Coffee Ingestion Suppresses Hyperglycemia in Streptozotocin-Induced Diabetic Mice

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(Received December 5, 2016)

Summary Coffee consumption reduces the risk of type 2 diabetes in humans, but the mechanism remains unclear. In this study, we investigated the effect of coffee on pancreatic β-cells in the induction of diabetes by streptozotocin (STZ) treatment in mice. We examined the effect of coffee, caffeine, or decaffeinated coffee ingestion on STZ-induced hyperglycemia. After STZ injection in Exp. 1 and 2, serum glucose concentration and water intake in coffee ingestion (Coffee group) tended to be lowered or was significantly lowered compared to those in water ingestion (Water group) instead of coffee. In Exp. 1, the values for water intake and serum glucose concentration in caffeine ingestion (Caffeine group) were similar to those in the Water group. In Exp. 2, serum glucose concentrations in the decaffeinated coffee ingestion (Decaf group) tended to be lower than those in the Water group. Pancreatic insulin contents tended to be higher in the Coffee and Decaf groups than in the Water group (Exp. 1 and 2). In Exp. 3, subsequently, we showed that coffee ingestion also suppressed the deterioration of hyperglycemia in diabetic mice which had been already injected with STZ. This study showed that coffee ingestion prevented the development of STZ-induced diabetes and suppressed hyperglycemia in STZ-diabetic mice. Caffeine or decaffeinated coffee ingestion did not significantly suppress STZ-induced hyperglycemia. These results suggest that the combination of caffeine and other components of decaffeinated coffee are needed for the preventive effect on pancreatic β-cell destruction. Coffee ingestion may contribute to the maintenance of pancreatic insulin contents.

Key Words streptozotocin, diabetes, hyperglycemia, coffee, caffeine

In 2015, 415 million people worldwide were estimated to be affected by type 2 diabetes (1). The International Diabetes Federation estimates that this will rise to 642 million individuals by the year 2040, and warns that the prevalence of type 2 diabetes will increase. These increases are caused by changes in environmental factors such as quantity and quality of food and exercise, and therefore modification of environmental factors can contribute to the prevention of type 2 diabetes.

In humans, many studies have revealed an association between coffee consumption and decreased risk of type 2 diabetes. A meta-analysis of 28 studies published between 1966 and 2013 showed that daily consumption of six cups of coffee was associated with a 33% lower risk of diabetes, and that coffee consumption dose-dependently reduced the risk of type 2 diabetes (2). In addition, the reduction in type 2 diabetes risk was observed with both, caffeinated and decaffeinated coffee. However, the mechanism of the anti-diabetic effect of coffee remains unclear.

Previously we reported that coffee had an anti-diabetic effect in both spontaneous and diet-induced type 2 diabetes in animal models (3, 4). In KK-A^y mice, which spontaneously develop type 2 diabetes, coffee ingestion suppressed hyperglycemia, hyperinsulinemia, hyperlipidemia, and hepatic steatosis (3). In C57BL/6J mice fed a high-fat diet, coffee ingestion also improved insulin sensitivity (4). Furthermore, we found that coffee increased the activation of Akt in insulin signaling in the liver and skeletal muscle (5). Insulin resistance and impaired insulin secretion are associated with the development of type 2 diabetes. We revealed that coffee ingestion improved insulin resistance in two type 2 diabetes animal models.

Streptozotocin (STZ) is well known as a chemical compound that induces diabetes in experimental animals, and is also a potent alkylating agent that directly damages cellular DNA (6–8). STZ is taken into the pancreatic β-cells via glucose transporter 2 (GLUT2) (9) where the N-methyl-N-nitrosourea moiety causes alkylation of the DNA strand, especially at the O6 position of guanine (6). Therefore, STZ directly induces DNA strand breaks that stimulate nuclear poly (ADP-ribose) polymerase and cause depletion of intracellular NAD (8). Depletion of NAD, a coenzyme in energy metabolism, leads to loss of
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Coffee ingestion can significantly improve hyperglycemia and insulin resistance in STZ-diabetic mice. This study aimed to investigate the protective effect of coffee ingestion on pancreatic β-cells in animals with STZ-induced diabetes. We performed Experiments 1 to 3 to analyze the preventive, suppressive, and curative effects of coffee ingestion on STZ-induced diabetes.

**Materials and Methods**

**Animals.** Four-week-old male C57BL/6j mice (Japan SL C, Inc., Shizuoka, Japan) were purchased and maintained at a controlled temperature of 23±2°C and 55±5% humidity with a 12-h light/dark cycle. The mice were allowed free access to water and a standard CE-2 laboratory diet (CLEA Japan, Inc., Tokyo, Japan) for 3 d before the start of the experiment. The animal care and experimental procedures were approved by the Animal Research Committee of Nagoya University (approval no. 2013022804, 2014021206, 2015022601) and were conducted according to the Regulations for Animal Experiments at Nagoya University.

We performed Exp. 1 to analyze the preventive effect of coffee or caffeine ingestion on the development of hyperglycemia after STZ injection. Acclimatized 4-wk-old mice were assigned to four groups and given water without STZ (Control group, 6 mice), water with STZ (Water group, 10 mice), diluted black coffee (Coffee group, 10 mice), or a caffeine solution (200 mg/L; Caffeine group, 10 mice). After 9 d, the Water, Coffee, and Caffeine groups were subjected to 8 h of food deprivation before they were intraperitoneally injected with STZ solution (175 mg/kg body weight) at 5 p.m. (designated as Day 0). The Control group was intraperitoneally injected with citrate buffer instead of STZ. Blood samples were collected from the tail vein at Day 0 (before STZ injection), and then at 3, 5, 8, and 12 d after STZ injection, and serum glucose concentration was measured.

Experiment 2 was performed to analyze the preventive effect of coffee or decaffeinated coffee ingestion on the development of hyperglycemia after STZ injection. Acclimatized 4-wk-old mice were assigned to three groups and given water (Water group, 15 mice), diluted black coffee (1:1.5 black coffee:water, Coffee group, 15 mice), or decaffeinated coffee (1:1.5 decaffeinated coffee:water, Decaf group, 15 mice). After 10 d, the Water, Coffee, and Decaf groups were subjected to 8 h of food deprivation before they were intraperitoneally injected with STZ solution (165 mg/kg body weight) at 5 p.m. (designated as Day 0). Blood samples were collected from the tail vein at Day 0 (before STZ injection), and then at 2, 5, 8, and 12 d after STZ injection, and the serum glucose concentration was measured.

Experiment 3 was performed to analyze the suppressive effect of coffee ingestion on the deterioration of hyperglycemia in STZ-diabetic mice. In Exp. 3, acclimatized 8-wk-old mice were intraperitoneally injected with STZ solution (150 mg/kg body weight). Eight days after STZ injection, serum glucose concentration was measured and diabetic mice were defined as having a serum glucose concentration exceeding 300 mg/dL (Day 0). The diabetic mice were divided into two groups and given water (Water group, 10 mice) or diluted black coffee (Coffee group, 12 mice). Animals were kept in individual cages, and fed the standard laboratory diet. Blood samples were collected from the tail vein on Days 0, 4, 6, 10, 13, and 17 after the mice were divided into the Water or Coffee groups, and the serum glucose concentration was measured.

The coffee, decaffeinated coffee, and caffeine solutions were prepared every 2nd day. Unfiltered coffee and decaffeinated coffee were stored at −20°C until needed. Food was removed 1 h before blood samples were collected from the tail vein at 10 a.m. The mice were killed by decapitation at the end of the experiment (between 10 a.m. and 12 p.m.), and the blood was collected and assayed for serum insulin concentration. The pancreases were removed and weighed. Fifty milligrams of pancreatic tissue was immediately frozen in liquid nitrogen and stored at −80°C. Collected blood was kept at room temperature for 15 min to allow coagulation. The serum was obtained from the coagulated blood by centrifugation at 2,430 ×g for 10 min at 4°C and stored at −30°C prior to analysis.
Materials. Regular canned black coffee and decaffeinated black coffee were provided by Pokka Sapporo Food & Beverage Ltd. (Aichi, Japan) and used after 2.5-fold dilution with water. In diluted black coffee, the concentrations of caffeine, chlorogenic acid, and trigonelline were 178, 62, and 94 mg/L, respectively. In diluted decaffeinated black coffee, the concentrations of caffeine, chlorogenic acid, and trigonelline were 6.7, 95, and 118 mg/L, respectively. Caffeine and STZ were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). STZ solutions (175 mg/kg body weight or 165 mg/kg body weight) were dissolved in sodium citrate buffer (0.05 M, pH 4.5) and injected into mice within 5 min.

Analysis of serum glucose, serum insulin, and pancreatic insulin. Serum glucose was measured by the glucose oxidase method using a Glucose C-test assay kit (Wako Pure Chemical Industries). Pancreatic tissue was homogenized with 23.5 : 75.0 : 1.5 water : ethanol : 1 N HCl, to extract pancreatic insulin into the solvent, a portion of which was diluted for pancreatic insulin analysis. A commercially available ELISA kit was used to determine both serum and pancreatic insulin concentration (Mori-naga Seikagaku, Kanagawa, Japan).

Cell viability assay in MIN6 cells. The mouse insulinoma cell line (MIN6) was a gift from Jun-ichi Miyazaki, Osaka University, Japan (26). MIN6 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Wako #044-29765) supplemented with 10% heat inactivated (fetal bovine serum, FBS), 0.1 mM 2-mercaptoethanol, and penicillin/streptomycin in humidified 5% CO₂ at 37°C. For cell viability assays, MIN6 cells (25,000 cells/well) were seeded in 96-well plates. After 2 d of culture, coffee (1, 3, or 5%) or nicotinamide (10 mM) was added to the medium. Eighteen hours after addition of coffee or nicotinamide, STZ at a final concentration of 2.5 mM was added to the culture medium. After cell culture with STZ for 6 h, the media were removed. Cell Counting Kit-8 solution (Dojindo, Japan) was added into the wells, and the plates were incubated in humidified 5% CO₂ at 37°C for 4 h.

Statistical analysis. Data were expressed mean±SE.
In body weight, food intake, water intake, and serum glucose concentration of Exp. 1, 2, and 3, data were analyzed by two-way ANOVA. In Exp. 1 and 2, if the interaction effect of two components (ingestion×time) was significant by two-way ANOVA, subsequent Tukey-Kramer’s tests were used to compare the means of ingestion groups at each time point. In Exp. 3, if the interaction effect of two components (ingestion×time) was significant by two-way ANOVA, Student’s t-tests were subsequently used to compare the means of ingestion groups at each time point. In serum insulin concentration and pancreatic insulin contents of Exp. 1 and 2, one-way ANOVA and subsequent Tukey-Kramer’s tests were used to compare the means among the ingestion groups. In serum insulin concentration and pancreatic insulin contents of Exp. 3, a Student’s t-test was used to compare the means between the Water and Coffee groups. In Exp. 4, data were analyzed by one-way ANOVA and subsequent Dunnett’s tests. Differences with p<0.05 were regarded as significant. Statistical analyses were performed using StatView version 5.0 software (SAS Institute, Cary, NC).

RESULTS

The preventive effect of coffee or caffeine ingestion on development of hyperglycemia after STZ injection (Exp. 1)

In Exp. 1, the Water group, the Coffee group, or the Caffeine group ingested water, black coffee, or decaffeinated coffee for 9 d before STZ injection, respectively. At Day 0 (before STZ injection), there were no significant differences in body weight, food intake, water intake, or serum glucose concentration among the groups (Fig. 1A, B, C, D). After STZ injection, body weight in the Coffee and Water groups tended to be lower, and that in the Caffeine group was significantly lower than that in the Control group (Fig. 1A). Food intake in the Water group was significantly higher than in the Control group without STZ at Days 8 and 12. Food intake tended to be higher in the Coffee and Caffeine groups than in the Control group after Day 8 (Fig. 1B). After Day 5, water intake in the Water group began to increase compared to that in the Control group (Fig. 1C). At the end of experiment, compared to the Control group, water intake in the Caffeine and Coffee groups was 3-fold and 2-fold higher,
respectively. On Day 2, serum glucose concentration in the Caffeine group tended to be higher compared to that in the Coffee and Control groups (Fig. 1D). The Caffeine group showed similar values for body weight, food intake, water intake, and serum glucose concentration to the Water group (Fig. 1A, B, C and D). The trends in water intake in all groups during the course of experiment were similar to the trends observed for serum glucose concentrations (Fig. 1C and D). At the end of the experiment, serum insulin concentration in the Water and Caffeine groups tended to be lower than that in the Control group (Fig. 1E). The trends in pancreatic insulin contents across the groups were similar to those observed for serum insulin concentrations, and indicated that coffee ingestion partially prevented decreases in pancreatic insulin contents caused by STZ treatment, but not significantly (Fig. 1F). In contrast, caffeine ingestion did not prevent a decrease in pancreatic insulin contents during STZ treatment.

The preventive effect of coffee or decaffeinated coffee ingestion on development of hyperglycemia after STZ injection (Exp. 2)

In Exp. 2, we analyzed the preventive effect of coffee or decaffeinated coffee on the development of STZ-induced diabetes. The Water group, the Coffee group, and the Decaf group ingested water, black coffee, and decaffeinated coffee for 10 d before STZ injection, respectively. There were no significant differences in body weight among the groups (Fig. 2A). Food intake in the Coffee group on Day 12 was significantly suppressed compared to that in the Water group (Fig. 2B). Water intake in the Water group was significantly higher than in the Coffee group on Days 5, 9, and 12 (Fig. 2C). On Day 12, food intake and water intake in the Decaf group tended to be lower than that in Water group (Fig. 2B and C). At all time points after Day 2, the Coffee group showed significantly lower serum glucose concentrations compared to the Water group, which showed marked hyperglycemia. (Fig. 2D). Compared to the Water group, the serum glucose concentration in the Decaf group tended to be lower at Day 12. There was no difference in serum insulin concentrations among the 3 groups (Fig. 2E). Pancreatic insulin contents tended to be higher in the Coffee and Decaf groups compared to the Water group (Fig. 2F).

The suppressive effect of coffee ingestion on the deterioration of hyperglycemia in STZ-diabetic mice (Exp. 3)

In Exp. 3, we injected STZ in 8-wk-old mice without
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STZ-induced diabetes is caused by pancreatic β-cell death and decreased pancreatic insulin content. In this study, coffee ingestion protected pancreatic β-cells against STZ cytotoxicity and maintained pancreatic insulin contents.

We previously reported that coffee ingestion suppressed hyperglycemia in KK-A^y mice, an animal model that spontaneously develops type 2 diabetes (3). Coffee or caffeine ingestion (250 mg/L) in drinking water suppressed hyperglycemia, inflammatory changes in adipose tissues, and development of fatty liver (3). In C57BL/6J mice fed a high-fat diet, coffee or caffeine (200 mg/L) also improved glucose tolerance, insulin sensitivity, and inflammatory changes in adipose tissues (4). Thus, caffeine ingestion improved the insulin resistance and contributed to the suppression of type 2 diabetes in both models. In addition, caffeine was shown to be one of the main effectors of these changes included in coffee.

Previously it has been reported that pre-treatment with caffeine (intraperitoneal injection, 100 mg/kg body weight) can partially suppress the increases in blood glucose levels and decreases in pancreatic insulin content caused by STZ toxicity in rats (27). Therefore, we expected that coffee or caffeine ingestion would prevent STZ-induced diabetes. In this study, we found that continuous coffee ingestion could prevent STZ-induced diabetes (Figs. 1 and 2). However, caffeine ingestion did not prevent the development of hyperglycemia (Fig. 1). In contrast, food intake, water intake, serum glucose concentration, and pancreatic insulin content in the Decaf group showed intermediate values between those in the Water and Coffee groups (Figs. 2B, C, D, E). Caffeine (27), chlorogenic acid (28), and trigonelline (29), which are major ingredients of coffee, have been shown to affect STZ-induced diabetes or STZ-induced pancreatic β-cell death. Kagami et al. suggested that caffeine administration prevented STZ-induced pancreatic β-cell death by inhibiting poly-(ADP-ribose) polymerase (PARP) activity (27). The caffeine metabolites 1,7-dimethylxanthine, 3-methylxanthine, and 1-methylxanthine are inhibitors of PARP-1 (30). Chlorogenic acid, the ester of caffeic acid with quinic acid, was previously shown to promote insulin secretion and suppress hyperglycemia in STZ-diabetic rats (28). Trigonelline can ameliorate hyperglycemia in diabetic rats induced by STZ injection with a high-carbohydrate/high-fat diet (29). Moreover, ferulic acid, also found in coffee, can suppress increased blood glucose concentration in STZ-induced diabetes (31). At present, we speculate that additive/synergistic effects between caffeine and the other compounds in decaffeinated coffee are needed to maintain pancreatic insulin content in STZ-treated mice.

In Exp. 1 and 2, coffee ingestion was continued for the entire experimental period. However, in Exp. 3, we examined whether coffee ingestion could ameliorate hyperglycemia induced by STZ (Fig. 3D). We found that coffee ingestion after the development of STZ-induced hyperglycemia could suppress hyperglycemia. In all three experiments, coffee intake tended to increase pan-

**DISCUSSION**

This study demonstrated that coffee ingestion had a preventive effect on the development of STZ-induced diabetes, and suppressed STZ-induced hyperglycemia.

**Fig. 4.** The effect of coffee treatment on STZ-induced cell death. MIN6 cells were treated with nicotinamide or coffee solution. After 18 h, citrate buffer (control) or 2.5 mM STZ were added and incubated for 6 h. The cell viability of the control was normalized to 100%. Three independent replicates were performed, and data is presented as the mean±SE. ** indicates p<0.01 compared with the control (no STZ) group.
creatic insulin contents compared to the Water group (Fig. 3F). It has been suggested that coffee ingestion can suppress STZ-induced decreases in pancreatic insulin content. In contrast, pancreatic α-cells, which do not express GLUT2, are not damaged by STZ injection. Recently, STZ and alloxan injection was reported to cause hyperglycemia by elevating serum glucagon concentration (32, 33). In addition, STZ-induced β-cell destruction in glucagon receptor knockout mice does not cause insulin-deficient type 1 diabetes (32). We cannot exclude the possibility that coffee prevents hyperglycemia by suppressing glucagon secretion in STZ-treated mice.

In Exp. 4, we examined the protective effect of coffee against STZ-induced β-cell death in vitro. The caffeine concentration in the medium with the addition of 1% coffee was 22.9 μM. In humans, the serum caffeine concentration has been reported to be about 30 μM an hour after drinking a cup of coffee (34). Therefore, the coffee concentrations used here in the MIN6 cell viability experiment were at physiological levels. However, the addition of 1–5% coffee could not protect against STZ-induced cell death. We also assessed several compounds found in coffee, including caffeine and trigonelline, but neither increased cell viability (data not shown). Caffeine absorbed from the intestine is dimethylated by hepatic enzymes in vivo, and is metabolized to xanthines, including theophylline, paraxanthine and theobromine (35). Chlorogenic acid, an ester of caffeic acid and quinic acid, is metabolized by gut microflora into aromatic acid metabolites (36). In future, it would be interesting to assess whether metabolites of the compounds found in coffee have a protective effect on pancreatic β-cells during treatment with STZ. On the other hands, there is a possibility that the compounds of coffee indirectly act on the protection of pancreatic β-cells via an extrapancreatic effect. It has been shown that chlorogenic acid inhibits the activity of glucose-6-phosphate translocase 1 (37, 38). This inhibition leads to delayed glucose absorption in the gastrointestinal tract, which promotes GLP-1 secretion at the distal region of the small intestine. In STZ-induced diabetic rats, it was reported that the enhancement of the plasma GLP-1 level increased pancreatic insulin contents and suppressed hyperglycemia via the stimulation of islet neogenesis and β-cell survival (39). These implied that the enhancement of incretin secretion caused by coffee ingestion might be involved in the protective effect of coffee on hyperglycemia in this study.

In conclusion, continuous coffee ingestion prevented the development of STZ-induced diabetes. The combination of caffeine and decaffeinated coffee was needed to produce this preventive effect. Moreover, in mice developing STZ-induced hyperglycemia, coffee ingestion also suppressed hyperglycemia. Coffee ingestion may contribute to protection of pancreatic β-cells and maintenance of pancreatic insulin contents in both type 1 and type 2 diabetes.

Acknowledgments
This work was supported by a grant from the All Japan Coffee Association to F.H.

REFERENCES
14) Khaldi MZ, Guiot Y, Gilon P, Henquin JC, Jonas JC. 2004. Increased glucose sensitivity of both triggering and amplifying pathways of insulin secretion in rat islets cul-
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