

Differences in the effects of Kenyan, Tanzanian, and Ethiopian coffee intake on interstitial glucose levels measured by FreeStyle Libre: A pilot case study

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ABSTRACT

Background: Although generally considered part of a healthy diet, coffee consumption has been suspected to be associated with elevated epinephrine levels and increasing insulin resistance.

Objectives: We studied the effects of the intake of 3 different types of coffee (Tanzanian, Ethiopian, and Kenyan) on postprandial interstitial glucose levels.

Method: Interstitial glucose levels were measured every 15 minutes using the FreeStyle Libre glucose monitoring system (Abbott Diabetes Care Ltd, Witney, United Kingdom) in each individual after drinking coffee compared with when not consuming coffee.

Results: Unlike Tanzanian and Ethiopian coffees, Kenyan coffee suppressed the increase of postprandial interstitial glucose levels. Kenyan coffee beans contain less anhydrous caffeine and more chlorogenic acid than Tanzanian and Ethiopian coffee beans. These findings may explain the different effects of these coffee types on postprandial interstitial glucose levels. Furthermore, Kenyan coffee beans inhibited α -glucosidase activity, which may partially explain why Kenyan coffee reduces postprandial interstitial glucose levels.

Conclusions: Coffee is widely consumed as a beverage worldwide, and our findings suggest that patients with diabetes mellitus may benefit from drinking Kenyan coffee because of its ability to reduce postprandial interstitial glucose levels. (*Curr Ther Res Clin Exp.* 2020; 81:XXX–XXX)

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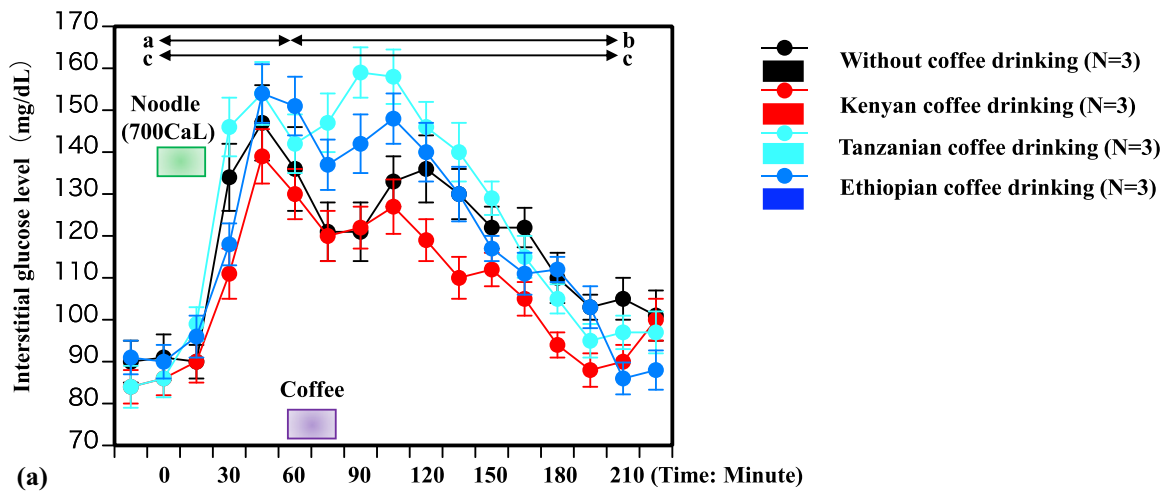
Introduction

Coffee is among the most widely consumed beverages globally. Epidemiologic studies have shown that coffee intake is inversely associated with chronic diseases such as cardiovascular diseases, diabetes, and Parkinson disease, as well as liver, colorectal, and endometrial cancers.¹ Prospective studies have shown that coffee drinking is consistently inversely associated with all-cause and cause-specific mortalities.¹ These findings were incorporated

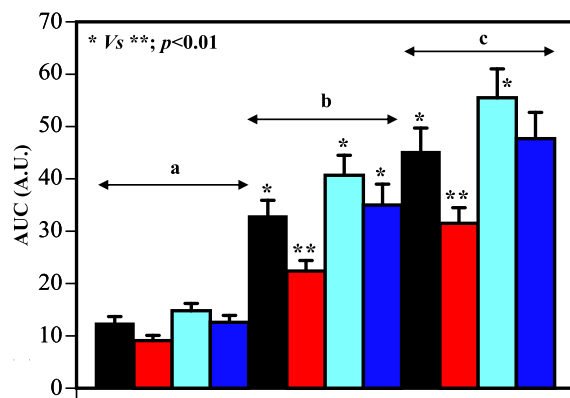
into the 2015 US Dietary Guidelines Advisory Committee Report, which states that moderate coffee consumption of up to 5 8-oz cups per day may form part of a healthy diet. However, the effects of coffee intake have raised some concerns because coffee is a major source of caffeine, which increases plasma glucose levels by elevating epinephrine levels and increasing insulin resistance.² In this pilot study, we compared the effect of 3 different coffee beverages (Kenyan, Tanzanian, or Ethiopian) on postprandial glucose levels by measuring interstitial glucose levels every 15 minutes using the FreeStyle Libre glucose monitoring system (Abbott Diabetes Care Ltd, Witney, United Kingdom) in a single individual without diabetes, compared with no coffee consumption after meal.

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(a)



(b)



(c) Kenya

Tanzania

Ethiopia

Figure 1. Effect of coffee on postprandial interstitial glucose levels. (A) The y-axis shows the postprandial interstitial glucose levels measured every 15 minutes using the FreeStyle Libre glucose monitor (Abbott Diabetes Care Ltd, Witney, United Kingdom), whereas the x-axis shows time (in minutes). The light green square indicates when the meal was consumed and the purple square indicates when the coffee was consumed. The black line with circles represents the data obtained when no coffee was consumed (control); the red line with circles represents Kenyan coffee consumption, light blue line with circles represents Tanzanian coffee consumption, and blue line with circles reflects Ethiopian coffee intake. (B) The black bar represents the data obtained when no coffee was consumed (control); the red bar represents Kenyan coffee consumption, the light blue bar represents Tanzanian coffee intake, and the blue bar represents Ethiopian coffee intake. The area under the curve (AUC) of the experiment with Kenyan coffee consumption was significantly smaller than those of the experiments involving Tanzanian, Ethiopian, or no coffee consumption ($P < 0.01$) in periods b and c. Period a represents the time lapsing from baseline until the beginning of Kenyan, Tanzanian, or Ethiopian coffee intake, whereas period b represents the period reflecting the time lapsing from the beginning of coffee drinking to the end of the experiment. Period c represents periods a + period b and thereby reflects the entire experimental period. Statistical significance was observed between * and ** ($P < 0.01$). Each experiment was repeated 3 times. (C) Photographs of the coffee beans used in the present study.

Case Presentation

A member in our laboratory volunteered to be studied and provided informed consent. This healthy volunteer was a nonsmoking man, aged 62 years, with a body mass index of 25.8 and a normal glucose tolerance at 75 g oral glucose tolerance test. Until the end of the study, he maintained his lifestyle and body weight throughout and fasted approximately 12 hours before each experiment.

The present study comprised 5 groups of experiments, which were repeated 3 to 5 times on different days over 3 months. In part 1, the volunteer consumed udon noodles as a test meal

(~700 kcal) within 20 minutes; subsequently, interstitial glucose levels were determined every 15 minutes from 30 minutes before to 240 minutes after meal consumption using FreeStyle Libre.³ Udon noodles consist of 143 g carbohydrates (572 kcal), 20.9 g protein (83.2 kcal), and 2.35 g fat (21.5 kcal). The volunteer was required to drink coffee (700 mL) within 20 minutes approximately 1 hour after starting the test meal. We tested 3 types of coffee beans (Kenyan, Tanzanian, and Ethiopian) in the present study. Kenyan coffee beans were purchased from O8COFFEE (Akita, Japan), whereas Tanzanian and Ethiopian beans were purchased from Japan Denali Coffee (Maebashi, Japan). We se-

Effect of coffee on insulin signal and α -glucosidase activity

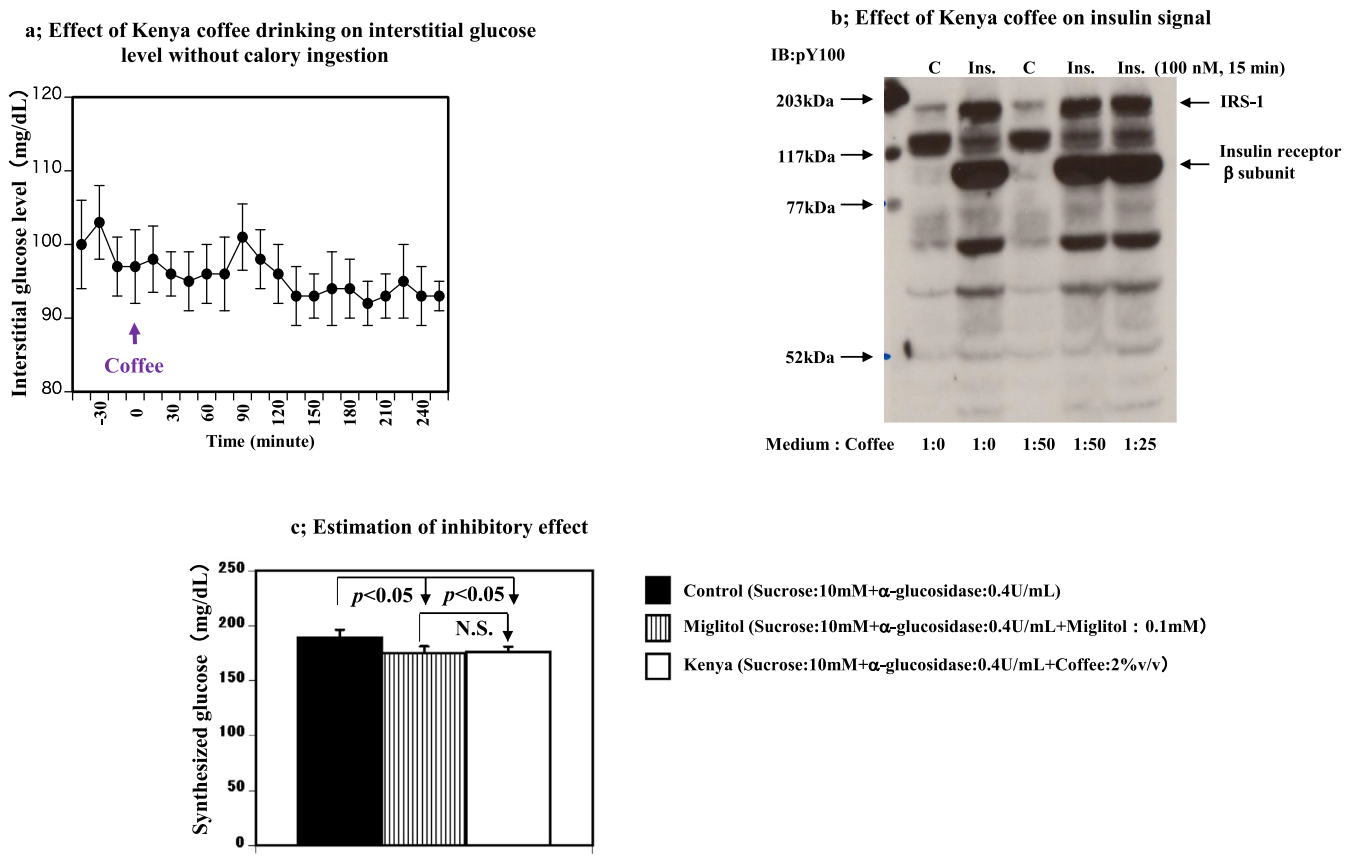


Figure 2. Effect of coffee on insulin signaling and α -glucosidase activity. (A) Effect of Kenyan coffee intake on interstitial glucose levels with no prior calory intake. The y-axis shows the interstitial glucose levels measured using the FreeStyle Libre glucose monitor (Abbott Diabetes Care Ltd, Witney, United Kingdom), and the x-axis shows time (in minutes). (B) The effect of Kenyan coffee intake on insulin signaling. Kenyan coffee was added to the culture medium at ratios of 1:0, 1:50, and 1:25 (medium:coffee) as indicated in the bottom of the picture. Chinese hamster ovary cells expressing human insulin receptors (CHOIR cells) were stimulated with or without 100 nM human insulin (Ins) for 15 minutes. Tyrosine phosphorylated IRS-1 and insulin receptor β -subunits detected by phosphotyrosine blotting are indicated by arrows. The experiments were conducted independently in triplicate. (C) Estimated inhibitory effect of coffee on α -glucosidase activity. The y-axis shows the levels of synthesized glucose. The closed column represents the control sample (assay conditions: 10 mM sucrose, 0.4 U/mL α -glucosidase); the column with vertical stripes represents the miglitol sample (assay conditions: 10 mM sucrose, 0.4 U/mL α -glucosidase, 0.1 mM miglitol), and the open column represents Kenyan coffee (assay conditions: 10 mM sucrose, 0.4 U/mL α -glucosidase, 2% v/v Kenyan coffee). The experiments were repeated independently four times. The synthesized glucose levels were compared with those of the control sample and statistically analyzed.

lected coffee beans with similar roasted properties as shown in Figure 1C. Coffee beans (21 g) were ground using an electric coffee mill and brewed in 700 mL water using an electric coffee maker.

In part 2, we examined whether the Kenyan coffee used in the present study contained an insulin-like substance. First, the volunteer drank Kenyan coffee without consuming the meal then fasted for 4 hours to see whether hypoglycemia developed. Subsequently, it was tested whether Kenyan coffee could affect insulin signaling by adding Kenyan coffee to the culture medium of Chinese hamster ovary cells expressing human insulin receptors (CHOIR cells) in either 1:25 or 1:50 dilution, followed by Western blotting. Levels of tyrosine-phosphorylated IRS-1 and insulin receptor β -subunit by insulin stimulation (100 nM, for 15 minutes) were estimated in the absence and presence of Kenyan coffee in the culture medium by Western blotting. The CHOIR cells were obtained as previously described.⁴ The cells were maintained in a minimal amount of Eagle's medium containing nucleotides supplemented with 10% fetal bovine serum. Whole cell extracts were prepared as previously described,⁴ and samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophore-

sis and Western blot analysis (Enhanced Chemiluminescence Detection Kit, Cytiva, Tokyo, Japan) using a phosphotyrosine-100 monoclonal antibody (Santa Cruz Biotechnology, Inc, Dallas, Texas). The experiment was repeated 3 times. Besides these experiments, any potential inhibitory effect on the α -glucosidase activity of Kenyan coffee was examined by following a previously reported procedure.⁵

In part 3, we tested the effects of drinking Kenyan coffee on postprandial interstitial glucose levels after simultaneous consumption of the test meal and Kenyan coffee. In the present study, the volunteer was requested to consume the test meal and the Kenyan coffee within 20 minutes. Next, interstitial glucose levels were measured every 15 minutes for a total of 270 minutes using FreeStyle Libre as described above. Part 3 experiments were repeated 5 times.

In part 4, we compared the uric acid levels of the volunteer after drinking Tanzanian coffee and after drinking Kenyan coffee. The volunteer was first asked to drink Tanzanian coffee every day for 2 months, and thereafter, the volunteer was requested to switch and drink Kenyan coffee every day for the following 2 months. Serum uric acid levels were measured once a month.

In part 5, the content of chlorogenic acid and anhydrous caffeine in Kenyan, Tanzanian, and Ethiopian coffee beans was determined by Japan Food Research Laboratories (Tokyo, Japan). Each measurement was repeated 3 times.

Data are presented as means (SD). Statistical analysis was conducted using SPSS version 10.0 (IBM-SPSS, Inc, Armonk, New York), and a 2-tailed P value < 0.05 was considered statistically significant.

Results

Effect of coffee consumption on interstitial glucose level

No changes in postprandial interstitial glucose levels were observed until consumption of either Kenyan, Tanzanian, or Ethiopian coffee (designated as period a in Figure 1A). During period b, which was the time passing from the beginning of coffee consumption until the end of the experiment (designated as period b), postprandial interstitial glucose levels did not differ among no coffee consumption or drinking Tanzanian or Ethiopian coffee. Conversely, consumption of Kenyan coffee significantly suppressed the increase in postprandial interstitial glucose levels observed in period b compared with Tanzanian or Ethiopian coffee consumption or no coffee consumption (Figure 1A). The differences in interstitial glucose levels were represented as the area under the curve (AUC, the trapezoid area was drawn using baseline data every 15 minutes, and each area was mathematically calculated by following the formula) in bar graphs and statistically quantitated (Figure 1B). The AUC pertaining to Kenyan coffee was significantly smaller than AUCs pertaining to Tanzanian, Ethiopian, or no coffee ($P < 0.01$) during periods b and c (period c = period a + period b).

Effect of Kenyan coffee on insulin signaling and α -glucosidase activity

Because interstitial glucose levels decreased after Kenyan coffee consumption, we examined whether the Kenyan coffee might contain an insulin-like substance. To explore this possibility, the volunteer drank Kenyan coffee without consuming any meals and then fasted for 6 hours. Because hypoglycemia did not occur within the observation period, it was suggested that Kenyan coffee did not contain any insulinogenic substances (Figure 2A).

Next, we examined whether Kenyan coffee directly affects insulin signaling. As shown in Figure 2B, tyrosine-phosphorylated IRS-1, and insulin receptor β -subunit levels by insulin stimulation were similar in samples with and without the addition of coffee in the culture medium (Figure 2B). These results further suggested that Kenyan coffee did not directly affect insulin signaling.

Next, as we considered the possibility that Kenyan coffee decreased glucose absorption at the small intestine level, we examined whether Kenyan coffee had an inhibitory effect on α -glucosidase activity by following a previously reported procedure.⁵ In this assay, we used miglitol as a positive control to validate the inhibitory effect of Kenyan coffee on α -glucosidase activity. Kenyan coffee decreased α -glucosidase activity, similar to that seen by miglitol (Figure 2C).

Effect of Kenyan coffee on interstitial glucose levels

Because drinking Kenyan coffee appeared to decrease the interstitial glucose levels by inhibiting α -glucosidase activity, we tested the effects of drinking Kenyan coffee on postprandial interstitial glucose levels after simultaneous consumption of the test meal and Kenyan coffee; hence, we wished to examine whether a larger effect compared with that of the experimental condition shown in Figure 1 could be observed. As predicted, Kenyan coffee intake significantly and greatly reduced the postprandial interstitial glucose

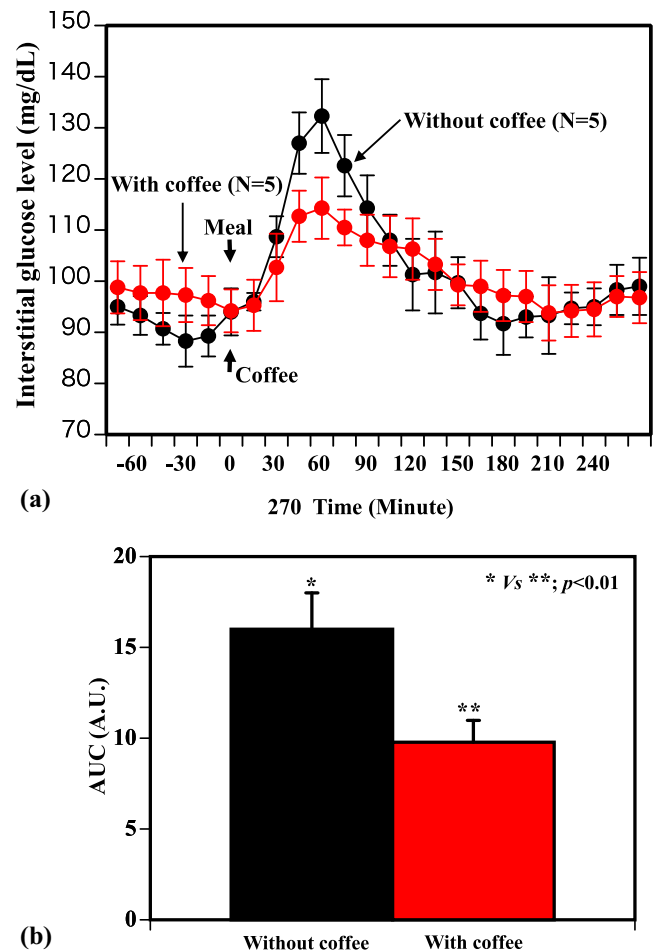


Figure 3. Effect of Kenyan coffee on postprandial interstitial glucose levels. (A) The y-axis shows the postprandial interstitial glucose levels measured every 15 minutes using the FreeStyle Libre glucose monitoring system (Abbott Diabetes Care Ltd, Witney, United Kingdom), and the x-axis shows time (in minutes), with and without coffee consumption. The red line with circles represents Kenyan coffee intake (experiments were repeated independently 5 times), and the black line with circles represents no Kenyan coffee intake (experiments were repeated independently 3 times). (B) This bar graph represents the area under the curve (AUC) of the line graph shown in Figure 3A (trapezoid area was drawn using baseline every 15 minutes, and each area was mathematically calculated by using the formula). The black bar represents the condition with no coffee intake, and the red column represents the condition with coffee intake. Error bars represent the standard deviation. The AUC of the condition with no coffee intake was statistically larger than that of the condition with coffee intake ($P < 0.01$).

levels compared with the experiment in which no coffee had been consumed (Figure 3A). The differences in interstitial glucose levels were represented as the AUC (the trapezoid area was drawn using baseline every 15 minutes, and each area was mathematically calculated by using the formula) in bar graphs and statistically quantitated (Figure 3B). It was confirmed that consumption of Kenyan coffee significantly reduced postprandial interstitial glucose levels compared with the condition in which no coffee was consumed ($P < 0.01$).

Effect of Kenyan coffee intake on uric acid levels

Consuming excessive amounts of caffeine reportedly increases uric acid levels.⁸ Therefore, we speculated that we could confirm that the measured caffeine content of Kenyan coffee was lower than that of Tanzanian coffee by comparing uric acid levels of the volunteer after drinking Tanzanian coffee and after drinking Kenyan coffee, respectively. As shown in Figure 4, uric acid lev-

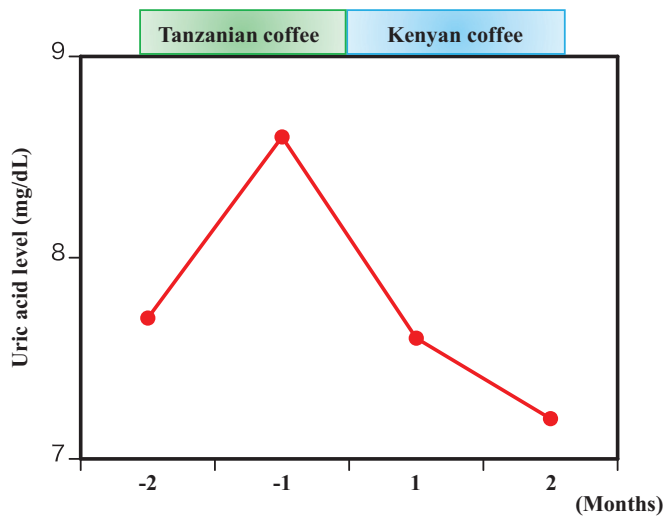


Figure 4. Effect of Kenyan coffee intake on uric acid levels. We compared uric acid levels of the volunteer after drinking Tanzanian and Kenyan coffee, respectively, and the change in uric acid levels is shown. In the present study, the volunteer was asked to drink Tanzanian coffee every day for 2 months, and thereafter, the volunteer was requested to switch to drinking Kenyan coffee every day for the following 2 months. Serum uric acid levels were measured once a month. The y-axis shows the uric acid levels measured once a month, and the x-axis shows the time (in months). Green rectangle = Tanzanian coffee consumption; blue rectangle = Kenyan coffee consumption.

els were markedly lower after drinking Kenyan coffee compared with after drinking Tanzanian coffee. Thus, these data are consistent with the finding that the caffeine content of Kenyan coffee is lower than that of Tanzanian coffee.

Estimation of chlorogenic acid and anhydrous coffee content

The mean (SD) chlorogenic acid content of Kenya coffee was 0.69 (0.04) g/100 g, Tanzania coffee was 0.27 (0.0435) g/100 g, and Ethiopian coffee was 0.33 (0.05) g/100 g. The anhydrous caffeine content of Kenya coffee was 1.12 (0.08) g/100 g, Tanzania coffee was 1.35 (0.09) g/100 g, and Ethiopian coffee was 1.47 (0.07) g/100 g.

Discussion

Coffee is among the most widely consumed beverages globally. Prospective studies have shown that coffee drinking is consistently inversely associated with all-cause and cause-specific mortalities.¹ However, the effects of coffee intake have raised some concerns because coffee is a major source of caffeine, which increases plasma glucose levels by elevating epinephrine levels and increasing insulin resistance.² Therefore, in this pilot study, we compared the effect of 3 different coffee beverages (Kenyan, Tanzanian, or Ethiopian) on postprandial glucose levels by measuring interstitial glucose levels every 15 minutes using the FreeStyle Libre glucose monitoring system in a single individual without diabetes, compared with no coffee consumption after meal.

Although interstitial glucose levels tend to correlate well with plasma glucose levels,⁹ we could not find literature to report the possibility about interference of caffeine with flash glucose monitoring system and we could not examine and rule out that possibility in this pilot study. Thus, we have to pay attention about accuracy of measurement values when we handle flash glucose monitoring systems.¹⁰

Nevertheless, to the best of our knowledge, this pilot study is the first to use the FreeStyle Libre glucose monitoring system to

Table 1

Estimation of chlorogenic acid and anhydrous caffeine contents. Chlorogenic acid (g/100 g) and anhydrous caffeine (g/100 g) levels measured in Kenyan, Tanzanian, and Ethiopian coffee beans are shown. Measurements were repeated 3 times and the results are represented as mean (SD).

	Kenya	Tanzania	Ethiopia
Chlorogenic acid (g/100 g)	0.69 (0.04)	0.27 (0.035)	0.33 (0.05)
Anhydrous caffeine (g/100 g)	1.12 (0.08)	1.35 (0.09)	1.47 (0.07)

examine the effects of drinking Kenyan, or Tanzanian, or Ethiopian coffee on postprandial interstitial glucose levels. Drinking Kenyan coffee reduced the postprandial interstitial glucose levels compared with drinking Tanzanian, Ethiopian, or no coffee (Figure 1). We used coffee beans roasted to a similar extent to minimize the effect of temperature on coffee bean content during roasting (Figure 1C).

To search for the mechanism, first, we examined whether the Kenyan coffee might contain an insulin-like substance. To explore this possibility, the volunteer drank Kenyan coffee without consuming any meals and then fasted for 6 hours. Because hypoglycemia did not occur within the observation period, it was suggested that Kenyan coffee did not contain any insulinogenic substances (Figure 2A). Second, we examined whether Kenyan coffee directly affects insulin signaling. As shown in Figure 2B, tyrosine-phosphorylated IRS-1, and insulin receptor β -subunit levels by insulin stimulation were similar in samples with and without the addition of coffee in the culture medium (Figure 2B). These results further suggested that Kenyan coffee did not directly affect insulin signaling. Third, we considered the possibility that Kenyan coffee decreased glucose absorption at the small intestine level and we examined whether Kenyan coffee had an inhibitory effect on α -glucosidase activity. We found that Kenyan coffee decreased α -glucosidase activity, similar to that seen by miglitol (Figure 2C). Next, to identify the mechanism by which Kenyan coffee decreased α -glucosidase activity, we measured the chlorogenic acid levels in Kenyan, Tanzanian, and Ethiopian coffee beans because chlorogenic acid was reported to decrease plasma glucose levels by reducing α -glucosidase activity.⁵ We found that Kenyan coffee beans contained the highest chlorogenic acid levels among the 3 types of coffee beans tested (Table 1). We also evaluated the anhydrous caffeine content, as anhydrous caffeine in coffee beans increases plasma glucose levels by elevating catecholamine levels and exacerbating insulin resistance.^{6,7} Kenyan coffee beans had the lowest anhydrous caffeine content among the 3 types of coffee beans tested. Consistent with these data, drinking Kenyan coffee reduced uric acid levels compared with drinking Tanzanian coffee.

These results may explain the decrease in postprandial interstitial glucose levels observed after Kenyan coffee intake. However, further studies are required to determine the exact underlying mechanisms involved to elucidate the differences between Kenyan, Tanzanian, and Ethiopian coffee beans and their effects on postprandial interstitial glucose levels.

Our study has several limitations. First, the results were obtained from a single participant, which limits the generalizability of the results. The study might also exhibit selection bias due to the ethnicity of the participant. However, despite the single-sample study design, we could compare and evaluate the characteristics of each coffee bean type. Therefore, the present study can be viewed as a pilot study and an incentive to compare the effects of the different types of coffee on plasma glucose levels. Further studies involving a larger number of participants are required to evaluate the reproducibility of our findings.

Conclusions

Although coffee is enjoyed by both healthy people and patients with diabetes mellitus worldwide, to compare and select suitable coffee beans may be a useful idea for maintaining good plasma glucose control.

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Conflicts of Interest

Miglitol was provided by Sanwa Kagaku Kenkyusho Co, Ltd (Nagoya, Aichi, Japan). The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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