was a rapid rise in plasma insulin in both CGA- and placebo-fed rats following mixed-meal administration (Fig. 2B). Insulin concentration peaked at 15 min in both treatment groups, and dropped steadily thereafter. Although the rise in blood glucose was attenuated by CGA, no corresponding declines in plasma insulin were observed between groups at any time point or for the AUC over 180 min (Table 3). Although blood glucose concentrations did not return to baseline after 180 min, plasma insulin reached a plateau 45 min after meal ingestion. NEFA concentration did not change after oral gavage in either treatment group (Fig. 2D). In the fasted state, NEFA concentrations are typically elevated, as glucose stores are depleted. Postprandially, endogenous fatty acids decrease as circulating blood glucose increases. Despite changes in glucose, the blood levels of NEFA we saw were not affected by CGA treatment (Table 3).

Gastric emptying

Gastric emptying was assessed with the administration and subsequent appearance in the blood of acetaminophen. Plasma levels of acetaminophen peaked 15–30 min after the meal gavage, and steadily decreased thereafter in both the CGA and placebo treatment groups (Fig. 3). Although several factors can affect gastric emptying, including GLP-1 secretion, no significant differences in the concentration of plasma acetaminophen were observed over the 180 min of study (p = 0.667). The lack of changes in gastric emptying between the groups suggests that any differences observed in the blood measures of interest can be attributed to the ab-
Table 3. Total area under the curve (AUC) for blood glucose, plasma insulin, NEFA, GIP, GLP-1, and acetaminophen in rats fed placebo or CGA in a mixed meal.

<table>
<thead>
<tr>
<th></th>
<th>Placebo AUC</th>
<th>CGA AUC</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose, mmol·L⁻¹ × 3 h</td>
<td>1360.5±25.9</td>
<td>1255.6±28.0</td>
<td>0.021</td>
</tr>
<tr>
<td>Plasma insulin, pg·mL⁻¹ × 3 h</td>
<td>91 298.0±10 308.0</td>
<td>94 932.0±15 004.0</td>
<td>&gt;0.050</td>
</tr>
<tr>
<td>Plasma NEFA, mmol·L⁻¹ × 3 h</td>
<td>125.2±11.3</td>
<td>125.3±16.2</td>
<td>&gt;0.050</td>
</tr>
<tr>
<td>Plasma GIP, pg·mL⁻¹ × 3 h</td>
<td>30 510.0±3683.0</td>
<td>20 678.0±2694.0</td>
<td>0.029</td>
</tr>
<tr>
<td>Plasma GLP-1, pmol·L⁻¹ × 3 h</td>
<td>1015.0±79.1</td>
<td>948.8±119.3</td>
<td>&gt;0.050</td>
</tr>
<tr>
<td>Plasma acetaminophen, μg·mL⁻¹ × 3 h</td>
<td>3400.5±429.1</td>
<td>3173.3±541.8</td>
<td>0.667</td>
</tr>
</tbody>
</table>

Note: Data represent mean ± SE, n = 12. Differences are considered statistically significant if p < 0.05.

Fig. 2. Plasma glucose-dependent insulintropic peptide (GIP), glucagon-like peptide-1 (GLP-1), insulin, and nonesterified free fatty acid (NEFA) in rats fed placebo or CGA with a mixed meal. (A) Plasma GIP response; (B) plasma insulin response; (C) plasma GLP-1 response; (D) plasma NEFA response. Results represent the mean ± SE, n = 12. *, Difference between placebo and CGA at 30 min (p = 0.036) and 60 min (p = 0.006).
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presence or presence of CGA, rather than to alterations in nutrient arrival to the gut.

**NCI-H716 in vitro study**

**GLP-1 secretion**

This experiment indirectly explored the ability of CGA to inhibit glucose uptake by measuring GLP-1 secretion without the confounding effect of altered GIP levels. Meat hydrolysate was used as a positive control, as it has previously been shown to promote GLP-1 secretion (Reimer et al. 2001). Our results corroborate this finding; meat hydrolysate significantly increased GLP-1 secretion, compared with control ($p < 0.05$). CGA had no effect on GLP-1 secretion, regardless of the concentration tested or the amount of glucose present (Figs. 4A and B).

**Discussion**

Although the protective effect of coffee on the development of T2D is well established, the mechanism of action has not been clarified. This study examined the role of CGA, the main polyphenol found in coffee, in altering glucose concentration and incretin response. Employing a conscious animal model, we show that oral CGA (120 mg·kg$^{-1}$) intake in combination with a mixed meal has a negligible impact on the rate of gastric emptying, attenuates postprandial blood glucose levels with no corresponding decrease in insulin response, and alters GIP but not GLP-1 secretion in rats. Additionally, we found no alteration in GLP-1 release in NCI-H716 colon cells incubated with CGA with or without glucose.

To understand the mechanisms by which CGA mediates blood glucose response, gastric emptying was evaluated. The possibility that CGA delays the rate of gastric emptying has not been considered in previous studies. In addition, data on polyphenolic intake and gastric emptying are scarce, with one study finding that ferulic acid, another coffee-related compound, increases the rate of gastric emptying and gastrointestinal transit time (Badary et al. 2006). Acetaminophen administration allows for indirect measure of gastric emptying, as it is not absorbed in the stomach but is rapidly absorbed in the small intestine (Hatanaka et al. 1994; Porsgaard et al. 2003). Thus, novel findings from this study show that CGA does not alter the rate of gastric emptying when consumed with a meal.

Despite no differences in gastric emptying, we found that acute CGA consumption lowers the postprandial rise in blood glucose concentration, compared with placebo treatment. Although blood glucose can be affected by several factors, including glucose production in the liver and muscle uptake, absorption of intact CGA is very low, with effects on muscle and (or) liver from an acute dose likely to be insignificant. In this experiment, the results are most likely due to a reduction in the rate of glucose absorption from a standardized meal, which was composed of rapidly digestible carbohydrates. Thus, our results show that CGA may have effectively lowered the glycemic index of the meal administered. A low-glycemic diet is one of the recommendations to prevent or treat T2D, as it helps prevent chronic hyperglycemia (Brand-Miller et al. 2003; Neff 2003). It is possible that the chronic intake of CGA lowers glucose absorption when consumed with or close to a meal, reducing the risk of T2D. To examine the mechanism by which CGA administration lowers blood glucose response, the incretin hormones GIP and GLP-1 were assessed. Previous work on the impact of coffee on these hormones in humans has shown variable results (Greenberg et al. 2009; Johnston et al. 2003; Olthof et al. 2011). The lack of consistent findings may stem from individual variability, a factor that was minimized with the use of an isolated coffee component in an animal model in the our study.

We observed a large reduction in GIP secretion with CGA administration. Conversely, no differences were observed for GLP-1. The reduction in GIP may be one factor that explains, in part, the attenuated blood glucose rise observed in our study with CGA administration. Johnston et al. (2003) also noted a decrease in GIP and blood glucose following decaffeinated coffee consumption by human subjects, compared with controls, following an oral glucose tolerance test. However, a subsequent study administering decaffeinated coffee failed to observe differential changes in glucose and insulin levels, compared with placebo, despite a lower total GIP response (Greenberg et al. 2009). Additionally, the results of this study show no effect of GIP on NEFA levels following a meal. It has been speculated that GIP plays a role in postprandial regulation of triglycerides via adipocytes; however, no direct effects of GIP on NEFA concentration have been found in humans (Asmar et al. 2010).

Results of incretin analysis demonstrated an expected decrease in GIP secretion, whereas no increase in GLP-1 secretion with CGA administration was observed. GLP-1 production and secretion is chiefly located in distal segments of the gut, as opposed to the more proximal production of GIP (Baggio and Drucker 2007). Although reduced glucose absorption in the proximal gut could result in increased glu-
Cose presence further down in the lumen, nearly all sugars are absorbed in the upper small intestine (Wright et al. 2003). An increase in glucose presence in the duodenum and jejunum may, therefore, not cause a corresponding increase in GLP-1. Additionally, GIP stimulates GLP-1 secretion (Roberge and Brubaker 1993); therefore, a reduction in GIP secretion following CGA treatment could counteract any potential increase in GLP-1. To further explore the role of GLP-1 with CGA, a cell line was employed. NCI-H716 cells incubated with CGA showed no alteration in GLP-1 secretion, even among different glucose concentrations. This is in contrast to previous work, which showed that the addition of 3% and 10% glucose resulted in an approximately 1.5- and 2.7-fold increase, respectively, in GLP-1 secretion, compared with controls (Jang et al. 2007). The results indicate that, at the concentrations studied, intact CGA may be unable to alter glucose uptake in GLP-1-secreting cells. Although Welsch et al. (1989) found a significant decrease in glucose uptake with $10^{-3}$ mol·L$^{-1}$ 5-CGA, they used rat membrane vesicles, which contain absorptive and enteroendocrine cells. Thus, it may be that CGA affects glucose uptake in absorptive cells, rather than the GLP-1-secreting L-cells, although we did not see changes in GLP-1 secretion in our in vivo study.

Unexpectedly, we could not attribute differences in rat blood glucose with CGA treatment to changes in plasma insulin. No differences in insulin were observed between CGA and placebo treatment, suggesting that CGA does not directly alter insulin secretion. This result substantiates previous work in humans, which showed no differences in insulin AUC after caffeinated or decaffeinated coffee consumption, despite differences in GIP levels (Johnston et al. 2003). In contrast, the recent finding that decaffeinated coffee intake was positively associated with acute insulin response was not supported by our results; however, that was a cross-sectional study in which neither volume nor timing of coffee ingestion was recorded (Loopstra-Masters et al. 2011). Insulin is secreted by pancreatic β-cells in response to elevated blood glucose; incretins are thought to be responsible for 50%–70% of the insulin response following a meal (Baggio and Drucker 2007; Nauck et al. 1993). The relative contributions of GIP and GLP-1 to the stimulation of insulin secretion are debated, although it is clear that they each contribute (Elahi et al. 1994; Fehmann et al. 1989; Siegel et al. 1992). GIP circulates in higher concentrations than GLP-1, yet GLP-1 appears to be a more potent stimulator (Nauck et al. 1993; Vilsbøll et al. 2001). In our study, GLP-1 secretions were not altered with CGA treatment; however, GIP was significantly reduced.

The in vivo study employed a conscious, unrestrained animal model for blood collection. This allowed for more physiologically relevant data, as stress is known to alter insulin, glucose, and free fatty acid concentrations (Cyr et al. 2007; Remage-Healey and Romero 2001; Sapolsky et al. 2000). Additionally, we used a mixed-meal gavage, as opposed to an oral glucose tolerance test, as humans typically eat meals composed of several food groups, not single nutrients, such as caffeine and sugar.
as glucose. Although coffee consumption may not always be accompanied by a meal, CGA remains in the small intestine for up to 6 h after administration (Azuma et al. 2000). A dose comparable to that expected from usual daily coffee consumption in humans is 500–1000 mg·day⁻¹, or 7–20 mg·kg⁻¹·day⁻¹ (Clifford 1999). However, studies looking at CGA absorption and bioavailability in rats have used much higher doses, from ~25 to 285 mg·kg⁻¹·day⁻¹ (Azuma et al. 2000; Dupas et al. 2006; Gonthier et al. 2003). For this project, a median dose of 120 mg·kg⁻¹ was used to ensure that enough of the compound was present to have an acute effect on glucose absorption. Despite our positive findings, showing that a major constituent of coffee protects against hyperglycemia, there is evidence that caffeine, caffeinated coffee, and decaffeinated coffee impair whole-body insulin sensitivity (Graham et al. 2001; Greenberg et al. 2009). Additionally, results from chronic intake may differ from those found acutely, and timing of intake may be a confounder (Johnston et al. 2003; Loopstra-Masters et al. 2011; Olthof et al. 2011). These contradictions highlight the need for further study in this area.

In conclusion, CGA may have a protective effect on elevated blood glucose and reduce GIP secretion by slowing the rate of glucose appearance from the intestine into the circulation. Indeed, CGA may reduce the glycemic index of foods, especially if ingested prior to or with a meal. Since coffee is the main source of CGA, this compound may explain the risk reduction for T2D seen in coffee drinkers.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Lin Su, Olivia T. Brussels, and Megan C. Hallam. Funding: J.S. holds salary support awards from the Alberta Heritage Foundation for Medical Research, the Heart and Stroke Foundation of Canada, and the Canadian Diabetes Association. Author disclosures: The authors declare no conflicts of interest. Author contributions: J.M.T. and J.S. designed the research and wrote the paper. L.K.E. assisted with the surgeries and biochemical analysis. R.A.R. assisted with the incretin analysis and edited the manuscript. D.S.H. provided technical support and manuscript editing. J.S. had primary responsibility for the final content. All authors read and approved the final manuscript.

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