Study on the Postprandial Glucose Responses to a Chlorogenic Acid-Rich Extract of Decaffeinated Green Coffee Beans in Rats and Healthy Human Subjects

Kazuya Iwai1*, Yusaku Narita1, Taiji Fukunaga1, Osamu Nakagiri1, Tomoyasu Kamiya2, Motoya Ikeuchi2 and Yoshiaki Kikuchi3

1 R&D Center, UCC Ueshima Coffee Co. Ltd., 3-1-4 Zushi, Takatsuki-shi, Osaka 569-0036, Japan
2 Research and Development Division, Toyo Shinyaku Co. Ltd., 7-28 Yayoioka, Tosu, Saga 841-0005, Japan
3 Medical Corporation Shinanokai Samoncho Clinic, 6F Yotsuya Medical Building, 20 Samoncho, Shinjuku-ku, Tokyo 160-0017, Japan

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The objective of this investigation was to assess the antihyperglycemic effects of an extract of decaffeinated green coffee beans (EDGCB). Despite the potential properties attributed to this compound, little is known about its action in vivo. In rats, EDGCB significantly decreased postprandial blood glucose levels when administered with each of the 4 carbohydrate types (sucrose, maltose, glucose, and soluble starch). An optimum effect was observed when EDGCB was administered at the beginning of the carbohydrate challenge. In the clinical trial, plasma glucose levels were significantly reduced by administering doses of 100 and 300 mg EDGCB after ingestion of 200 g carbohydrate, particularly in subjects with a high glycemic response. No significant differences were observed in plasma insulin profiles, however, over the course of the experiment.

Keywords: coffee, chlorogenic acid, postprandial hyperglycemia, blood glucose

Abbreviations: AUC, area under the blood concentration-time curve; CQAs, caffeoylquinic acids; FQAs, feruloylquinic acids; diCQAs, dicaffeoylquinic acids; EDGCB, extract of decaffeinate green coffee beans; HPLC, high-performance liquid chromatography; IC50, half maximal inhibitory concentration; OGTT, oral glucose tolerance test; SD, Sprague Dawley

Introduction

The number of diabetic patients has been increasing, particularly in Asia, because of the prevalence of western lifestyle (Zimmet et al., 2001). In Japan, the number of people that are strongly suspected of having diabetes has increased from 6.9 to 8.9 million from 1997 to 2007, and the number of people possibly having diabetes has increased from 6.8 to 13.2 million during the same period (Wild et al., 2004; Kawamori, 2002). These figures are expected to rise most rapidly worldwide necessitating the consideration of management of diabetic complications as an important social issue (i).

Lee et al. (2011) reported that the prevalence of type 2 diabetes was substantially more common among Asians than among whites, whereas body mass index (BMI) was substantially lower in Asians than in whites. It is therefore postulated that impaired insulin secretion is the primary metabolic defect underlying glucose intolerance in the Japanese population (Kawamori, 2002; Kanauchi et al., 2002). Individuals who are not diabetic but have impaired insulin secretion may have serious diabetic complications in the future because they have no subjective symptoms. According to the International Diabetes Federation Guideline for Management of Post-meal Glucose (Ceriello and Colagiuri, 2008), 2-h postprandial plasma glucose levels should not exceed 7.8 mmol/L (140 mg/dL), as long as hypoglycemia is avoided. Namely, controlling postprandial plasma glucose is ultimately less expensive than treating the complications of diabetes.

There has recently been an increased interest in polyphenols, since some studies have suggested an association between the prevention of lifestyle diseases, such as type 2 diabetes, and consumption of polyphenols (Thompson et al., 1984; Bryans et al., 2007). For example, hydroxycinnamic acids, which are one of the main and most studied groups of polyphenols, are abundant in foods of plant origin and

*To whom correspondence should be addressed.
E-mail: kazuya-iwai@ucc.co.jp
are frequently found in green coffee beans (GCB). GCB are a rich source of chlorogenic acids, whose content is 3.5 – 7.5% (w/w-dry matter) in Coffea arabica and 7.0 – 14.0% (w/w-dry matter) in Coffea canephora (Ky et al., 2001). The chlorogenic acids in GCB consist of three main classes: caffeylquinic acids (CQAs) with three isomers (3-, 4-, and 5-CQA), dicaffeylquinic acids (diCQAs) with three isomers (3,4-, 3,5-, and 4,5-diCQA), and feruloylquinic acids (FQAs) with three isomers (3-, 4-, and 5-FQA). GCB contain considerable amounts of hydroxycinnamic acid derivatives (chlorogenic acids), including 4.3 – 7.2% CQAs, 0.3 – 1.2% FQAs, and 0.8 – 2.5% diCQAs (Clifford, 1985). During roasting, there is a progressive destruction and transformation of CGAs with approximately 8 – 10% being lost for every 1% loss of dry matter (Clifford, 1999).

A previous study demonstrated that chlorogenic acids are potent inhibitors of pancreatic alpha-glycosidase activity in vitro (Rohn et al., 2002; Ishikawa et al., 2007). Moreover, Welsch et al. (1989) reported that 1 mM chlorogenic acid reduced glucose uptake by approximately 80% in an in vitro brush border membrane system through dissipation of the Na⁺ gradient. Similarly, other studies have shown that chlorogenic acid or chlorogenic acid-rich coffee reduces blood glucose concentration in rats and humans, but these data were obtained from oral glucose tolerance tests (OGTTs) (van Dijk et al., 2009; Thom, 2007).

Despite the potential properties attributed to these compounds, little is known about their action in vivo. It is unclear whether chlorogenic acids suppress postprandial hyperglycemia after the intake of carbohydrates such as sucrose, maltose, starch, glucose, and other carbohydrate-rich foods. Moreover, in order to assess food as a physiological modulator, it is necessary to confirm the most effective time of administration.

The objective of this investigation was to assess the antihyperglycemic effects of an extract of decaffeinated green coffee beans (EDGCB), and its influence on postprandial glucose responses in rats and healthy human subjects.

**Materials and Methods**

**Sample preparation**

Coffee beans (Coffea canephora var. robusta), obtained from Vietnam, were used in this study. Caffeine and fat were first extracted from the ground coffee beans using a supercritical CO₂ extraction system after which DGCB were extracted with 56% (v/v) aqueous ethanol. The extracts were filtered, evaporated, and spray-dried. The chlorogenic acid content of EDGCB was determined by high-performance liquid chromatography (HPLC) analysis (Iwai et al., 2004; Narita and Inouye, 2011). In brief, the conditions were as follows: column, Inertsil ODS-3 4.6 mm i.d. × 15.0 cm (GL Sciences, Tokyo, Japan); column temperature, 35°C; mobile phase, solvents A (50 mM acetic acid in H₂O) and B (50 mM acetic acid in acetonitrile), gradient, 0 – 30.0 min, 5 – 20% (v/v) of B; 30.0 – 45.0 min, 20 – 35% (v/v) of B; 45.0 – 50.0 min, 35 – 80% (v/v) of B; 50.0 – 50.1 min, 80 – 5% (v/v) of B; 50.1 – 60 min, 5% (v/v) of B; injection volume, 10 μL; flow-rate, 1.0 mL/min; detection, UV 325-nm. The nutrient composition (carbohydrate, protein, fat, fiber, mineral, and energy), caffeine, and chlorogenic acid content of EDGCB are shown in Table 1. Nutrient analyses were performed by Japan Food Research Laboratories (Tokyo, Japan). Sucrose, maltose, and soluble starch were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**In vivo studies in Sprague Dawley (SD) rats**

All rat studies were conducted according to the Act on Welfare and Management of Animals law - Act No. 105 of October 1, 1973. Six-week-old specific pathogen-free male SD rats were purchased from Kyudo Co. Ltd. (Kumamoto, Japan) and acclimatized for 1 week in polycarbonate flat-bottomed cages. All animals were fed a laboratory diet (MF; Oriental Yeast, Tokyo, Japan) and given water ad libitum until the start of the experiment. After the acclimatization period, the rats were randomly divided into groups as required. Carbohydrate tolerance tests were conducted following 16-h food deprivation. After ingestion of carbohydrate, blood samples were collected from the tail vein and blood glucose levels were measured immediately before t = 0 and at t = 30, 60, and 120 min administration of EDGCB, using a disposable glucose sensor (Glutest Pro; Sanwa Chemical Research Co., Tokyo, Japan).

**Effects of EDGCB on glycemic response after carbohydrate (sucrose/maltose/soluble starch/glucose) administration**

**Table 1. Characterization of EDGCB.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<td>Water</td>
<td>3.1 g/100 g</td>
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<tr>
<td>Carbohydrate</td>
<td>15.2 g/100 g</td>
</tr>
<tr>
<td>Protein</td>
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<td>Fat</td>
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<tr>
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<td>3.2 g/100 g</td>
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<tr>
<td>Kahweol</td>
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<tr>
<td>Arsenic</td>
<td>&lt; 0.1 ppm</td>
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<tr>
<td>Stunnun</td>
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</tr>
<tr>
<td>Lead</td>
<td>0.07 ppm</td>
</tr>
<tr>
<td>Live bacteria</td>
<td>&lt; 300 CFU/g</td>
</tr>
<tr>
<td>Colibacillus form</td>
<td>(–)</td>
</tr>
<tr>
<td>Energy</td>
<td>14.3 kJ/g</td>
</tr>
</tbody>
</table>
tion} After acclimatization for 1 week and 16-h food deprivation, SD rats were randomly divided into 5 experimental groups; each group was comprised of 2 sets of 6 rats (n = 6 for the test sample administration and n = 6 for the control). EDGCB (500 mg/kg body weight) and carbohydrate (sucrose, maltose, soluble starch, or glucose; 2 g/kg body weight in 10 mL/kg water) were administered to groups receiving the test sample whereas the controls in each experimental group received the same volume of carbohydrate solution without EDGCB. The blank experimental group received only EDGCB (500 mg/kg in 10 mL/kg water).

Preparation of the 325-nm absorbent fraction and a nonabsorbent fraction from EDGCB  EDGCB was subjected to column chromatography to separate the samples into a 325-nm absorbent fraction and a nonabsorbent fraction. In brief, 5.81 g (dry weight basis) EDGCB was dissolved in a small amount of 0.05% (v/v) acetic acid. The solution was applied to a Toyopearl HW-40 column (290 × 20 mm), eluted with 0.05% acetic acid, 50% (v/v) methanol, and finally with 99.5% (v/v) methanol at 1.5 mL/min and scanned for absorbance at 325 nm. Furthermore, 325-nm absorbent fractions (F-2 and F-4) and nonabsorbent fractions (F-1, F-3, and F-5) were combined separately (Fig. 2). Subsequently, each fraction was concentrated under reduced pressure, then freeze-dried, and stored until use.

Effects of the 325-nm absorbent and nonabsorbent fractions on glycemic response  After an acclimatization period of 1 week and 16-h food deprivation, SD rats were randomly divided into 4 groups. Each group was comprised of a set of 6 rats (n = 6 for administration of EDGCB, n = 6 for administration of the 325-nm absorbent fraction, n = 6 for administration of the nonabsorbent fraction, and n = 6 for the control). The rats were administered sucrose (2 g/kg body weight in 10 mL/kg water) either alone or together with EDGCB (500 mg/kg body weight), the 325-nm absorbent fraction (235 mg/kg body weight) or the nonabsorbent fraction (305 mg/kg body weight). The chlorogenic acid content in the 325-nm absorbent fraction and the content of solids, with the exception of chlorogenic acids in the nonabsorbent fraction, were equivalent to those of EDGCB.

Effect of administration time  After an acclimatization period of 1 week and 16-h food deprivation, SD rats were randomly divided into 3 experimental groups. Each group was comprised of 2 sets of 6 rats (n = 6 for the EDGCB administration experiment and n = 6 for the control). A 500-mg/kg body weight dose of EDGCB (50 mg EDGCB dissolved in 1 mL distilled water) was administered orally to the EDGCB-treated group either 30 min prior to, simultaneously with or 5 min after a challenge of 2 g/kg body weight of sucrose (200 mg sucrose dissolved in 1 mL distilled water).

The control group was given 1 mL of distilled water instead of the EDGCB solution at each of the three time points. The effects of EDGCB on blood glucose levels were compared with increases observed in controls (sucrose challenge without EDGCB). Capillary blood glucose was measured prior to ingestion of EDGCB or control at t = 0, 30, 60, and 120 min after the sucrose challenge.

In vivo studies in healthy human subjects  This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board (chairperson, Tetsuo Hosoya, MD) of the Suda Clinic (Tokyo, Japan). Subject recruitment, blood sampling, blood pressure, pulse rate, body weight, height measurements, and medical examinations were carried out at the Samoncho Clinic (Tokyo, Japan: Main Investigator, Yoshiaki Kikuchi). Blood analyses were performed by Mitsubishi Chemical Medience Co. Ltd., (Tokyo, Japan). Subjects who either did not satisfy the selection criteria or met the exclusion criteria were excluded from the study. Inclusion criteria were as follows: 1) healthy men and women between 20 and 50 years of age; 2) individuals who were able to abstain from food before the experiment; 3) individuals who understood the purpose and procedures of the experiment; and 4) individuals who were considered suitable by the doctor. Exclusion criteria were as follows: 1) individuals who usually avoided breakfast; 2) individuals taking drugs that affect glucose levels; 3) individuals taking supplements that improve glucose levels; 4) individuals with serious complications or with a disease that required urgent treatment; 6) individuals with a chronic disease; 7) individuals with a digestive system disease and with a history of digestive organ surgery; 8) individuals with a medical record of drug or alcohol dependence; 9) pregnant women, women intending to become pregnant, and lactating women; 10) individuals participating or intending to participate in a drug administration test; and 11) individuals considered unsuitable by the doctor. Based on the results of recruitment, 45 subjects were selected for the study after clinical analysis.

Study schedule and method of ingestion  This study was a randomized, double-blind, placebo-controlled, single-ingestion cross-over clinical trial. The design and purpose of the study were explained to the subjects both orally and in writing. The controller, who did not directly participate in the examination, divided the subjects into 3 random groups. The tests were conducted 1 week apart, in the same time zone, with the same diet. The subjects were instructed to consume their normal quantities of food and drink, to exercise normally, and abstain from excessive eating, drinking, and exercising during the study period. All subjects were instructed
to finish dinner by 21:00 on the day before the test and drink only water between 21:00 and the test. On the test day, the subjects did not smoke 1 h before the test. The subjects ingested 2 pieces (total weight: 200 g) of Onigiri (a snack of rice formed into a triangle and wrapped in edible seaweed) together with the test beverage (either 100 or 300 mg EDGCB/200 mL water or 200 mL water alone as a control) within 10 min. The Onigiri were filled with salted salmon and salted edible kelp. The nutritional value of Onigiri was as follows: For salmon, energy, 774 kJ (185.0 kcal); carbohydrate, 38.9 g; protein, 5.2 g; and fat, 1.0 g; for edible kelp, energy, 757 kJ (180.9 kcal); carbohydrate, 40.6 g; protein, 3.8 g; and fat, 0.4 g. The test beverages and the placebo control were packaged by the controller such that the contents could not be identified by the staff or subjects.

Medical examination Following ingestion, antecubital venous blood samples were collected by repeated venipuncture to measure plasma glucose and serum insulin concentrations before the meal and at t = 30, 60, 90, and 120 min, where t = 0 was the starting point of test meal ingestion. All subjects were interviewed by the doctor just before the test and 120 min after the test had ended, in regards to their health and subjective symptoms. Samples collected for glucose and insulin analyses were immediately centrifuged, and the plasma was separated, aliquoted, and frozen at −20°C until analysis. Plasma glucose was measured by the glucokinase method. Insulin was determined from serum samples using a chemiluminescent immunoassay. These analyses were performed by Mitsubishi Chemical Medicine Corp. (Tokyo, Japan). The area under the blood concentration-time curve (AUC) for blood glucose and insulin was calculated according to the trapezoidal rule. The technicians who analyzed the plasma samples were blinded to the identity and treatment regimen of subjects, and the outcome was determined before statistical analysis.

Statistical analysis All data in the experiment are shown as the mean ± standard deviation. Differences observed among the groups at various time points during the experiment were subjected to a one-way analysis of variance followed by Dunnett’s multiple comparison test. Statistical comparisons between the 2 groups were analyzed using the independent samples t-test after confirming the equality of variance by the F-test for 2 samples. All statistical analyses were performed with SPSS 16.0J for Windows (SPSS Inc., Chicago, IL, USA), and the level of significance was set at under 5% by a 2-tailed test.

Results

Effects of EDGCB on glycemic response after carbohydrate (sucrose/maltose/soluble starch/glucose) administration Figure 1 shows the change in blood glucose levels in rats after oral administration of 2 g/kg carbohydrate (sucrose, maltose, soluble starch, or glucose) with or without EDGCB. When 500 mg/kg EDGCB was simultaneously administered orally with sucrose (Fig. 1A), maltose (Fig. 1B), soluble starch (Fig. 1C), or glucose (Fig. 1D), the blood glucose levels 30 min after administration (5.93 ± 0.67 mmol/L, 7.17 ± 0.87 mmol/L, 6.92 ± 0.80 mmol/L, and 8.04 ± 0.98 mmol/L, respectively) were significantly lower compared to controls (8.11 ± 1.01 mmol/L, 8.42 ± 0.85 mmol/L, 8.24 ± 0.75 mmol/L, and 9.60 ± 1.07 mmol/L, respectively) (P < 0.01, P < 0.05, P < 0.05, and P < 0.01, respectively). In addition, a dose of 500 mg/kg EDGCB significantly (P < 0.05) decreased the postprandial rise in blood glucose 60 min following administration of maltose (Fig. 1B) or glucose (Fig. 1D) (6.49 ± 0.65 mmol/L and 6.27 ± 0.47 mmol/L, respectively) compared with the control (7.19 ± 0.40 mmol/L and 7.42 ± 0.58 mmol/L, respectively). EDGCB had no effect on the glucose tolerance curve when administered alone (Fig. 1E).

Preparation of a 325-nm absorbent and nonabsorbent fractions from EDGCB Figure 2 shows the elution pattern of the 325-nm absorbent fraction on a Toyopearl HW-40 column. In this experiment, the yields of the 325-nm absorbent and nonabsorbent fractions were 45.1% (2.62 g) and 51.6% (3.00 g), respectively, and 96.7% (5.62 g) of the total solids in the initial EDGCB sample (5.81 g) was recovered. Chlorogenic acid content and isomeric composition of the initial EDGCB is compared with its retention at the 325-nm absorbent fraction and the nonabsorbent fraction in Table 2. HPLC analysis of the 325-nm absorbent and nonabsorbent fractions indicated that chlorogenic acids were rarely found in the nonabsorbent fraction (0.04%), while 73.3% of chlorogenic acids were included in the 325-nm absorbent fraction. Total chlorogenic acids (CQAs, FQAs, and diCQAs) recovered from the 325-nm absorbent fraction amounted to 84.2% (1.92 g) of those in the initial EDGCB (2.28 g). As shown in Table 2, the isomeric ratios of chlorogenic acids in the 325-nm absorbent fraction were very similar to the initial EDGCB ratios.

Effects of the 325-nm absorbent and nonabsorbent fractions on glycemic response In the sucrose-loading test (Fig. 3), EDGCB (500 mg/kg) and the 325-nm absorbent fraction (235 mg/kg) significantly reduced (P < 0.05) the elevation of blood glucose levels at 30 min (5.26 ± 0.34 mmol/L and 5.08 ± 0.59 mmol/L, respectively) compared to the control group (6.95 ± 0.57 mmol/L). In addition, glucose tolerance curves were very similar in both groups. In contrast, the nonabsorbent fraction (305 mg/kg) had no effect on blood glucose levels.

Effect of administration time The effect of 3 different
Fig. 1. Effects of EDGCB on glycemic response after administration of carbohydrate (sucrose/maltose/soluble starch/glucose) to SD rats (n = 6). (A) EDGCB with sucrose, (B) EDGCB with maltose, (C) EDGCB with soluble starch, (D) EDGCB with glucose, (E) blank. *P < 0.05, **P < 0.01 versus control. (Student’s t-test). SD rats were deprived of food for 16 h and then administered sucrose, maltose, soluble starch, or glucose (2 g/kg) without EDGCB (●) or with (○) 500 mg/kg EDGCB. A blank group was untreated (●) or administered EDGCB (500 mg/kg in 10 mL/kg water) (○) only.

Fig. 2. Elution pattern of EDGCB on a Toyopearl HW-40 column. Toyopearl HW-40 column chromatography (290 × 20 mm) was performed at a flow rate of 1.5 mL/min, as described in the text. The 325-nm absorbent fractions (F-2 and F-4; 2.62 g) and nonabsorbent fractions (F-1, F-3, and F-5; 3.00 g) were combined separately, and each fraction was concentrated and freeze-dried.
levels at 30 min (5.23 ± 0.44 mmol/L and 5.51 ± 0.65 mmol/L, respectively) were significantly reduced (P < 0.01 and P < 0.05, respectively) compared to the levels in the control group (7.34 ± 0.44 mmol/L and 6.51 ± 0.56 mmol/L respectively). In addition, simultaneous administration of EDGCB with sucrose significantly decreased (P < 0.05) the postprandial increase in blood glucose at 60 min (5.36 ± 0.30 mmol/L) compared with that in the control group (6.01 ± 0.45 mmol/L). AUC was also significantly (P < 0.01) decreased (51.6 ± 8.9 mg∙h/dL) when EDGCB was administered simultaneously with the sucrose challenge, compared with that in the control group (75.8 ± 8.5 mg∙h/dL). There were, however, no other significant differences observed. The reductions in average AUC were 3.3%, 32.0%, and 1.1% with administration times of 0 min (simultaneously with sucrose challenge), 30 min before, and 5 min after the challenge, respectively.

In vivo studies in healthy human subjects
Forty-five subjects were selected and randomly assigned to treatment groups. The doctor confirmed that no subjects were in ill health during the test and none of the randomly assigned subjects dropped out of the trial. Data from 1 subject were not included in the efficacy-evaluable population, however, due to difficulty with sampling during the test. Moreover, subjects having a blood glucose level increase less than 20 mg/dL (1.11 mmol/L; 2 men and 1 woman) were excluded from statistical analyses. Of the efficacy-evaluable population (41 subjects), 22 were men and 19 were women. They had a mean age of 34.8 ± 8.0 years, a mean body weight of 60.4 ± 8.9 kg, BMI of 22.0 ± 3.1 kg/m², 24.5 ± 8.3% body fat, fasting glucose of 4.88 ± 0.36 mmol/L, systolic blood pressure of 110.5 ± 11.6 mmHg, and 58.8 ± 9.0 mmHg diastolic blood pressure. The postprandial glucose and insulin response under test conditions are shown in Table 3A. Plasma glucose levels at 30 min (5.23 ± 0.44 mmol/L and 5.51 ± 0.65 mmol/L, respectively) were significantly reduced (P < 0.01 and P < 0.05, respectively) compared to the levels in the control group (7.34 ± 0.44 mmol/L and 6.51 ± 0.56 mmol/L respectively). In addition, simultaneous administration of EDGCB with sucrose significantly decreased (P < 0.05) the postprandial increase in blood glucose at 60 min (5.36 ± 0.30 mmol/L) compared with that in the control group (6.01 ± 0.45 mmol/L). AUC was also significantly (P < 0.01) decreased (51.6 ± 8.9 mg∙h/dL) when EDGCB was administered simultaneously with the sucrose challenge, compared with that in the control group (75.8 ± 8.5 mg∙h/dL). There were, however, no other significant differences observed. The reductions in average AUC were 3.3%, 32.0%, and 1.1% with administration times of 0 min (simultaneously with sucrose challenge), 30 min before, and 5 min after the challenge, respectively.

Fig. 3. Effects of EDGCB, the 325-nm absorbent fraction, and another fraction on blood glucose levels (mmol/L) in sucrose-administered rats. Sucrose at 2 g/kg body weight was administered orally to male SD rats either alone (●) or with EDGCB (○), the 325-nm absorbent fraction (△), or another fraction (▲) at 500 mg/kg, 235 mg/kg, or 305 mg/kg, respectively. Blood samples were obtained at 0, 30, 60, and 120 min after treatment. Values (mmol/L) are means with standard deviations depicted by vertical bars (n = 6). In each sampling period, means followed by different letters are significantly different (*P < 0.05; a repeated analysis of variance followed by the Dunnett’s multiple comparison test).

Table 2. Chlorogenic acid content and isomeric composition of EDGCB, 325 nm absorbent fraction and non-absorbent fraction indicated in Fig. 2.

<table>
<thead>
<tr>
<th>compd.</th>
<th>Chlorogenic acids content (% dry basis)</th>
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<tbody>
<tr>
<td></td>
<td>EDGCB</td>
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<tr>
<td>3-CQA</td>
<td>5.0</td>
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<tr>
<td>4-CQA</td>
<td>5.8</td>
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<tr>
<td>5-CQA</td>
<td>13.9</td>
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<td>4,5-diCQA</td>
<td>2.6</td>
</tr>
<tr>
<td>total</td>
<td>39.2</td>
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</table>

* Values within parentheses represent percent retention compared to EDGCB.
CQA, cafeoylquinic acid; FQA, feruloyl quinic acid; diCQA, dicaffeoylquinic acid; ND, not detected.

In vivo studies in healthy human subjects
Forty-five subjects were selected and randomly assigned to treatment groups. The doctor confirmed that no subjects were in ill health during the test and none of the randomly assigned subjects dropped out of the trial. Data from 1 subject were not included in the efficacy-evaluable population, however, due to difficulty with sampling during the test. Moreover, subjects having a blood glucose level increase less than 20 mg/dL (1.11 mmol/L; 2 men and 1 woman) were excluded from statistical analyses. Of the efficacy-evaluable population (41 subjects), 22 were men and 19 were women. They had a mean age of 34.8 ± 8.0 years, a mean body weight of 60.4 ± 8.9 kg, BMI of 22.0 ± 3.1 kg/m², 24.5 ± 8.3% body fat, fasting glucose of 4.88 ± 0.36 mmol/L, systolic blood pressure of 110.5 ± 11.6 mmHg, and 58.8 ± 9.0 mmHg diastolic blood pressure. The postprandial glucose and insulin response under test conditions are shown in Table 3A. Plasma glucose
levels vary according to the individual. We therefore performed statistical analysis using the group with the highest mean postprandial glucose level 30 min after consumption of the loading diet (placebo food). This high glycemic-response group (n = 18; 10 men and 8 women) had an average age of 35.1 ± 8.5 years, weighed 58.9 ± 9.2 kg, had BMI of 21.4 ± 3.0 kg/m², 23.6 ± 8.1% body fat, fasting glucose of 4.89 ± 0.36 mmol/L.

Table 3. Changes in postprandial glucose, insulin response and glucose AUC after ingestion of EDGCB.

<table>
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<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>0</th>
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<th>60</th>
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<th>120</th>
<th>Glucose AUC 0 – 120 min (mmol · min/L)</th>
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<tbody>
<tr>
<td>Control</td>
<td>Glucose (mmol/L)</td>
<td>5.13 ± 0.27</td>
<td>8.60 ± 0.85</td>
<td>7.98 ± 1.69</td>
<td>6.94 ± 1.24</td>
<td>6.50 ± 1.11</td>
<td>879.68 ± 105.49</td>
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<tr>
<td></td>
<td>Insulin (mU/mL)</td>
<td>3.9 ± 1.6</td>
<td>40.5 ± 22.2</td>
<td>33.7 ± 13.5</td>
<td>27.7 ± 13.8</td>
<td>22.6 ± 10.4</td>
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<tr>
<td>100 mg EDGCB</td>
<td>Glucose (mmol/L)</td>
<td>5.14 ± 0.30</td>
<td>7.98 ± 1.07</td>
<td>7.27 ± 1.27</td>
<td>6.45 ± 1.07</td>
<td>6.20 ± 0.82</td>
<td>821.26 ± 93.60*</td>
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<td></td>
<td>Insulin (mU/mL)</td>
<td>4.0 ± 1.6</td>
<td>39.3 ± 23.5</td>
<td>33.3 ± 14.6</td>
<td>26.3 ± 14.0</td>
<td>24.4 ± 11.0</td>
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<tr>
<td>300 mg EDGCB</td>
<td>Glucose (mmol/L)</td>
<td>5.01 ± 0.32</td>
<td>7.91 ± 0.77</td>
<td>7.64 ± 1.52</td>
<td>6.72 ± 1.39</td>
<td>6.27 ± 0.91</td>
<td>837.22 ± 97.74</td>
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<td></td>
<td>Insulin (mU/mL)</td>
<td>3.6 ± 1.5</td>
<td>40.9 ± 27.9</td>
<td>33.4 ± 15.2</td>
<td>26.6 ± 11.0</td>
<td>21.7 ± 11.7</td>
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</table>

Data are means ± SD. Significant difference was shown with repeated ANOVA followed by Dunnet’s multiple comparison. *p<0.05, **p<0.01 vs. control. (A) All subjects (n = 41), (B) High glycemic response group (n = 18).

Fig. 4. Time profiles of blood glucose concentrations according to differences in timing of EDGCB administration in relation to the beginning of sucrose administration. (A) EDGCB −30 min, (B) EDGCB 0 min, (C) EDGCB +5 min. Control (●); EDGCB (○). *P < 0.05, **P < 0.01 versus control (Student’s t-test).
0.36 mmol/L, 111.7 ± 11.5 mmHg systolic blood pressure, and 57.6 ± 8.4 mmHg diastolic blood pressure. Changes in postprandial glucose, insulin responses, and glucose AUC after ingestion of the loading diet with or without EDGCB in this group are shown in Table 3B. Plasma glucose after 30 min was significantly (P < 0.01) lower after ingestion of both EDGCB-containing beverages (7.98 ± 1.07 mmol/L, 100 mg; and 7.91 ± 0.77 mmol/L, 300 mg) compared to the controls (8.60 ± 0.85 mmol/L). The plasma glucose AUC from 0 to 120 min was significantly reduced following ingestion of 100-mg EDGCB (P < 0.05) compared with the control. No significant differences were observed in plasma insulin profiles throughout the course of the experiment.

Discussion

It is well known that dietary carbohydrate is not absorbed directly from the intestine unless it has been subjected to the action of saliva and pancreatic alpha-glycosidase. The inhibition of alpha-glycosidase activity would therefore be an effective approach to control hyperglycemia (Matsui et al., 2007). Acarbose, Voglibose, and Migritol, specific pancreatic alpha-glycosidase inhibitors, are used clinically to prevent hyperglycemia (van de Laar et al., 2005). There has recently been an increased interest in natural and nutraceutical products for diabetes control. The efficacy of “Foods for specified health uses” (FOSHU: foods containing ingredients with functions for health and officially approved to claim its physiological effects on the human body) products for postprandial glucose control has also been evaluated (Deguchi et al., 1998; Fujita et al., 2001a; Fujita et al., 2001b; Hosoda et al., 2003; Kodama et al., 2005; Seri et al., 2005; Okuma, 1990).

As shown in Table 1, EDGCB contains a high concentration of chlorogenic acids (approximately 40%). In contrast, coffee beans are a major source of caffeine. According to Keijzers et al. (2002) caffeine can decrease insulin sensitivity by increasing the concentration of serum epinephrine. In a recent report, administration of caffeine increased blood glucose concentrations during the day and augmented postprandial glucose levels when habitual coffee drinkers with type 2 diabetes were monitored during daily life (Lane et al., 2007). In contrast, other studies reported that caffeine substantially lowered the risk of clinical type 2 diabetes (Debrah et al., 1996; Watson and Kerr, 1999; Watson et al., 2000; van dam and Feskens, 2002). Although the influence of caffeine in normal adults and diabetics has been studied, the results are clearly controversial.

The most prevalent phenolic compounds in food are hydroxycinnamic acids, and coffee is the major source of chlorogenic acids, which are hydroxycinnamic acid derivatives (Clifford, 2000). It is well known, however, that terpenoids, cafestol, and kahweol, which are found in coffee beans, increase low-density lipoprotein (Bradbury, 2006). We therefore removed the caffeine and terpenoids from green coffee beans using a supercritical CO2 extraction process to obtain EDGCB that could be used in the development of a functional food.

In our study in SD rats, we investigated whether EDGCB could control intestinal glucose absorption in whole animals, and if so, whether it does so by blocking carbohydrate degradation by alpha-glycosidase or intestinal glucose absorption. We tested 4 types of carbohydrates (sucrose, maltose, soluble starch, and glucose), and the results showed that EDGCB significantly reduced plasma glucose levels following oral administration of all 4 test compounds.

Our findings, together with those of published studies, showed that EDGCB reduced postprandial blood glucose levels even after administration of glucose itself. These data substantiate the hypothesis that chlorogenic acids, which are the main physiological functional components in EDGCB, mediate the reduction in postprandial blood glucose levels through the suppression of intestinal glucose transport and inhibition of alpha-glycosidase (sucrase, maltase) and alpha-amylase activity.

In our previous in vitro study (Kamitani et al., 2009), the activities of amylolytic enzymes were inhibited depending on the concentration of EDGCB, in which the proportion of hydroxycinnamic acid derivatives (CQAs, diCQAs, and FQAs) was 63.1 – 85.8%. Therefore, unknown active components in EDGCB may have acted additively or synergistically against postprandial hyperglycemia. For this reason, in the present study we examined whether EDGCB contained active components other than hydroxycinnamic acid derivatives, and evaluated the postprandial blood glucose-lowering effects of the 325-nm absorbent fraction and the nonabsorbent fraction (235 mg/kg and 305 mg/kg, respectively) in rats. Our results strongly suggest that the mechanism for the antihyperglycemic effect of EDGCB in the intestine is inhibition or retardation of the action of alpha-glycosidase by CQAs, FQAs, and diCQAs, since the nonabsorbent fraction did not affect postprandial hyperglycemia.

No studies to date have investigated the optimum time for administration of chlorogenic acids in relation to a carbohydrate challenge. We hypothesized that further reductions in postprandial blood glucose could be achieved according to the time of administration. Deguchi et al. (2002) reported that the postprandial blood glucose level in mice was significantly decreased by oral administration of guava leaf extract, a therapeutic alpha-glycosidase inhibitor, 30 min before a sucrose challenge. Administration of EDGCB prior to a sucrose challenge.
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challenge, however, did not produce a similar effect but more closely resembled the inhibition of postprandial blood glucose by acarbose, the most widely investigated alpha-glycosidase inhibitor (Rosak et al., 1995). While the mechanisms for these observations are unknown, it is conceivable that the effect of EDGCB depends on chlorogenic acid molecules reaching the upper small intestine at the same time as the intestinal contents. When EDGCB was administered 30 min prior to the sucrose challenge, this ideal substrate/inhibitor relationship clearly did not exist. Indeed, our finding that administration of EDGCB 0 or 5 min after the sucrose challenge had favorable effects, supports this interpretation.

In our previous study (Narita and Inouye, 2009; Narita and Inouye, 2011), we investigated the inhibition of porcine pancreas alpha-amylase (PPA) isozymes PPA-I and PPA-II by 8 types of cinnamic acid derivatives in green coffee beans. All CQAs, FQAs, and diCQAs showed mixed-type inhibition against PPA with $K_i > K_i'$, indicating that these compounds bind to the enzyme-substrate complex more tightly than to the enzyme. These results might have been associated with effects of timing of EDGCB administration on postprandial hyperglycemia, which led us to suggest that EDGCB may exert its optimum effect when administered with the first mouthful of a meal (or shortly after a meal).

We further examined whether this postprandial hyperglycemia-suppressive effect of EDGCB could be observed in humans. It is known that rats and humans do not always respond similarly, however, the results of the present clinical trial demonstrated that ingestion of either 100 mg (40 mg as chlorogenic acids) or 300 mg (120 mg as chlorogenic acids) EDGCB together with a carbohydrate-rich meal significantly reduced postprandial glucose levels albeit not in a dose-dependent manner. Although 100 and 300 mg of EDGCB decreased postprandial plasma glucose significantly compared with placebo at 30 min in the group of high-glycemic response subjects, there were no significant differences in response between the doses. Similarly, a dose dependent response was not observed for indigestible dextrin (Kawasaki et al., 2000; Kishimoto et al., 2000; Shioda et al., 2001), Salacia reticulata (Kitabayashi et al., 2008), and D-psicose (Hayashi et al., 2010). Although these responses are not uncommon, confirmatory evidence is needed from further clinical trials to determine the effective dose for EDGCB. EDGCB was effective in lowering plasma glucose levels in a group whose peak postprandial glucose level was higher than the mean value. This may be explained by the study design. In our study, the subjects were non-diabetic healthy adults who had adequate pancreatic function.

Hydroxycinnamic acid derivatives in EDGCB interact with the Na+-dependent glucose transporter as antagonist-like molecules and possibly play a role in controlling dietary glucose uptake in the intestinal tract (Ishikawa et al., 2007; Welsch et al., 1989). We observed no differences in the plasma insulin profiles in the present study. Van Dijk et al. (2009) reported that, compared to a placebo, ingestion of 5-CQA (1 g) led to significantly lower insulin concentrations 15 min after an oral glucose tolerance test (OGTT) but did not significantly reduce insulin AUC values. In the current study, we obtained similar results, although we did not measure plasma insulin levels at 15 min. We did, however, observe an elevation of the insulinogenic index [(insulin 30 min-insulin 0 min) / (glucose 30 min-glucose 0 min)]: 0.70 (control), 0.75 (100 mg dose), and 0.79 (300 mg dose) in healthy human subjects ($n = 41$) and 0.58 (control), 0.69 (100 mg dose), and 0.71 (300 mg dose) in the high-glycemic response group ($n = 18$). Olthof et al. (2011) tested the hypothesis that coffee consumption reduces the risk of type 2 diabetes by inducing incretin hormone secretion, which increases insulin secretion, by measuring the acute effects of decaffeinated coffee and coffee components on incretin concentrations. They found, however, that compared to the placebo, decaffeinated coffee, chlorogenic acid, and trigonelline did not significantly affect the levels of incretin hormones, glucagon-like peptide-1 (GLP-1), or glucose-dependent insulino tropic polypeptide (GIP) in an OGTT. These findings do not support the hypothesis that coffee and its components acutely improve glucose tolerance through incretin hormone secretion. In vivo data on the effects of chlorogenic acid on incretin concentrations are largely lacking. Further investigation is needed to elucidate the relationship between chlorogenic acid intake, incretin action, and insulin secretion.

We previously reported on the relationships between 8 cinnamic acid derivatives in EDGCB and half-maximal inhibitory concentration (IC$_{50}$) values against the amylolytic enzymes maltase, sucrase, and alpha-amylase (Kamitani et al., 2009). In that study, by comparing IC$_{50}$ values against maltase, sucrase, and alpha-amylase, we showed that FQAs lowered the inhibitory activity to less than half that of CQAs, which in turn lowered the inhibitory activity to less than half that of diCQAs. These differences in inhibitory activity could be related to the bioavailability and metabolism of ingested FQAs, CQAs and diCQAs that seem to play crucial roles in lowering postprandial blood glucose levels. It is known that chlorogenic acids are quickly absorbed in rats and humans. Lafay et al. (2006a) demonstrated gastric absorption by infusing chlorogenic acid into the ligated stomach of food-deprived rats. After a 30 min infusion, intact chlorogenic acid was found in the gastric vein and aorta, showing that it is quickly absorbed in the rat stomach. In addition, 5-CQAs and caffeic acid were also absorbed in the small intestine.
of rats, preferentially in the jejunum, and net absorption accounted for 8% of the perfused 5-CQAs (Lafay et al., 2006b). With regard to the bioavailability of chlorogenic acids in humans, it was reported that in healthy subjects, more than 30% (33.1 ± 23.1%) of ingested cinnamic acid moieties, including metabolites, were recovered in plasma with peak levels being observed 0.5 to 8 h after the consumption of 2 capsules of green coffee extract (Svetol; 400 mg) (Farah et al., 2008). Since up to 0.5 h was required to achieve a post-prandial blood glucose level, we presume that complicated events, such as inhibition of amylolytic enzymes by chlorogenic acids, suppression of glucose uptake by chlorogenic acids, and absorption of chlorogenic acids, occur at the same time on the intestinal brush border membrane. Monteiro et al. (2007) reported that the major chlorogenic acids present in coffee are differentially absorbed and/or metabolized in humans. The molar ratio of CQAs: diCQAs was 12.2 in the brewed coffee in that study, whereas in plasma ranged from 0.6 to 2.9, which shows that diCQAs, which contribute considerably to the inhibitory activity of EDGCB against amylolytic enzymes, are more easily absorbed than CQAs. Thus, one-third of ingested chlorogenic acids that are absorbed could have biological effects in the blood, while the unabsorbed remnant could have biological effects in the intestine; interindividual variability in the absorption ratio is large and this may be attributed to interindividual differences in digestive transit time, preferential site of absorption, and metabolism of chlorogenic acids. Further studies are necessary to elucidate the exact mechanism of action of EDGCB on the intestinal brush border membrane.

In conclusion, EDGCB suppresses postprandial hyperglycemia after carbohydrate loading (sucrose, maltose, soluble starch, glucose, and carbohydrate-rich food) in vivo. The results from the present study showed that EDGCB may inhibit amylolytic enzyme activity and attenuate intestinal glucose absorption. These findings point to EDGCB as a compound of interest with regard to reducing the risk of developing type 2 diabetes, and could motivate a renewed interest in health-conscious food, soft drinks, and supplements.

References
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