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Effects of Coffee on Inflammatory Cytokine Gene Expression in Mice Fed High-Fat Diets

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In order to investigate the risk-reducing effects of coffee in metabolic syndrome, we performed a study in mice fed a high-fat diet with added coffee and analyzed gene expression in liver and adipose tissues using cDNA microarray. Male C57BL/6J mice were raised for 8 weeks on either a normal diet (N group), a high-fat diet (HF group), or a high-fat diet with 1.1% decaffeinated (HF+DC group) or 1.1% caffeine-containing instant coffee (HF+CC group). The body weights of mice in the HF+DC and HF+CC groups were mostly intermediate between the N and HF groups, even if there were no difference in the amount of diet consumption in each group. Mesenteric fat weight was lower in the HF+DC group than in the HF group ($p < 0.05$) and tended to become lower in the HF+CC group than in the HF group. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were significantly lower in the HF+DC and HF+CC groups than in the HF group ($p < 0.05$). Inflammatory cytokine interleukin (IL)-1β gene expression in liver was up-regulated in the HF group and significantly down-regulated in the HF+DC and HF+CC groups ($p < 0.01$), while MCP-1 gene expression in white adipose tissue was also significantly suppressed in the HF+DC group ($p < 0.01$). The induction of these anti-inflammatory responses by coffee consumption may contribute to reducing the risks of metabolic syndrome.

KEYWORDS: Coffee; high-fat diet; gene expression; inflammatory cytokine; mouse

INTRODUCTION

The metabolic syndrome, a cluster of conditions including insulin resistance, hypertension, and dyslipidemia, is highlighted as a common basis for type 2 diabetes and arteriosclerotic vascular diseases. The proportion of the population with metabolic syndrome, defined by centrally distributed obesity, decreased plasma HDL cholesterol, hypertriacylglycerolemia, hypertension, and hyperglycemia (*1*), is high in male adults, and it reaches 12% of Japanese men (*2*). The most common feature of the metabolic syndrome is accumulation of intra-abdominal visceral fat. Recently, adipose tissue was recognized not merely as a fat-storing tissue, but it plays a central role in secreting a variety of adipocytokines, whose impaired regulation causes metabolic syndrome-related pathogenesis (*3*).

In recent years, rates of obesity and type 2 diabetes have been increasing globally, in parallel with changes in human environment, behavior, and lifestyles (*4*, *5*). Type 2 diabetes is often part of a much broader underlying disorder characterized by the metabolic syndrome. A series of epidemiological studies in Holland, Finland, Sweden, the United States, and Japan have indicated that coffee consumption reduced the risk of type 2 diabetes (*6*−*13*). However, the mechanism behind the anti-diabetic effect of coffee and its association with metabolic syndrome are not fully understood. Whereas coffee is a major source of caffeine whose acute administration induces insulin resistance and impairs glucose tolerance (*14*), coffee intake is sometimes inversely associated with the risk of type 2 diabetes and improves glucose tolerance as shown by some epidemiological studies (*6*, *15*, *16*). It is suggested that induction of complete tolerance to caffeine by its long-term intake may cancel the adverse effect (*17*), with positive results of reducing the risk by thermogenesis and stimulation of lipolysis (*18*, *19*). Decaffeinated coffee has actually been found to have an anti-diabetic effect (*20*). Also coffee is rich in antioxidant polyphenols including chlorogenic acids (*21*). A link between diabetes and oxidative stress has thus been proposed (*22*). These characteristics of coffee may contribute to risk reduction of type 2 diabetes, having potential to affect the development of metabolic syndrome.

In this study, we performed an animal study using high-fat diets with caffeinated or decaffeinated coffee, and measured

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gene-expression in liver and adipose tissues, to investigate anti-metabolic syndrome effects of coffee.

MATERIALS AND METHODS

Animals and Experimental Design. The experiments were conducted with 5-week-old C57BL/6J mice (SPF, Charles River Laboratories Japan, Inc.) acclimatized for 12 days before the start of the experiment. First, mice were individually raised under an SPF condition and then divided into groups of 8 animals, which were submitted to *ad libitum* feeding for 8 weeks on a normal diet (N group), a high-fat diet (HF group), a high-fat diet with 1.1% freeze-dried decaffeinated coffee (HF+DC group), or a high-fat diet with 1.1% freeze-dried coffee containing 3% caffeine (HF+CC group) (**Table 1**). Decaffeinated coffee was prepared using green coffee beans decaffeinated by extraction with water and processed in the same manner as the caffeinated coffee.

Biochemical Tests. On the first day following the end of the administration period, the animals were fasted for 6 h and then anesthetized with an intra-abdominal dose of sodium pentobarbital. Blood samples were taken from the abdominal aorta, and sera were obtained by centrifugation. An Olympus AU400 automatic biochemical analyzer was used to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-globulin, total cholesterol, triacylglycerides, HDL cholesterol, LDL cholesterol, glucose, and insulin levels, and an ELISA method was employed to measure adiponectin levels. Part of the liver samples were pulverized with a homogenizer and then used for measurement of triacylglycerides and cholesterol levels.

cDNA Microarray. After the mice were dispatched by blood-letting, the liver, epididymal adipose tissue (white adipose tissue), scapular adipose tissue (brown adipose tissue), and gastrocnemius muscle were sampled. Then, each sample was weighed, and an aliquot was subjected to RNA extraction and purification using Isogen RNA extraction formula (Nippon Gene Co., Ltd.) and an RNEasy kit (Qiagen). Gene expression analysis was performed with DNA chips in cooperation with the Life Science Group at Hitachi, Ltd. For the four organs, liver, white adipose tissue, brown adipose tissue, and gastrocnemius muscle, DNA array analysis was conducted using total RNA extracted from a pool of equal weights of organs. The DNA chips used were Whole Mouse Genome Oligo Microarrays (Agilent Technologies, Tokyo), and competitive hybridization was performed by fluorescent labeling of the organs of the HF group with Cy3 as control samples and of the other organs with Cy5. The microarray data were analyzed using GeneSpring software. We investigated 21 875 genes that had signal intensities over a specified value, excluding parts that were duplicated on the chip. Changes in gene expression were regarded as significant when the expression quantity was more than twice or less than half of the one from the HF group.

Real-Time Polymerase Chain Reaction (PCR). Interleukin (IL)-1β and monocyte chemotactic protein (MCP)-1 in liver and white adipose tissues were chosen for further analysis to quantify the individual RNA levels by the RT-PCR method. The phosphoglycerate kinase 1 (Pgk1) gene was used as an internal control gene after confirming that the variations in DNA arrays were small if any. RNAs extracted with the Isogen reagent (Nippon gene) were reverse transcribed using oligo (dT) primer and SuperScript II (Gibco BRL) as described by the manufacturer. TaqMan Gene Expression Assays (Applied Biosystems Inc., Foster City, CA), which contain primers and probes for each gene (Assay ID; IL-1β, Mm00434228_m1; MCP-1, Mm00441242_m1; Pgk1, Mm00435617_m1) in 20× mix, were used for real-time PCR. PCR reactions were conducted with 25 μL of a mixture consisting of approximately 500 ng of cDNA, 12.5 μL of 2× Universal Master Mix, and 1.25 μL of 20× mix, applying the following conditions: 50 °C for 2 min, 95 °C for 10 min, followed by 40 PCR cycles at 95 °C for 15 s and 60 °C for 1 min.

Statistics. The results are presented as mean value ± standard deviation (SD). Data analysis was carried out with SPSS 11.0J software (SPSS Japan Inc., Tokyo) using the Wilcoxon rank sum test. The difference between means was considered significant at $p < 0.05$.

RESULTS

Body Weight and Blood Biomarkers. Diet intake, body weights, internal organ weights, and biochemical data on blood and liver properties are shown in **Table 2**. No differences in diet consumption were observed among the three high-fat diet groups, HF, HF+DC, and HF+CC groups. Compared with mice in the N group, those in the HF group had significantly greater body weight on day 29 of feeding, and on day 57, the weights of liver, white adipose tissue, mesenteric adipose tissue, and brown adipose tissue were significantly higher in the HF group than in the N group. Also, the blood AST and ALT levels and the liver homogenate triacylglycerides and cholesterol levels were all significantly higher in the HF group than in the N group. The total blood cholesterol levels in HF group were higher than those in the N group, but the HDL levels were also significantly higher, while triacylglycerides were lower.

Table 1. Diet Composition

ingredient	normal diet (N), %	high-fat diet (HF), %	HF + decaffeinated coffee (HF+DC), %	HF + caffeinated coffee (HF+CC), %
casein	20.00	20.00	20.00	20.00
sucrose	39.944	39.944	38.844	38.844
decaffenated instant coffee	0.00	0.00	1.10	0.00
soluble coffee	0.00	0.00	0.00	1.10
corn starch	9.80	0.00	0.00	0.00
alpha corn starch	13.20	0.00	0.00	0.00
beef fat	0.00	14.00	14.00	14.00
lard	0.00	14.00	14.00	14.00
soy bean oil	7.00	2.00	2.00	2.00
cellulose powder	5.00	5.00	5.00	5.00
mineral mix AIN-93G	3.50	3.50	3.50	3.50
vitamin mix AIN-93G	1.00	1.00	1.00	1.00
choline bitartrate	0.25	0.25	0.25	0.25
tertiary hydroquinone	0.0014	0.0060	0.0060	0.0060
L-cystine	0.30	0.30	0.30	0.30
total	100.00	100.00	100.00	100.00

The body weights of mice in the HF+DC and HF+CC groups were mostly intermediate between those of the HF and N groups, showing that the consumption of coffee suppressed the weight gain of mice receiving the high-fat diet by approximately 50%, but this reduction was not statistically significant. The body weight per weight of diet consumption was significantly lower in the HF+DC and HF+CC groups than in the HF group ($p < 0.05$). The weight of mesenteric fat was significantly lower in the HF+DC group than in the HF group ($p < 0.05$) and also tended to be lower in the HF+CC group. In the HF+DC and HF+CC groups, there were significant reductions in the levels of AST ($p < 0.05$ and $p < 0.01$, respectively) and ALT ($p < 0.01$) as indicators for liver function. Parameters such as blood glucose, insulin, and adiponectin did not differ among the four groups.

Gene Expression. High-fat diet caused changes in gene clusters with gene ontologies such as immune response, inflammatory response, lipid metabolism, and complement activation (**Figure 1**). Similar patterns of changes in gene ontology were found for liver in the HF+DC and HF+CC groups compared with the N group, indicating that inflammatory responses were down-regulated for the groups consuming coffee. Overall changes in gene ontology of the other tissues were similar between the N and HF+DC group, whereas some, such as cytokine signaling pathway in mesenteric adipose tissue and complementary activation in white adipose tissue, were different in the HF+CC compared with the N and HF+DC groups. The expression of genes related to the metabolic syndrome up-regulated or down-regulated compared with expression in the HF group are summarized in **Table 3**. Inflammatory cytokines and chemokines such as MCP-1 in liver and adipose tissues were suppressed in the HF+DC and HF+CC groups, as

Table 2. Diet Consumption, Body weight, and Blood analysis data in Mice Fed Normal and High-Fat Diet with or without Caffeinated or Decaffeinated Coffee[a]

	N (n = 8)	HF (n = 7)	HF+DC (n = 8)	HF+CC (n = 8)
diet consumption, g/day	3.33 ± 0.21[b]	2.66 ± 0.10	2.58 ± 0.21	2.65 ± 0.19
water consumption	3.18 ± 0.53	2.89 ± 0.53	3.00 ± 0.91	2.81 ± 0.29
body weight (BW), g				
day 1	21.6 ± 1.0	21.5 ± 0.9	21.4 ± 1.0	21.6 ± 0.9
day 15	24.8 ± 1.0	25.7 ± 1.4	24.8 ± 1.5	25.2 ± 1.6
day 29	27.3 ± 1.6[c]	30.0 ± 2.4	28.0 ± 2.4	28.6 ± 2.5
day 43	29.9 ± 2.3[c]	34.1 ± 3.0	31.4 ± 3.0[d]	31.9 ± 3.3
day 57	31.6 ± 2.6[b]	37.2 ± 2.9	34.3 ± 3.8[d]	34.9 ± 3.8
BW (day 57)/diet consumption, g/g	9.64 ± 0.72[b]	14.19 ± 0.60	13.34 ± 0.54[c]	13.31 ± 0.86[c]
weight gain (WG), g/day	0.18 ± 0.04[b]	0.28 ± 0.05	0.23 ± 0.07[d]	0.24 ± 0.07
WG/diet consumption, mg/g	53.7 ± 10.9[b]	105.9 ± 15.9	87.3 ± 23.4	89.2 ± 20.7
weight of organ (day 57), g				
liver	1.16 ± 0.11[b]	1.34 ± 0.10	1.20 ± 0.15[d]	1.23 ± 0.17[d]
white adipose tissue	1.18 ± 0.34[b]	2.01 ± 0.50	1.74 ± 0.60	1.68 ± 0.51
mesenteric adipose tissue	0.45 ± 0.12[b]	0.72 ± 0.15	0.53 ± 0.20[c]	0.58 ± 0.22
brown adipose tissue	0.12 ± 0.03[b]	0.20 ± 0.06	0.16 ± 0.03	0.15 ± 0.08
blood analysis (day 57)				
AST, IU/L	39.0 ± 1.3[b]	55.5 ± 9.5	47.9 ± 8.5[c]	41.3 ± 7.6[b]
ALT, IU/L	14.3 ± 2.3[b]	36.6 ± 10.1	25.2 ± 9.9[b]	18.1 ± 4.1[b]
γ-GTP, IU/L	e	e	e	e
triacylglycerides, mg/dL	19.9 ± 3.0[b]	13.1 ± 3.4	15.7 ± 2.7	14.7 ± 3.2
total cholesterol, mg/dL	130 ± 12[b]	169 ± 20	161 ± 31	157 ± 22
LDLC, mg/dL	4 ± 1	5 ± 2	4 ± 1	5 ± 1
HDLC, mg/dL	68 ± 5[b]	76 ± 5	74 ± 8	76 ± 8
glucose, mg/dL	237 ± 49	268 ± 29	273 ± 35	286 ± 56
insulin, ng/mL	3.08 ± 2.67	2.60 ± 0.95	2.63 ± 1.61	3.82 ± 1.90
adiponectin, μg/mL	24.1 ± 1.4	23.9 ± 2.6	23.8 ± 4.4	23.8 ± 5.3
liver homogenate (day 57)				
triacylglycerides, mg/g	2.80 ± 0.56[c]	33.60 ± 6.07	30.23 ± 11.31	20.80 ± 8.27
total cholesterol, mg/g	0.53 ± 0.15[c]	1.35 ± 0.13	1.53 ± 0.13	1.28 ± 0.10

[a] Values are expressed as mean ± SD. [b] $p < 0.01$ compared with HF diet group. [c] $p < 0.05$ compared with HF diet group. [d] $p < 0.10$ compared with HF diet group. [e] Not detected.

well as the N group. Only in mesenteric adipose tissue of the HF+CC group, increase in gene expression of tumor necrosis factor (TNF)-α, an inflammatory cytokine, was observed. Changes in 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and synthase and leptin recepter, found in liver from the N group, were not observed in the HF+DC and HF+CC groups. Lecithin-cholesterol acyltransferase (LCAT) and glucose transporter (GLUT), down-regulated in white adipose tissue from the HF+DC group, was up-regulated in the HF+CC group. Relative gene expression for IL-1β in liver and MCP-1 in white adipose tissue in the HF+DC group was significantly reduced ($p < 0.01$, **Figure 2**). In the HF+CC group, hepatic IL-1β and MCP-1 were significantly reduced ($p < 0.01$ and $p < 0.05$, respectively, **Figure 2**).

DISCUSSION

To investigate the antimetabolic syndrome effects of coffee and understand the mechanisms of these effects, we performed an animal study using mice fed high-fat diets with coffee. Our results showed that the consumption of decaffeinated or caffeinated coffee suppressed the weight increase especially in the adipose tissue that resulted from a high-fat diet. Mice fed high-fat diet for 8 weeks in this study gained approximately 18% more weight than those fed a normal diet, suggesting that the high-fat diet in this study could lead to not severe but mild obesity. The consumption of coffee suppressed approximately one-half the weight increase resulting from the high-fat diet. The amount of coffee consumed in this study was 1.1% in the animal diet. It is comparable to approximately 2 1/2 cups (5 g of solid) of coffee consumption per day in humans, based on the assumption that a Japanese adult consumes 500 g total solids from the diet per day. The average consumption of coffee by Japanese adults is about 200 mL per day (about 1 1/2 cups), and approximately 25% of the population consume 2 1/2 cups or more (*23*). Therefore, the 1.1% coffee in the animal diet used in this study is in the range of common use in human society, and thus the results showing that the coffee consumption halved the weight gain caused by high-fat diet is relevant.

The gene expression analysis in adipose tissue, liver, and muscle showed that there were large variations in immunity-related gene expression. In particular, the groups fed on coffee diets exhibited significant reductions in the expression of chemokines, such as MCP-1, produced in adipocytes, and inflammatory cytokine IL-1β produced in macrophages. The expression of such genes was up-regulated in the group fed on the high-fat diet. It is known that the basic pathology of the metabolic syndrome includes a mild inflammatory response throughout the whole body, with reduced production of anti-inflammatory cytokines and increased production of inflammatory cytokines in obese adipose tissue (*24*, *25*). In obese states, fat cells are swollen and accompanied by increased macrophages in adipose tissue (*26*). Macrophages in adipose tissue reduce in parallel with reductions in the amount of body fat as a result of diet therapy in humans (*27*). MCP-1 is a chemokine that causes migration of monocytes (macrophages) and lymphocytes, and has a major effect on the initiation of inflammation and insulin resistance by increased gene expression earlier than the other inflammation markers (*28*−*30*). Increase of MCP-1 gene expression in adipose tissue also plays a key role in hepatic steatosis, insulin resistance, and infiltration of macrophages into body tissues that accompany obesity (*31*, *32*). It is still unknown whether the anti-inflammatory effect of coffee directly caused reduction of weight gain in the high-fat diet mice. Caffeine and tea catechin, a polyphenol different from coffee, are known to increase in energy expenditure (*33*, *34*). In this study, we observed no major changes in

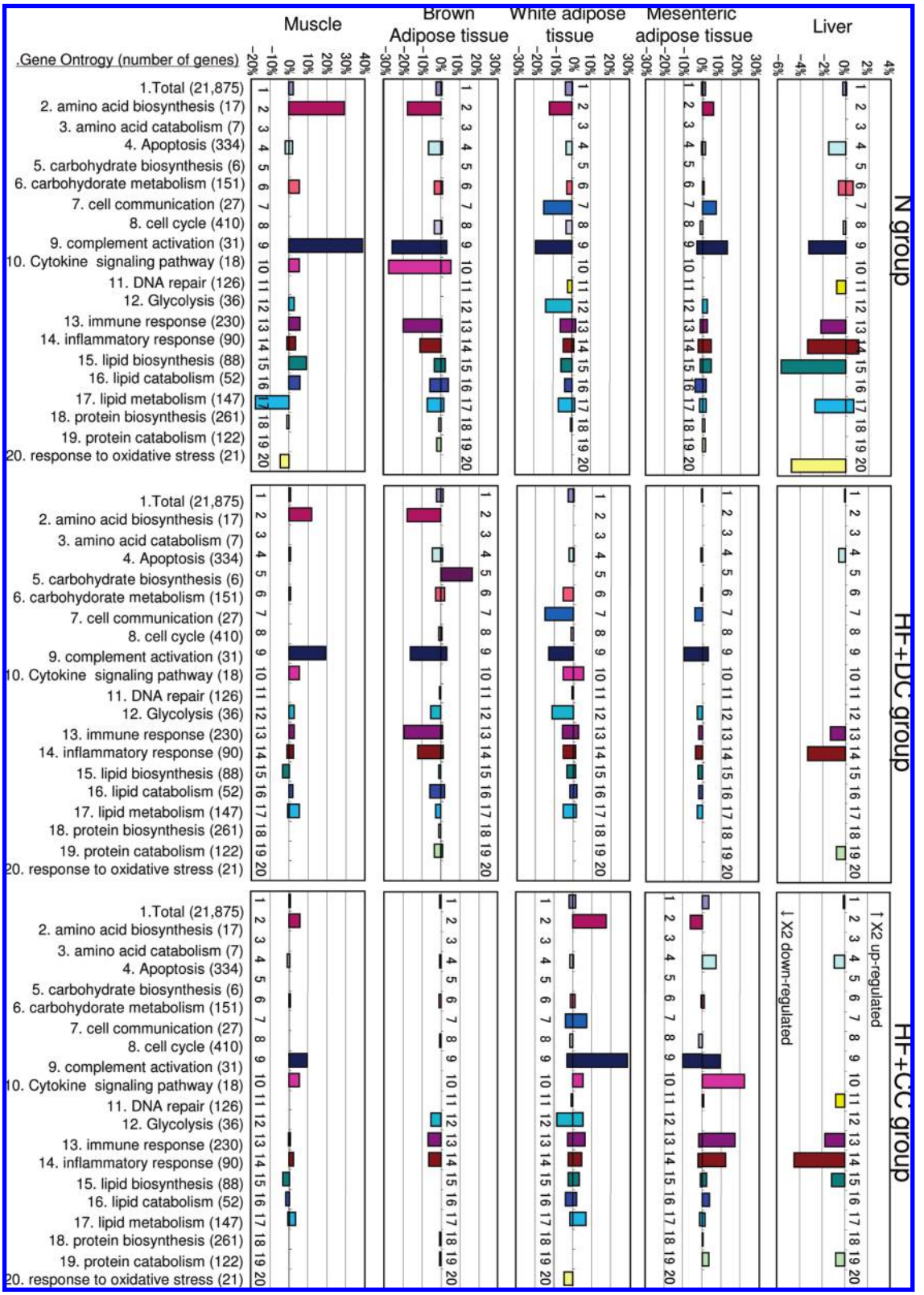

Figure 1. Up- and down-regulated genes (%) at Gene Ontology (Biological Process) with 2-fold or greater differences.

UCP-1 in brown adipose tissue, which has a key function for thermogenesis. UCP-1 in mesenteric adipose tissue was down-regulated by coffee intake; however, the impact for thermogenesis would be negligible. Absorption of fat and glucose may be surpressed by polyphenols (*35*, *36*). Coffee, at least, triggered reduction of weight in high-fat diet fed animals possibly through energy balance or anti-inflammatory effect.

The reduced expression of these inflammatory cytokines in mice fed on a high-fat diet with coffee should be important for considering the mechanism of the effect of coffee on reducing the risk of type 2 diabetes, for which inflammation contributes to the onset of the syndrome (*37*). Insulin resistance is a basic feature of type 2 diabetes, and it is likely that the suppression of chemokine expression by coffee may contribute to suppressing its onset.

Table 3. Expression of Metabolic Syndrome-Related Genes Showing at Least 2-Fold Difference from the HF Group

	N					HF+DC					HF+CC				
	liver	mesenteric adipose tissue	white adipose tissue	brown adipose tissue	muscle	liver	mesenteric adipose tissue	white adipose tissue	brown adipose tissue	muscle	liver	mesenteric adipose tissue	white adipose tissue	brown adipose tissue	muscle
up-regulated >2×	leptin R				GLUT2 LCAT				insulin R	GLUT2		TNF	GLUT2 LCAT		GLUT2
down-regulated <1/2×	HMG-CoA red. HMG-CoA syn.1 IL1-β MCP-1 UCP-2	UCP-1	MCP-1 UCP-1 IL1R II	MIP-1aR MCP-1 MCP-1R UCP-2 leptin		IL1-β MCP-1	GLUT2 UCP-1	GLUT2 IL1-β LCAT MCP-1	MCP-1 MIP-1aR	leptin UCP-1	IL1-β MCP-1	UCP-1	IL1-β	MCP-1	

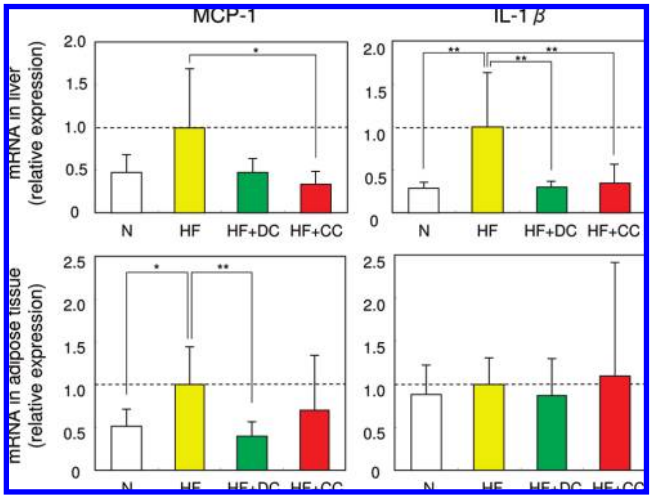

Figure 2. mRNA levels of MCP-1 and IL-1β genes in liver and white adipose tissue of C57BL/6J mice fed a normal diet (N), a high-fat diet (HF), or a high-fat diet with decaffeinated coffee (HF+DC) or caffeinated coffee (HF+CC): $*$ indicates $p < 0.05$, and $**$ indicates $p < 0.01$.

For clinical confirmation, several epidemiological studies have shown that coffee consumption improves sensitivity to insulin (*38*−*40*). In this study, suppression of anti-inflammatory cytokines took place with decaffeinated coffee, which had an even stronger effect than caffeinated coffee. Since the effects of suppression of type 2 diabetes are observed whether or not the coffee is caffeinated (*20*), caffeine may not exert any detrimental effect on the suppression of diabetes. Chlorogenic acid, a major coffee polyphenol, shows anti-inflammatory effect in rats (*41*) and may help reduce intestinal glucose uptake in humans (*36*). Since the combination of obesity and insulin resistance is a precursor state for liver dysfunction, it is thought that the improvement of liver function may be mediated by the anti-inflammatory effects of coffee on obese adipose tissue. We observed a pronounced reduction of blood hepatic enzymes, AST and ALT, which are well-known indicators for damage of liver, in caffeinated and decaffeinated coffee diet groups. Epidemiological studies have also shown that coffee consumption is effective in reducing the risk of liver cirrhosis and liver cancer (*39*, *40*, *42*−*45*), and it has been pointed out that chlorogenic acids may also contribute to liver protection and anticancer effects of coffee (*42*). Further studies are required to show how noncaffeine compounds in coffee such as chlorogenic acids contribute to the antidiabetic effect as well as the liver protection effect.

In conclusion, coffee consumption suppressed weight increase of adipose tissue in mice fed on a high-fat diet, and down-regulated gene expression for the inflammatory cytokines. Our findings in this study suggest that coffee, whether caffeinated or decaffeinated, has an anti-inflammatory action on liver and adipose tissue, which may contribute to its antimetabolic syndrome effects.

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