JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 5597–5603 5597
DOI:10.1021/jf904062c

Coffee and Caffeine Ameliorate Hyperglycemia, Fatty Liver, and Inflammatory Adipocytokine Expression in Spontaneously Diabetic KK-A^y Mice

Rie Yamauchi,[†] Misato Kobayashi,[†] Yuji Matsuda,[†] Makoto Ojika,[†] Shigeru Shigeoka,[§] Yuko Yamamoto,[#] Yoshie Tou,[#] Takashi Inoue,[#] Takao Katagiri,[#] Atsushi Murai,[†] and Fumihiko Horio*,[†]

[†]Department of Applied Molecular Bioscience, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan, [§]Department of Advanced Bioscience, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan, and [#]Product Research and Development Department, Pokka Corporation, Aichi 481-8515, Japan

Epidemiological surveys have demonstrated that habitual coffee consumption reduces the risk of type 2 diabetes. The aim of this work was to study the antidiabetic effect of coffee and caffeine in spontaneously diabetic KK-A^y mice. KK-A^y mice were given regular drinking water (controls) or 2-fold diluted coffee for 5 weeks. Coffee ingestion ameliorated the development of hyperglycemia and improved insulin sensitivity. White adipose tissue mRNA levels of inflammatory cytokines (MCP-1, IL-6, and TNFα), adipose tissue MCP-1 concentration, and serum IL-6 concentration in the coffee group were lower than the control group. Moreover, coffee ingestion improved the fatty liver. Caffeine ingestion as drinking water also caused an amelioration of hyperglycemia and an improvement of fatty liver. These results suggest that coffee exerts a suppressive effect on hyperglycemia by improving insulin sensitivity, partly due to reducing inflammatory cytokine expression and improving fatty liver. Moreover, caffeine may be one of the effective antidiabetic compounds in coffee.

KEYWORDS: Coffee; caffeine; diabetes; blood glucose; insulin; mice

INTRODUCTION

Type 2 diabetes is a chronic disease associated with high rates of morbidity and premature mortality. An alarming increase in the prevalence of type 2 diabetes is expected, and the need for preventive action is widely acknowledged. While increased physical activity and restriction of energy intake can substantially reduce the incidence of type 2 diabetes, insight into the role of other lifestyle factors may contribute to additional prevention strategies for type 2 diabetes.

Coffee is among the most widely consumed beverages in the world. In 2002, results from a Dutch cohort study (*1*) demonstrated that higher coffee consumption was associated with a lower risk of type 2 diabetes. This finding was confirmed in subsequent studies (*2*, *3*). Recently, much attention has been focused on this topic, and it has been hotly discussed (*4*).

Coffee contains numerous substances; among them, caffeine, chlorogenic acid, quinides, trigonelline, and lignan have been shown to affect glucose metabolism in animals or metabolic studies (*5*−*11*). Nonetheless, it has not been determined which of these substances is the main antidiabetic compound in coffee. The physiological effects of caffeine have been widely studied (*12*). It is well-known that caffeine acts as an antagonist of adenosine receptors. Adenosine is an inhibitory neuromodulator involved in sleep−wake regulation, and caffeine promotes wakefulness by acting as an antagonist of adenosine A_{2A} receptors (*13*). Moreover, some studies have reported that caffeine induces energy expenditure by increasing thermogenesis (*5*, *14*). From this point of view, it is expected that caffeine prevents the development of obesity and, thereby, has an antidiabetic effect. On the other hand, some compounds other than caffeine in coffee might have an antidiabetic effect; several cohort studies have reported that decaffeinated coffee consumption reduces the risk of type 2 diabetes as well (*15*−*17*).

The potential for suppression of hyperglycemia through a common beverage such as coffee could be exploited as a strategy to prevent type 2 diabetes. Thus, an experimental study using a spontaneously diabetic animal model would be invaluable for examining the antidiabetic potency of coffee. Nonetheless, few such studies have been performed. In this study, we used KK-A^y mice that spontaneously develop type 2 diabetes to investigate the preventive effect of coffee on the development of hyperglycemia. The KK-A^y mouse is a polygenic model for type 2 diabetes; it shows obesity due to hyperphagia and develops hyperglycemia at 6 weeks of age. Hyperinsulinemia, hypertriglyceridemia, and fatty liver are also observed. Hence, the hyperglycemia of KK-A^y mice is mainly due to the insulin resistance in peripheral tissues. In addition, KK-A^y mice have been reported to show altered production of adipocytokines in adipose tissue compared to nondiabetic control mice. For example, the production of adiponectin, which is

*Corresponding author (telephone +81-52-789-4075; fax +81-52-789-4075; e-mail horiof@agr.nagoya-u.ac.jp).

Published on Web 04/21/2010

an adipocytokine improving insulin sensitivity in peripheral tissues, is decreased in KK-A^y mice (*18*). The production of monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor α (TNF-α), which are adipocytokines inducing insulin resistance in peripheral tissues, is elevated in KK-A^y mice. These alterations in adipocytokine production could contribute to the incidence of insulin resistance in KK-A^y mice.

In this study, we first examined whether the ingestion of coffee would prevent the development of hyperglycemia in KK-A^y mice. Second, we investigated the mechanism of action of coffee on the pathogenesis of type 2 diabetes in KK-A^y mice. Subsequently, to identify the compound contributing the antihyperglycemic effect, we attempted to clarify the preventive effect of caffeine on the development of hyperglycemia. The results show that coffee and caffeine have antidiabetic effects and suggest that coffee and caffeine ameliorate insulin resistance in KK-A^y mice.

MATERIALS AND METHODS

Chemicals. Black regular canned coffee was a gift from Pokka Corp. (Aichi, Japan). Coffee was used after 2-fold dilution with water. The concentrations of caffeine, chlorogenic acid, and trigonelline in the diluted coffee used in this experiment were 290, 190, and 230 mg/L, respectively. The caffeine used in experiment 2 was purchased from Wako Pure Chemical (Tokyo, Japan).

Animals. Four-week-old male KK-A^y mice (CLEA, Tokyo, Japan) were used for all experiments and were maintained at a controlled temperature of 23 ± 3 °C and 55 ± 5% humidity on a 12 h light/dark cycle. The mice were allowed free access to water and a standard laboratory diet (CE-2; CLEA Japan, Inc.) for 3 days before the experiments were begun. The composition of the diet was as follows: protein, 254 g/kg; fat, 51 g/kg; non-nitrogenous substances, 506 g/kg; crude fiber, 35 g/kg; crude ash, 67 g/kg; energy, 15.2 MJ/kg; sufficient minerals and vitamins to maintain the health of mice.

In experiment 1, mice were divided into two groups and given water (control group, 11 mice) or diluted black coffee (black coffee/water = 1:1) (coffee group, 10 mice) as drinking water. Coffee solutions was prepared every second day. Nondiluted coffee was stored at −20 °C until the dilution. Mice were allowed free access to drinking water and diet (CE-2; CLEA) for 5 weeks. During the course of the experiment, blood samples were collected from the tail vein once a week for measurement of the serum glucose concentration. The blood samples were collected from mice at 10:00 a.m., 1 h after the removal of diet. At the end of the experiment, the mice were killed by decapitation (between 10:00 a.m. and 12:00 p.m.), and the blood was collected. Using these blood samples, serum insulin, triglyceride (TG), and total cholesterol (TC) were measured. The liver, white adipose tissues (WAT) (subcutaneous, epididymal, mesenteric, and retroperitoneal fat tissues), and interscapular brown adipose tissue (BAT) were removed. Subcutaneous fat tissue was defined as fat pads below the root of the forefoot on the side of the body. They were then immediately frozen using liquid nitrogen and kept at −80 °C until use. The collected blood was kept at room temperature for 15 min for coagulation. Then, the serum was obtained from the coagulated blood by centrifugation at 2430*g* for 10 min at 4 °C. The serum was kept at −30 °C prior to use. We conducted the same experiment using KK-A^y mice twice. In the second experiment, we performed only the measurement of nonfasting blood glucose concentration and the insulin tolerance test.

In experiment 2, 10 mice were divided into two groups and given water (control group, 5 mice) or caffeine solution (250 mg of caffeine/L) (250CA group, 5 mice) as drinking water. Caffeine solution was prepared every second day. The other experimental procedures were the same as in experiment 1.

The animal care and experimental procedures were approved by the Animal Research Committee of Nagoya University and were conducted according to the Regulations for Animal Experiments at Nagoya University.

Analysis of Metabolic Parameters. Serum glucose was measured by the assay kit using glucose oxidase method, Glucose CII-test (Wako Pure Chemical). Serum triglycerides and cholesterol concentrations were measured by the assay kit using the glycerol-3-phosphate oxidase method, Triglyceride-E test (WAKO Pure Chemical), and the cholesterol oxidase method, Cholesterol-E test (Wako Pure Chemical), respectively.

Commercially available ELISA kits were used for determining the serum concentrations of insulin (Morinaga Seikagaku, Kanagawa, Japan), MCP-1 (R&D Systems, Minneapolis, MN), and interleukin-6 (Millipore, Bedford, MA).

For determination of the MCP-1 concentration in epididymal WAT, tissue (0.4 g) was homogenized with PBS that contained protease inhibitor (Complete Mini; Roche Diagnostics, Tokyo, Japan). After centrifugation at 2000*g* for 10 min at 4 °C, the clear aqueous phase under the top lipid layer were carefully collected into another tube. This aqueous phase were centrifuged again at 2000*g* for 10 min at 4 °C. The clear supernatant was assayed for the presence of MCP-1 by using an ELISA kit (Pierce Biotechnology, Rockford, IL).

Insulin Tolerance Test (ITT). An ITT was performed at 5 weeks after the start of the experiment with the following protocol. After a 15 h fast, blood samples were collected from the tail vein (fasting blood sample, 0 min sample in ITT). Then, human insulin (Humulin; 0.67 unit/kg of BW; Eli Lilly, Kobe, Japan) solution was injected intraperitoneally. Blood samples were collected from the tail vein at 30, 60, 120, and 150 min after the injection, and the serum glucose concentration was measured.

Hepatic Lipid Analysis. Frozen livers were homogenized with chloroform/methanol (2:1), and liver lipids were extracted into organic solvents. A portion of this extract was dried, and the hepatic contents of TG, TC, and phospholipids (PL) were measured by means of a Triglyceride E-Test, Cholesterol E-Test, and phospholipid C-Test (Wako Pure Chemical), respectively. This extract was also used to measure the total lipids content according to the method of Folch et al. (*19*).

RNA Preparation and Expression Level Analysis. Total RNA was extracted from frozen tissues using TRIzol reagent (Invitrogen, Carlsbad, CA). Then, it was treated with DNase using a Turbo DNA-free kit (Ambion, Austin, TX). cDNA was synthesized using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantification of gene expression was measured by real-time PCR that was performed with an ABI 7300 real-time PCR System using the PCR Master Mix Reagent and probes (TaqMan Gene Expression Assays; Applied Biosystems). The level of each mRNA was normalized to that of the corresponding 18S rRNA.

Statistical Analysis. All results are expressed as the means ± SEM. Phenotypic data were statistically analyzed by either Student's *t* test or Welch's test. When the variances of each group were equal, mean values were compared using the former test. When the variances of each group were unequal, significance of differences was determined using the latter test. Values of $p < 0.05$ were considered to be statistically significant (Stat View; SAS Institute, Cary, NC).

RESULTS

Effects of Coffee Ingestion on Blood Glucose Concentration and Lipid Metabolism in KK-A^y Mice (Experiment 1). In experiment 1, 4-week-old KK-A^y mice ingested coffee or water as their drinking water for 5 weeks. The body weight, food intake, and tissue weights are shown in **Table 1**. The final body weight did not differ between the control and coffee groups. The food intake (on days 11−13 and 25−27) was also not different between these two groups. Coffee ingestion reduced subcutaneous or retroperitoneal fat tissue weight, but did not affect epididymal or mesenteric fat tissue or interscapular BAT weights. The liver weight in the coffee group was significantly lower than that in the control group. As shown in **Figure 1A**, the blood glucose concentration in the control group gradually increased during the course of the experiment, reaching a maximum of 30.2 ± 1.5 mmol/L. After the second week, the blood glucose concentrations in the coffee groups were significantly lower than the respective values in the control group. Finally, blood glucose concentration in the coffee group (19.6 ± 1.7 mM) exhibited a 30% decrease compared with that in the control group (28.1 ± 1.5 mM) (**Figure 1A**).

The nonfasting serum insulin concentrations at the end of the experiment in the coffee and control groups were not significantly

Table 1. Initial and Final Body Weights, Tissue Weights, Serum Components, Inflammatory Markers, and Liver Lipid Contents of KK-A^y Mice Treated with Water or Coffee for 5 Weeks (Experiment 1)[a]

parameter	control (n = 11)	coffee (n = 10)	p value
body weight (g)			
initial	19.2 ± 0.3	19.0 ± 0.4	>0.1
final	40.4 ± 1.0	38.5 ± 0.6	>0.1
food intake (g/100 g of BW/day)			
days 11−13	18.6 ± 0.4	18.6 ± 0.3	>0.1
days 25−27	16.8 ± 0.4	15.8 ± 0.4	0.0658
tissue weights (g/100 g of BW)			
liver	6.40 ± 0.13	5.57 ± 0.10*	<0.0001
subcutaneous fat	1.80 ± 0.11	1.46 ± 0.09*	0.0259
epididymal fat	3.52 ± 0.10	3.25 ± 0.11	0.0768
retroperitoneal fat	0.90 ± 0.04	0.75 ± 0.02*	0.0025
mesenteric fat	1.84 ± 0.09	1.70 ± 0.06	>0.1
interscapular BAT	0.35 ± 0.03	0.38 ± 0.02	>0.1
serum components			
nonfasting insulin (final; ng/mL)	12.89 ± 2.61	9.51 ± 1.64	>0.1
nonfasting TG (mmol/L)	4.20 ± 0.31	3.39 ± 0.16*	0.0346
nonfasting TC (mmol/L)	3.50 ± 0.24	4.05 ± 0.19	>0.1
inflammatory markers			
serum MCP-1 (pmol/L)	3.35 ± 0.28	2.57 ± 0.22	0.0726
serum IL-6 (pg/mL)	5.34 ± 1.21	2.54 ± 0.50*	0.0478
MCP-1, epididymal fat (pmol/g of tissue)	0.053 ± 0.005	0.039 ± 0.002*	0.0163
liver lipids			
total lipids (mg/100 g of BW)	437 ± 33	320 ± 15*	0.0059
TG (μmol/100 g of BW)	179 ± 15	117 ± 10*	0.0035
TC (μmol/100 g of BW)	47.7 ± 1.5	43.5 ± 1.0*	0.0393
PL (μmol/100 g of BW)	141 ± 5	130 ± 3	>0.1

[a] Data are expressed as means ± SE; *, $p < 0.05$ versus the control group. BAT, brown adipose tissue; TG, triglyceride; TC, total cholesterol; PL, phospholipid; BW, body weight.

different (**Table 1**). To confirm the effect of coffee ingestion on insulin sensitivity, an ITT was performed 5 weeks after the start of the experiment. The glucose-lowering effect of insulin was greater in the coffee group compared to the control group, and the blood glucose concentration at 60 or 150 min after the injection in the coffee group was significantly lower than the respective value in the control group (**Figure 1B**).

The serum TG and TC concentrations, liver total lipids content, liver TG content, liver TC content, and liver PL content in experiment 1 are shown in **Table 1**. The serum TG concentration was lower in the coffee group than the control group. On the other hand, the serum TC concentration did not differ between the control and coffee groups. The liver total lipids content, TG content, and TC content were significantly lower in the coffee group than in the control group. Liver PL content did not differ between these two groups.

Alteration of Aipocytokine mRNA Levels in the Epididymal Fat Tissue by Coffee Ingestion (Experiment 1). To investigate the mechanism by which coffee improves insulin sensitivity, we measured epididymal fat tissue mRNA levels of adipocytokines that are known to influence insulin sensitivity. The results are shown in **Figure 2A**. MCP-1, TNF-α, and IL-6 are adipocytokines that cause insulin resistance in peripheral tissues. MCP-1, TNF-α, and IL-6 mRNA levels in the coffee group were significantly lower than the respective levels in control group. The mRNA level of F4/80, one of the marker molecules of macrophages, was lower in the coffee group than in the control group. Adiponectin and leptin are adipocytokines that improve insulin sensitivity. Adiponectin and leptin mRNA levels did not differ between the control and coffee groups. The serum concentration of MCP-1 in the coffee group tended to be lower than that in the control group (Table**1**), but not significantly so. The MCP-1 concentration in epididymal fat tissue and serum IL-6 concentration were significantly lower in the coffee group compared with the respective values in the control group (**Table 1**).

Alteration of Gene Expression in the Liver by Coffee Ingestion (Experiment 1). Coffee ingestion decreased the liver TG content compared with that in the control group (**Table 1**). We measured the hepatic levels of the mRNA of genes relating to fatty acid synthesis, fatty acid oxidation, and insulin signaling, and the results are shown in **Figure 2B**. Sterol regulatory element binding protein-1 (SREBP-1) is indicated to up-regulate the expression of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), which are regulatory enzymes of fatty acid synthesis (*20*). Conversely, SREBP-1 down-regulates the expression of insulin receptor substrate-2 (IRS-2), that is, the intracellular substrate of phosphorylation by insulin action (*21*). As shown in **Figure 2B**, the SREBP-1 and FAS mRNA levels in the coffee group were lower than those in the control group. The hepatic mRNA level of ACC was not different between the control and coffee groups. The hepatic IRS-2 mRNA level in the coffee group was higher than that in the control group. On the other hand, peroxisome proliferator activator receptor α (PPAR-α) is a transcriptional factor that up-regulates the expression of carnitine palmitoyltransferase-1 (CPT-1), the regulatory enzyme in fatty acid β-oxidation. Hepatic PPAR-α and CPT-1 mRNA levels did not differ between the control and coffee groups.

Antidiabetic Action of Caffeine as an Effective Compound in Coffee (Experiment 2). To investigate the antidiabetic action of caffeine in coffee, we treated KK-A^y mice with caffeine solution (250 mg/L) as drinking water in experiment 2. The caffeine concentration (250 mg/L) was comparable to the concentration in the diluted coffee solution used in experiment 1.

The final body weight and food intake (on days 11−13 and 25−27) did not differ between the control and caffeine groups (**Table 2**). However, the liver, subcutaneous fat, epididymal fat, or retroperitoneal fat weights in the 250 mg of caffeine/L (250CA)

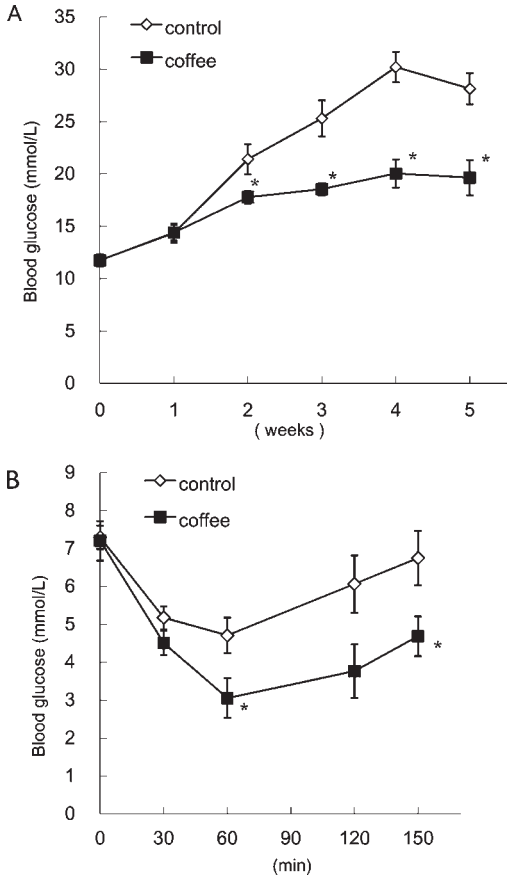

Figure 1. Time course of blood glucose concentration and insulin sensitivity in the control and coffee groups (experiment 1): (**A**) time course of blood glucose concentration of KK-A^y mice in the control (n = 11) or coffee (n = 10) group; (**B**) glucose-lowering effect of insulin in the insulin tolerance test in KK-A^y mice after 5 weeks of treatment with water or coffee. Data are the means ± SE; ∗, $p < 0.05$ versus the control group.

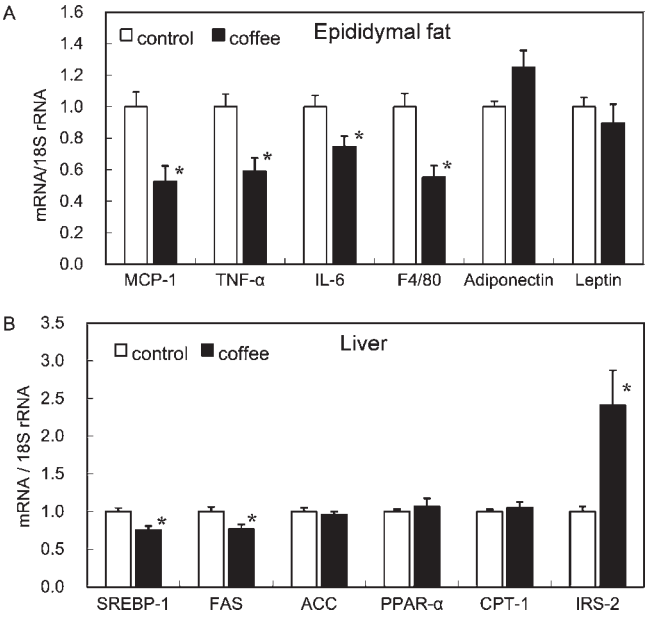

Figure 2. Epididymal fat tissue and hepatic mRNA levels: (**A**) epididymal fat tissue mRNA levels of the indicated genes determined by real-time RT-PCR (results were expressed as fold change versus the control group set to 1 unit); (**B**) hepatic mRNA levels of the indicated genes determined by real-time RT-PCR (results were expressed as fold change versus the control group set to 1 unit). Mice in the control (n = 11) or coffee (n = 10) group were treated with water or coffee, respectively, for 5 weeks. Data are the means ± SE; ∗, $p < 0.05$ versus the control group.

group were significantly lower than the respective values in the control group. The blood glucose concentration during the course of the experiment is shown in **Figure 3**. Hyperglycemia developed in the control group. In the 250CA group, the blood glucose concentration tended to be lower than the control group at all time points after the second week of the experiment. The blood glucose concentration in the 250CA group on day 21 was significantly lower than the respective value in the control group. At 5 weeks, blood glucose concentration in the 250CA group (15.7 ± 2.6 mM) exhibited a 25% decrease compared with that in the control group (21.7 ± 2.3 mM) (**Figure 3**). The nonfasting serum insulin concentration was not significantly different between two groups at the end of the experiment (**Table 2**). These results show that caffeine ingestion, like coffee ingestion, ameliorates the development of hyperglycemia.

The serum TG and TC concentrations in the 250CA group were significantly lower than those in the control group (**Table 2**). The hepatic total lipids and TG contents were significantly decreased in the 250CA group compared with the control group. Thus, caffeine ingestion seems to ameliorate the development of hypertriglyceridemia and fatty liver.

The adipocytokine mRNA levels in epididymal fat tissue in the control and 250CA groups are shown in **Figure 4A**. MCP-1 and F4/80 mRNA levels in the 250CA group were lower than those in the control group. The TNF-α, IL-6, and adiponectin mRNA levels were not different between the two groups. The leptin mRNA level was significantly lower in the 250CA group than in the control group. Caffeine ingestion did not significantly decrease the serum concentration of MCP-1. However, the MCP-1 concentration in epididymal fat tissue in the 250CA group was significantly lower than that in the control group (**Table 2**). The

Table 2. Initial and Final Body Weights, Tissue Weights, Serum Components, Inflammatory Markers, and Liver Lipid Contents of KK-A^y Mice Treated with Water or 250 mg/L (250CA) Caffeine Solution for 5 Weeks (Experiment 2)*

parameter	control (n = 5)	250CA (n = 5)	p value
body weight (g)			
initial	19.8 ± 0.3	19.9 ± 0.3	>0.1
final	40.0 ± 0.7	38.6 ± 1.2	>0.1
food intake (g/100 g of BW/day)			
days 11−13	18.3 ± 0.8	18.0 ± 0.4	>0.1
days 25−27	16.0 ± 1.1	13.1 ± 1.2	>0.1
tissue weights (g/100 g of BW)			
liver	6.09 ± 0.14	5.38 ± 0.22*	0.0253
subcutaneous fat	1.74 ± 0.06	1.38 ± 0.10*	0.0141
epididymal fat	3.79 ± 0.14	3.34 ± 0.13*	0.0447
retroperitoneal fat	0.91 ± 0.03	0.75 ± 0.03*	0.0049
mesenteric fat	1.53 ± 0.09	1.45 ± 0.07	>0.1
interscapular BAT	0.41 ± 0.03	0.44 ± 0.03	>0.1
serum components			
nonfasting insulin (ng/mL)	8.42 ± 2.01	5.62 ± 1.53	>0.1
nonfasting TG (mmol/L)	4.98 ± 0.34	3.07 ± 0.43*	0.0083
nonfasting TC (mmol/L)	4.95 ± 0.18	3.81 ± 0.34*	0.0214
inflammatory markers			
serum MCP-1 (pmol/L)	3.75 ± 0.41	2.99 ± 0.40	>0.1
MCP-1, epididymal fat (pmol/g of tissue)	0.046 ± 0.004	0.031 ± 0.003	0.0101
liver lipids			
total lipid (mg/100 g of BW)	448 ± 28	343 ± 19*	0.0144
TG (μmol/100 g of BW)	114 ± 9	81 ± 8*	0.0251
TC (μmol/100 g of BW)	49.5 ± 1.3	45.3 ± 2.3	>0.1
PL (μmol/100 g of BW)	147 ± 6	143 ± 6	>0.1

* Data are expressed as means ± SE; *, $p < 0.05$ versus the control group. BAT, brown adipose tissue; TG, triglyceride; TC, total cholesterol; PL, phospholipid; BW, body weight.

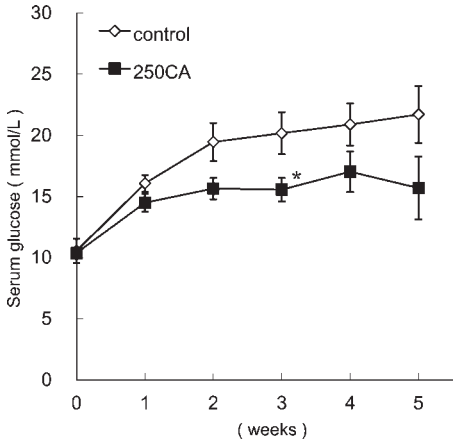

Figure 3. Time course of blood glucose concentration in the control and 250CA groups (experiment 2). Data are the means ± SE; *, $p < 0.05$ versus the control group.

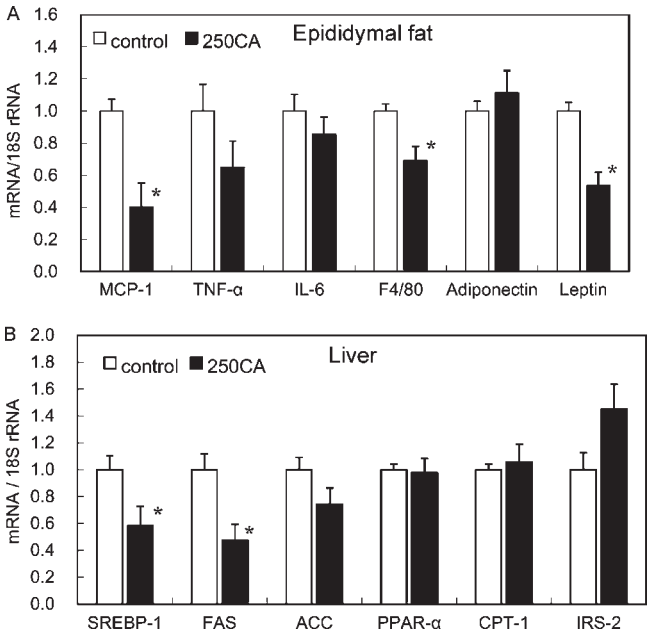

Figure 4. Epididymal fat tissue and hepatic mRNA levels.: (**A**) epididymal fat tissue mRNA levels of the indicated genes determined by real-time RT-PCR (results were expressed as the fold change versus the control group set to 1 unit); (**B**) hepatic mRNA levels of the indicated genes determined by real-time RT-PCR (results were expressed as fold change versus the control group set to 1 unit). Mice in the control ($n = 5$) or 250CA ($n = 5$) groups were treated with water or 250 mg/L caffeine solution, respectively, for 5 weeks. Data are the means ± SE; *, $p < 0.05$ versus the control group.

change in epididymal fat tissue MCP-1 concentration paralleled the change in the MCP-1 mRNA level in epididymal fat tissue by caffeine ingestion.

As shown in **Figure 4B**, in the liver, SREBP-1 and FAS mRNA levels were significantly lower in the 250CA group compared with the control group. The ACC, PPAR-α and CPT-1 mRNA levels were not different between these two groups. The IRS-2 mRNA level tended to be increased by caffeine treatment, but not significantly. Thus, the alterations of mRNA levels observed in the livers of mice treated with caffeine were almost similar to those observed in the mice treated with coffee.

DISCUSSION

In this study, we demonstrated that coffee ingestion ameliorated the development of hyperglycemia in KK-A^y mice, which spontaneously develop type 2 diabetes. KK-A^y mice develop hyperglycemia and hyperinsulinemia mainly due to insulin resistance in peripheral tissues. The antidiabetic effect of coffee observed in this study was not due to the reduction of food intake or body weight loss of KK-A^y mice. From the results in the ITT, coffee ingestion enhanced the insulin sensitivity in KK-A^y mice. It is considered that the improvement of insulin resistance by coffee ingestion contributes to its suppressive effect on hyperglycemia.

The expression of inflammatory cytokines, such as TNF-α, MCP-1, and IL-6, in adipose tissue is up-regulated in obesity and type 2 diabetes, suggesting that these adipocytokines contribute to insulin resistance (*22*, *23*). TNF-α is indicated to phosphorylate serine/threonine residue of IRS-1 through the activation of JNK (c-jun N-terminal kinase), IKKβ (inhibitor of NF-κB kinase β), PKC (protein kinase C), or ERK (extracellular signal-related kinase) (*24*). Phosphorylation of IRS-1 by TNF-α interferes with the subsequent insulin-stimulated tyrosine phosphorylation of IRS-1, leading to insulin resistance. MCP-1 is a significant signal that triggers macrophage infiltration into adipose tissues and that leads to inflammation (*25*, *26*). The expression of MCP-1 in adipocytes is up-regulated by TNF-α or the mitogen-activated protein kinase (MAPK) pathways (*27*). In addition, MCP-1 has been reported to play a crucial role in the development of insulin resistance in various tissues by acting through its receptor, chemokine C−C motif receptor 2 (CCR2) (*28*). IL-6 up-regulates suppressor of cytokine signaling (SOCS) expression through activation of the Janus kinase-signal transduction and activator of transcription (JAK-STAT) pathway in liver and adipose tissues and inhibits insulin signaling (*29*, *30*). The expression of IL-6 is also induced by TNF-α. In this study, coffee ingestion significantly decreased MCP-1, TNF-α, and IL-6 mRNA levels in adipose tissue. A significant decrease of MCP-1 concentration in the adipose tissue, a nonsignificant tendency of decrease in serum MCP-1 concentration, and a significant decrease in serum IL-6 concentration were observed in the coffee group compared to the control group. Moreover, coffee ingestion decreased the adipose tissue mRNA level of F4/80, which is a macrophage marker. From these results, coffee ingestion lowers the production of the inflammatory adipocytokines and restrains adipose tissue inflammation, leading to the amelioration of insulin resistance in various tissues.

It has been elucidated that fatty liver causes insulin resistance in liver (*31*). Interestingly, in this study, coffee ingestion caused an improvement of dyslipidemia, which is one of the characteristic conditions of KK-A^y mice; that is, coffee ingestion improved fatty liver and reduced the serum TG concentration. Then, we examined alterations in the hepatic expression of genes involved in fatty acid synthesis and oxidation. Our results showed that the expression of genes related to fatty acid synthesis was decreased in the mice administered coffee. Expressions of the FAS and ACC genes are known to be up-regulated by the transcription factor SREBP-1 (*20*). In this study, the hepatic expression of the SREBP-1 and FAS genes was lowered by coffee ingestion. Although the expression of ACC was not changed by coffee ingestion, the reason for this insensitivity of ACC to coffee ingestion is not clear. On the other hand, coffee ingestion did not affect the hepatic expression of genes related to fatty acid oxidation. Thus, it is supposed that fatty acid synthesis was decreased by coffee ingestion, leading to the improvement of fatty liver. In addition, it has been reported that the expression of IRS-2 is down-regulated by SREBP-1 (*21*). In this study, we observed that the hepatic expression of the IRS-2 gene

was increased by coffee ingestion. Thus, it was considered that this increase in IRS-2 expression contributed to the improvement of hepatic insulin resistance in the coffee group. Ueki et al. (*32*) reported that SREBP-1 expression is increased by SOCS1 and SOCS3, the expressions of which are stimulated by inflammatory cytokines, especially by IL-6 (*33*). The result of Ueki et al. (*32*) and the present findings suggest that the decreased production of inflammatory cytokines in the adipose tissue by coffee ingestion causes the reduction of SREBP-1 expression in the liver via a reduction in SOCS expression.

Among the components included in coffee, we chose to test the efficacy of caffeine in reducing hyperglycemia. We found that caffeine was effective at ameliorating the development of hyperglycemia. It has been reported that caffeine induces thermogenesis and energy expenditure in humans (*5*). In animal experiments, some reports have shown that long-term consumption of caffeine decreases subcutaneous adipose tissue weight, blood TG concentration, and hepatic TG concentration (*34*, *35*). In this study, coffee and caffeine ingestion decreased subcutaneous, epididymal, and retroperitoneal fat tissue weights. Other studies have reported that intraperitoneal injection of caffeine increased uncoupling protein-1 (UCP-1) expression in brown adipose tissue, which provoke energy expenditure (*14*). However, in this study, coffee ingestion did not alter UCP-1 expression in interscapular BAT (data not shown). On the other hand, we observed that caffeine ingestion, as well as coffee ingestion, reduced the expressions of inflammatory cytokine genes in WAT and the hepatic expression of genes relating to fatty acid synthesis. Our results are supported by a previous study in which caffeine decreased TNF-α expression in a primary culture of human adipocytes (*36*). On the other hand, caffeine ingestion significantly decreased leptin expression in adipose tissue, but coffee ingestion did not. At present, we did not address the explanation of this difference in the action on leptin expression between caffeine and coffee. We are planning to clarify the action of caffeine on adipocytokine expression by using cultured adipocytes.

Previous epidemiological studies have demonstrated that not only coffee ingestion but also decaffeinated coffee ingestion correlates with a reduction in diabetes incidence (*15*−*17*). It has been reported that decaffeinated coffee ingestion might improve insulin sensitivity of skeletal muscle in rats (*37*). In the present study, the caffeine concentration in the drinking water used in experiment 2 was the same as that in the diluted coffee used in experiment 1. However, the effectiveness of the caffeine in experiment 2 was a little less than that of the diluted coffee. In addition, previous studies have reported that the coffee components except for caffeine have a beneficial effect on glucose metabolism in rats (*6*−*11*). Shearer et al. (*9*) reported the unique action of 3,4-diferuloyl-1,5-quinide, which is involved in roasted coffee at small concentration. In their study, the administration of this compound improved insulin action during hyperinsulinemic-euglycemic clamps in rats. In addition, in coffee there might exist compounds having synergistic or antagonistic action with caffeine action. Taken together, these results lead us to speculate that there are antidiabetic compounds in coffee other than caffeine. We are currently searching for these unidentified compounds.

In conclusion, we have demonstrated that coffee had an antidiabetic effect in spontaneously diabetic KK-A^y mice. It was suggested that this effect emerged partly via the reduced expression of inflammatory cytokines in white adipose tissue and partly via the amelioration of fatty liver. Moreover, our results indicated that caffeine is one of the most effective antidiabetic compounds in coffee. We hope to identify the target tissues upon which coffee or caffeine acts directly, as well as effective antidiabetic compounds other than caffeine. However, the present results suggest that coffee consumption may help to prevent type 2 diabetes and metabolic syndrome.

ABBREVIATIONS USED

ACC, acetyl-CoA carboxylase; BAT, interscapular brown adipose tissue; CCR2, chemokine C−C motif receptor 2; CPT-1, carnitine palmitoyltransferase-1; FAS, fatty acid synthase; IL-6, interleukin-6; IRS2, insulin receptor substrate-2; ITT, insulin tolerance test; MCP-1, monocyte chemoattractant protein 1; PL, phospholipids; PPAR-α, peroxisome proliferator activator receptor-α; SOCS, suppressor of cytokine signaling; SREBP-1, sterol regulatory element binding protein-1; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor α; UCP-1, uncoupling protein-1; WAT, white adipose tissues; 250CA, 250 mg of caffeine/L.

LITERATURE CITED

(1) van Dam, R. M.; Feskens, E. J. Coffee consumption and risk of type 2 diabetes mellitus. *Lancet* **2002**, *360*, 1477–1478.

(2) van Dam, R. M.; Hu, F. B. Coffee consumption and risk of type 2 diabetes. A systematic review. *JAMA*−*J. Am. Med. Assoc.* **2005**, *294*, 97–104.

(3) Huxley, R.; Lee, C. M.; Barzi, F.; Timmermeister, L.; Czernichow, S.; Perkovic, V.; Grobbee, D. E.; Batty, D.; Woodward, M. Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus: a systematic review with meta-analysis. *Arch. Intern. Med.* **2009**, *169*, 2053–2063.

(4) Reunanen, A.; Heliövaara, M.; Aho, K. Coffee consumption and risk of type 2 diabetes mellitus. *Lancet* **2003**, *361*, 702–704.

(5) Astrup, A.; Toubro, S.; Cannon, S.; Hein, P.; Breum, L.; Madsen, J. Caffeine: a double placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. *Am. J. Clin. Nutr.* **1990**, *51*, 759–767.

(6) Hemmerle, H.; Burger, H.-J.; Below, P.; Schubert, G.; Rippel, R.; Schindler, P. W.; Paulus, E.; Herling, A. W. Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. *J. Med. Chem.* **1997**, *40*, 137–145.

(7) Andrade-Cetto, A.; Wiedenfeld, H. Hypo-glycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rates. *J. Ethnopharmacol.* **2001**, *78*, 145–149.

(8) Rodriguez de Sotillo, D. V.; Hadley, M. Chlorogenic acid modifies plasma and liver concentrations of cholesterol, triacylglycerol, and minerals in (fa/fa) Zucker rats. *J. Nut. Biochem.* **2002**, *13*, 717–726.

(9) Shearer, J.; Farah, A.; de Paulis, T.; Bracy, D. P.; Pencek, R. R.; Graham, T. E.; Wasserman, D. H. Quinides of roasted coffee enhance insulin action in conscious rats. *J. Nutr.* **2003**, *133*, 3529–3532.

(10) Mishkinsky, J.; Joseph, B.; Sulman, F. G. Hypoglycaemic effect of trigonelline. *Lancet* **1967**, *16*, 1311–1312.

(11) Prasad, K.; Mantha, S. V.; Muir, A. D.; Westcott, N. D. Protective effect of secoisolariciresinol diglucoside against streptozotocin-induced diabetes and its mechanism. *Mol. Cell. Biochem.* **2000**, *206*, 141–150.

(12) Fredholm, B. B.; Battig, K.; Holmen, J.; Nehlig, A.; Zvartau, E. E. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol. Rev.* **1999**, *51*, 83–133.

(13) Huang, Z. L.; Qu, W. M.; Eguchi, N.; Chen, J. F.; Schwarzschild, M. A.; Fredholm, B. B.; Urade, Y.; Hayaishi, O. Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. *Nat. Neurosci.* **2005**, *8*, 858–859.

(14) Kogure, A.; Sakane, N.; Takakura, Y.; Umekawa, T.; Yoshioka, K.; Nishino, H.; Yamamoto, T.; Kawada, T.; Yoshikawa, T.; Yoshida, T. Effects of caffeine on the uncoupling protein family in obese yellow KK mice. *Clin. Exp. Pharmacol. Physiol.* **2002**, *29*, 391–394.

(15) Salazar-Martinez, E.; Willet, W. C.; Ascherio, A.; Manson, J. E.; Leitzmann, M. F.; Stampfer, M. J.; Hu, F. B. Coffee consumption and risk of type 2 diabetes mellitus. *Ann. Intern. Med.* **2004**, *140*, 1–8.

(16) Greenberg, J. A.; Axen, K. V.; Schnoll, R.; Boozer, C. N. Coffee, tea and diabetes: the role of weight loss and caffeine. *Int. J. Obes.* **2005**, *29*, 1121–1129.
(17) Van Dam, R. M.; Manson, J. E.; Willett, W. C.; Hu, F. B. Coffee, caffeine, and risk of type 2 diabetes. A prospective cohort study in younger and middle-aged U.S. women. *Diabetes Care* **2006**, *29*, 398–403.
(18) Yamauchi, T.; Kamon, J.; Waki, H.; Terauchi, Y.; Kubota, N.; Hara, K.; Mori, Y.; Ide, T.; Murakami, K.; Tsuboyama-Kasaoka, N.; Ezaki, O.; Akanuma, Y.; Gavrilova, O.; Vinson, C.; Reitman, M. L.; Kagechika, H.; Shudo, K.; Yoda, M.; Nakano, Y.; Tobe, K.; Nagai, R.; Kimura, S.; Tomita, M.; Froguel, P.; Kadowaki, T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat. Med.* **2001**, *7*, 941–946.
(19) Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1956**, *226*, 497–509.
(20) Horton, J. D.; Shah, N. A.; Warrington, J. A.; Anderson, N. N.; Park, S. W.; Brown, M. S.; Goldstein, J. L. Combined analysis of oligonucletide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12027–12032.
(21) Ide, T.; Shimano, H.; Yahagi, N.; Matsuzaka, T.; Nakakuki, M.; Yamamoto, T.; Nakagawa, Y.; Takahashi, A.; Suzuki, H.; Sone, H.; Toyoshima, H.; Fukamizu, A.; Yamada, N. SREBPs suppress IRS-2-mediated insulin signaling in the liver. *Nat. Cell Biol.* **2004**, *6*, 351–357.
(22) Hotamisligil, G. S.; Shargill, N. S.; Spiegelman, B. M. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* **1993**, *259*, 87–91.
(23) Kern, P. A.; Ranganathan, S.; Li, C.; Wood, L.; Ranganathan, G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E745–E751.
(24) Qi, C.; Pekala, P. H. Tumor necrosis factor-α-induced insulin resistance in adipocytes. *Proc. Soc. Exp. Biol. Med.* **2000**, *223*, 128–135.
(25) Suganami, T.; Nishida, J.; Ogawa, Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes. Role of free fatty acids and tumor necrosis factor α. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 2062–2068.
(26) Suganami, T.; Tanimoto-Koyama, K.; Nishida, J.; Itoh, M.; Yuan, X.; Mizuarai, S.; Kotani, H.; Yamaoka, S.; Miyake, K.; Aoe, S.; Kamei, Y.; Ogawa, Y. Role of the toll-like receptor 4/NF-κB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 84–91.
(27) Ito, A.; Suganami, T.; Miyamoto, Y.; Yoshimasa, Y.; Takeya, M.; Kamei, Y.; Ogawa, Y. Role of MAPK phosphatase-1 in the induction of monocyte chemoattractant protein-1 during the course of adipocyte hypertrophy. *J. Biol. Chem.* **2007**, *282*, 25445–25452.
(28) Kanda, H.; Tateya, S.; Tamori, Y.; Kotani, K.; Hiasa, K; Kitazawa, R.; Kitazawa, S.; Miyachi, H.; Maeda, S.; Egashira, K.; Kasuga, M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resisatance, and hepatic steatosis in obesity. *J. Clin. Invest.* **2006**, *116*, 1494–1505.
(29) Senn, J. J.; Klover, P. J.; Nowaki, I. A.; Zimmers, T. A.; Koniaris, L. G.; Furlanetto, R. W.; Mooney, R. A. Suppressor of cytokine signaling-3 (SOCS-3), a potencial mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J. Biol. Chem.* **2003**, *278*, 13740–13746.
(30) Shi, H.; Tzameli, I.; Bjørbæk Flier, J. S. Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. *J. Biol. Chem.* **2004**, *279*, 34733–34740.
(31) Marchesini, G.; Brizi, M.; Bianchi, G.; Tomassetti, S.; Bugianesi, E.; Lenzi, M.; McCullough, A. J.; Natale, S.; Forlani, G.; Melchionda, N. Nonalchoholic fatty liver disease. A feature of the metabolic syndrome. *Diabetes* **2001**, *50*, 1844–1850.
(32) Ueki, K.; Kondo, T.; Tseng, Y.-H.; Kahn, C. R. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10422–10427.
(33) Sabio, G; Das, M.; Mora, A.; Zhang, Z.; Jun, J. Y.; Ko, H. J.; Barrett, T.; Kim, J. K; Davis, R. J. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* **2008**, *322*, 1539–1543.
(34) Kobayashi-Hattori, K.; Mogi, A.; Matsumoto, Y.; Takita, T. Effect of caffeine on the body fat and lipid metabolism of rats fed on a high-fat diet. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 2219–2223.
(35) Inoue, H.; Kobayashi-Hattori, K.; Horiuchi, Y.; Oishi, Y.; Arai, S.; Takita, T. Regulation of the body fat percentage in development-stage rats by methylxanthine derivatives in a high-fat diet. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 1134–1139.
(36) Dray, C.; Daviaud, D.; Guigne, C.; Valet, P.; Castan-Laurell, I. Caffeine reduces TNFα up-regulation in human adipose tissue primary culture. *J. Physiol. Biochem.* **2007**, *63*, 329–336.
(37) Shearer, J.; Sellars, E. A.; Farah, A.; Graham, T. E.; Wasserman, D. H. Effects of chronic coffee consumption on glucose kinetics in the conscious rat. *Can. J. Physiol. Pharmacol.* **2007**, *85*, 823–830.

Received for review November 19, 2009. Revised manuscript received February 22, 2010. Accepted April 12, 2010. We thank the All Japan Coffee Association for financial support for this study.