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Coffee and Fitness—Coffee Suppresses Lipopolysaccharide-induced Liver Injury in Rats

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Summary To clarify the relationship between coffee and fitness, we investigated the effect of coffee on weight gain and total cholesterol as well as production of cytokines and activities of GOT (aspartate aminotransferase; EC 2.6.1.1.) and GPT (alanine aminotransferase; EC 2.6.1.2.) as injected lipopolysaccharides. Forty-eight male Wistar rats were divided into three dietary groups ($n=16$), which were fed a stock diet (control group), the diet supplemented with freeze-dried coffee of 6.2 g/kg (0.62% coffee group), and the diet supplemented with freeze-dried coffee of 13.6 g/kg (1.36% coffee group). It was confirmed by HPLC analysis that the serum caffeine concentrations in both coffee groups became significantly higher in 140 days after the start of feeding. No significant differences in body weight and serum cholesterol were found between the coffee groups and control group, though the coffee groups tended to be somewhat high at cholesterol level. Activities of serum GOT and GPT increased at 2 h after LPS injection, but in the coffee groups were significantly suppressed ($p<0.05$). However, the coffee feeding could not suppress the increases of serum cytokine (TNF-α and IL-6) levels. These results suggest that coffee may serve as a preventive against liver injury.

Key Words coffee, caffeine, LPS-liver injury, GOT, cytokines

Coffee is one of the most widely consumed psychoactive beverages in our society. It is a well-known and extensively utilized psychotropic agent with effects on mood, cognitive performance and motor activity. In fact, many investigators have clarified that caffeine, one of the main constituents of coffee, has a variety of pharmacological and cellular responses in a wide spectrum of biological systems (*1*). These include stimulation of the central nervous system and cardiac muscle, increased urinary output, and relaxation of smooth muscle. In addition to caffeine, coffee also contains a relatively large amount of tannin (chlorogenic acid), a tea catechin analogue, showing biological effects such as antioxidation, antimutation, anticarcinogenesis, antibiotic, antihypercholesterolemia, antihypertension, and anti-inflammatory actions (*2*). Accordingly, coffee must be a useful beverage for health. In contrast, some investigators have reported that caffeine and/or heavy coffee intake may serve as risk factors for lifestyle-related diseases including heart disease and osteoporosis as well as periodontal diseases (*3*, *4*), which are intimately associated with nutrition, exercise, alcohol, smoking, and several other lifestyle factors. In the present study, we investigated a relationship between coffee and fitness; namely, the effects of coffee intake on body weight gain and serum cholesterol, cytokine and activities of GOT or GPT levels in lipopolysaccharide (LPS)-injected rats.

Abbreviations: LPS, lipopolysaccharide; GOT, aspartate aminotransferase; GPT, alanine aminotransferase; MIF, macrophage migration inhibitory factor; TNF-α, Tumor necrosis factor-α; IL-6, Interleukin-6.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats of 8 weeks age were housed every four per wire cage and given free access to a stock diet (Nihon Nosan Corp., Japan) and tap water. After 14 days, rats were randomly divided into three dietary groups ($n=16$), which were fed a stock diet (control group), the diet supplemented with freeze-dried coffee of 6.2 g/kg (0.62% coffee group), and the diet supplemented with freeze-dried coffee of 13.6 g/kg (1.36% coffee group). The composition of the stock diet was as follows (g/kg): proteins 183, fats 43, fibers 50, minerals 61; soluble non-nitrogen substances 583, water 80. The coffee used was a freeze-dried one (Nescafe Excella L906320:16, Nestle Japan Ltd., Japan), which contained 2.5–5.0% caffeine (*5*). The rats were maintained on experimental diets and their body weights were measured every 7 days. After feeding the experimental diets for 140 days, the animals were divided into two groups, lipopolysaccharide (LPS; Escherichia coli 026 : B6; Sigma, St. Louis, MO, U.S.A.)-injection group ($n=8$) and non-injection group ($n=8$). LPS was injected intraperitoneally at a dose of 2 mg/kg of body weight. At 2 h after the LPS injection, blood

samples were taken by cardiac puncture under light diethyl-ether anesthesia and centrifuged at 2,000 rpm for 10 min to separate serum. Serum was stored at −30°C until assayed. The experimental design was approved by the Laboratory Animal Care Committee of the School of Dentistry, Hokkaido University.

Biochemical analysis. GOT (EC 2.6.1.1.), GPT (EC 2.6.1.2.), and total cholesterol were measured colorimetrically using kits (Wako Chemical, Japan). The amounts of leptin, TNF-α, and IL-6 of serum were measured using ELISA kits (Morinaga Seikagaku Institute, Japan and Wako Chemical, Japan). The amount of MIF in the serum was measured by ELISA, using an anti-rat MIF IgG polyclonal antibody, as described previously (*6*).

Assay of caffeine. One mL of serum was applied to an Octadecyl Speedisk^R column (10 μm, J. T. Baker Inc., NJ, U.S.A.) and caffeine was extracted with 400 μL of methanol. Prior to determination of caffeine by HPLC (ODS-120 T column, Toyo Soda Co., Japan), extracted samples were diluted ten times with a mobile phase consisting of CH_3OH/0.1% H_3PO_4 (3 : 7). HPLC analysis was carried out with the mobile phase at a flow rate of 1.0 mL/min and detection at 260 nm.

Statistic analysis For statistical calculation the Student's *t*-test was used. Data are given as mean and standard deviations (mean±SD). A significant difference was accepted when $p<0.05$.

RESULTS

Effects of coffee diets on body weight, leptin and total cholesterol

The results for body weight are shown in Fig. 1. During the period of the experiment, no significant differences in body weight change were found between coffee groups and the control group. However, there was a tendency for the 0.62% coffee group to lose weight after 91 days of feeding, though in the 1.36% coffee group it increased after 14 days. Next, we measured caffeine, leptin, and cholesterol levels in serum from non-injection groups, to clarify the relationship between coffee consumption and fitness. The coffee diets for 140 days resulted in a significant increase of caffeine concentrations in sera. As shown in Table 1, although caffeine in serum was not detected in rats fed the control diet, the 0.62% coffee group led to an increase in caffeine concentration to 0.56±0.10 μg/mL ($n=8$) and the 1.36% coffee group led to one of 1.78±0.24 μg/mL ($n=8$). These results were obtained from HPLC analysis, showing one single peak with a retention time of 6 min 10 sec for caffeine, as shown in Fig. 2. The increase of the serum caffeine concentration reflected increased serum cholesterol levels (95.9± 13.2 mg/dL in the control group; 104.1±17.9 mg/dL in the 0.62% coffee group, and 122.5±17.9 mg/dL in the 1.36% coffee group). However, there was no significant difference between the cholesterol levels in the control group and coffee groups. The level of serum leptin, one of the hormones attributed to food intake (*7*), was not different between any groups, as shown in

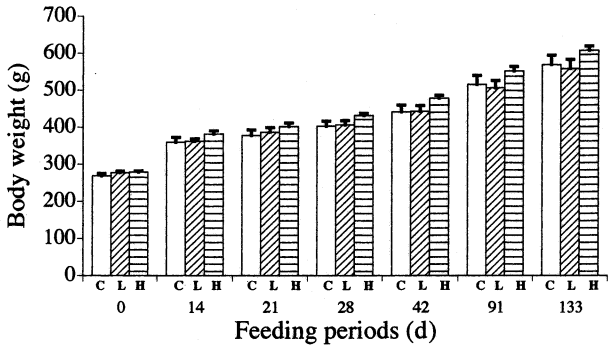

Fig. 1. Body weight in rats fed the control diet, 0.62% coffee diet, and 1.36% coffee diet. C, control group; L, 0.62% coffee group; H, 1.36% coffee group. $n=16$.

Table 1. Caffeine, leptin, and cholesterol levels in serum from rats fed the control-diet and coffee-diets

	Control group	0.62% coffee group	1.36% coffee group
Caffeine (μg/mL)	N.D.	0.50±0.10	1.78±0.24
Leptin (ng/mL)	5.18±2.51	5.84±2.25	4.64±1.06
Cholesterol (mg/dL)	95.9±13.2	104.1±17.9	122.5±10.4

The animals were housed four per wire cage and given free access to food and tap water. After feeding the experimental diets for 140 days, blood samples were taken by cardiac puncture under light diethyl-ether anesthesia. Serum caffeine levels were determined by HPLC, as described in Fig. 2. Serum leptin and cholesterol were estimated as described in the text. N.D., not detectable. $n=8$.

Table 1.

Effects of coffee diets on activities of GOT and GPT and cytokine levels in serum

As shown in Table 2, GOT activities in rat sera were 126.6 IU/L, 119.8 IU/L, and 130.9 IU/L in the three experimental diet groups, and GPT activities were 60.4 IU/L, 67.5 IU/L, and 60.0 IU/L. Therefore, the coffee groups did not affect serum activities of GOT and GPT. However, although activities of serum GOT and GPT were enhanced by LPS injection, the enhancement of these activities was significantly suppressed in the coffee groups ($p<0.05$). GOT activities of the coffee groups were 182.8±25.6 IU/L in the 0.62% coffee group and 188.6±60.9 IU/L in the 1.36% coffee group, whereas the control group was 265.9±53.8 IU/L. Similarly, GPT activities of the coffee groups were significantly suppressed as compared with the control group ($p<0.05$). However, no significant dose-dependent difference in the effect of coffee was observed between the two coffee groups. In general, it is well known that LPS injection induces inflammation, tissue injuries, and production of cytokines such as TNF-α and IL-6. Next, we measured serum cytokine levels in rats fed these experimental diets in order to clarify the relationship between coffee and cytokine production. As shown in Table 3, TNF-α and IL-6 were not detected (less than

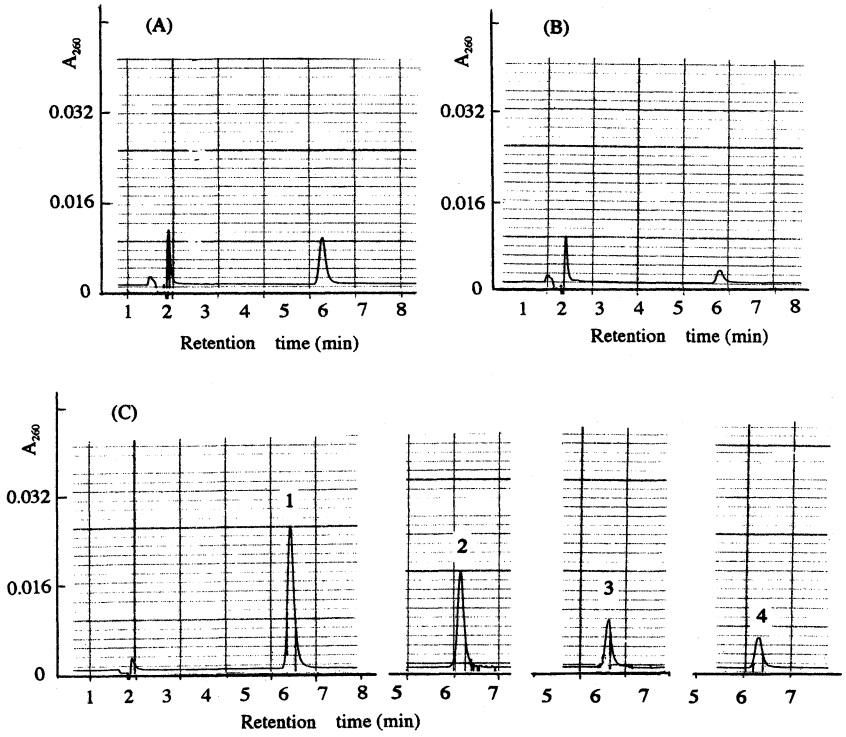

Fig. 2. Reverse-phase HPLC of caffeine and serum extracts from rats fed the control diet and coffee diets. A hundred μL of sample, which was pretreated with Octadecyl Speedisk^R column (10 μm, J.T.Baker Inc., NJ, USA), was injected into the reverse-phase column (ODS-120T column, Toyo Soda Co., Japan). The column was eluted with CH_3OH/0.1% H_3PO_4 (3 : 7) at a flow rate of 1.0 mL/min. (A) Serum extract from rat fed 1.36% coffee diet; (B) Serum extract from rat fed 0.62% coffee diet; (C) standard caffeine: 1; 142 ng, 2; 100 ng, 3; 50 ng, 4; 25 ng.

Table 2. Effect of coffee-diets on LPS-induced enhancement of GOT and GPT activities in rat serum

	GPT activity (IU/L)			GOT activity (IU/L)		
	Control group	0.62% coffee group	1.36% coffee group	Control group	0.62% coffee group	1.36% coffee group
Non-injection	60.4±10.4	67.5±9.3	60.0±14.2	126.6±45.8	119.8±20.3	130.9±19.2
LPS-injection	99.0±35.5	76.9±8.9*	68.3±133*	265.9±53.8	182.8±25.6*	188.6±60.9*

Experimental conditions were described in Table 1, except for that blood samples were taken by cardiac puncture from rats at 2 h after the LPS-injection. Serum GPT and GOT activities were estimated as described in the text. $n=8$. * $p<0.05$ vs. control group.

Table 3. Effect of coffee-diets on LPS-induced enhancement of cytokines in rat serum

	Control group	0.62% coffee group	1.36% coffee group
MIF (ng/mL)			
Non-injection	417.7±209.9	356.2±76.8	430.7±126.9
LPS-injection	427.1±53.0	451.8±110.9	457.1±118.5
TNF-α (ng/mL)			
Non-injection	N.D.	N.D.	N.D.
LPS-injection	4.6±3.8	5.7±4.1	4.1±2.4
IL-6 (ng/mL)			
Non-injection	N.D.	N.D.	N.D.
LPS-injection	12.6±4.2	11.4±3.9	12.9±4.6

Experimental conditions were described in Table 1, except for that blood samples were taken by cardiac puncture from rats at 2 h after the LPS-injection. Serum MIF, TNF-α, and IL-6 were estimated as described in the text. N.D., not detectable. $n=8$.

30 pg/mL) in the three experimental-diet groups. In contrast, MIF levels in sera of non-LPS injected rats from the three groups were 417.7 ng/mL, 356.2 ng/mL, and 430.7 ng/mL, which values were hardly increased by LPS injection. However, LPS injection led to increased serum levels of TNF-α (4.6±3.8 ng/mL in control group, 5.7±4.1 ng/mL in the 0.62% coffee group, and 4.1±2.4 ng/mL in the 1.36% coffee group) and IL-6 (12.6±4.2 ng/mL in control group, 11.4± 3.9 ng/mL in the 0.62% coffee group, and 12.9±4.6 ng/mL in the 1.32% coffee group), though no significant difference was observed between the control group and coffee groups.

DISCUSSION

The effects of coffee on health have been debated throughout the last four centuries since the Arab Abdelkader first wrote about the drink in 1587. Recently, many investigators have suggested that polyphenols in Japanese tea, such as tannins, flavonoids and caffeic acid derivatives, appear to protect human bodies against cardiovascular disease, liver disease, and malignancies (*2*). Regarding polyphenols, coffee contains tannins and caffeine, common constituents of Japanese teas. Therefore, coffee also should be expected to be a useful beverage for human health. According to the report of Cooper et al. (*8*), the median daily caffeine consumption of women was 210 mg (range 0–849.6 mg) from intakes of coffee, tea, and other caffeinated beverages. Using these data and assuming caffeine consumption of 150 mg/d and 300 mg/d in humans, therefore, total daily caffeine per rat was estimated to be 1 mg (0.62% coffee group) and 2 mg (1.36% coffee group), respectively. After 140 days' feeding of the coffee diets, assumed to be approximately 15 years for humans, the caffeine levels of rat sera were significantly increased by HPLC analysis. Interestingly, the increase of caffeine levels had already risen to approximately the same extent after 60 days as that of 140 days' feeding of the coffee diets, in the preliminary experiments. Accordingly, this study design is appropriate for investigating the effect of coffee consumption on fitness in vivo, even though coffee contains physiologically active components such as chlorogenic acids and trigonelline. In general, it is well known that caffeine increases the metabolic rate and lipolysis (*1*). The present study showed no significant difference in body weight change or cholesterol level between the control group and coffee groups, though there was no significant diffrence compared to the control group. These results were consistent with the reports of Miyake et al. (*9*). Next we investigated the preventive effect of coffee on LPS-induced liver injury in rats, measuring serum GOT and GPT activities together with cytokines levels. With regard to the LPS-induced cytokines, Givalois et al. have reported that the maximum peak of serum cytokines was at 1–2 h after injection of LPS into rats (*10*). In fact, our present study showed that TNF-α and IL-6, acts as a mediator for liver injury (*11*), markedly increased at 2 h after injection of LPS, but serum GOT and GPT levels increased only approximately two-fold compared to that of the control rats. Although hepatic injury in the present study model may be mild, it could be justified to investigate the effect of coffee on coupling action linked hepatic injury and cytokines such as IL-6, TNF-α, and MIF, which cross-talk via the cytokine network system in inflammatory responses (*12*). Consumption of 0.62% and 1.36% coffee groups for 140 days showed preventive effects on LPS-induced liver injury in rats. In fact, Sugiyama et al. recently reported similar results, showing that coffee suppressed D-galactosamine-induced liver injury in rats (*2*). However, unexpectedly, the coffee diets could not suppress elevation of serum cytokines after LPS injection. In general, in the signal transduction pathways triggered by LPS, it is thought that the agent mediates its transmembrane signal transduction by binding to a membrane protein, CD14, which leads to activation of a protein-tyrosine kinase (*13*). Therefore, it appears that coffee diets do not prevent either the binding of LPS to CD14 or any step of its signal transduction for activation of tyrosine kinase. Recently, Xiong et al. reported that acetoside, which contains a caffeoyl moiety as the chlorogenic acid of the antioxidant in coffee components, could block hepatic injury by LPS and D-galactosamine in mice, suggesting the involvement of reactive oxygen intermediates by TNF-α (*14*). In association with this finding, investigation on the preventive mechanism of coffee in the the liver injury is under way.

REFERENCES

1) Dews PB. 1982. Caffeine. *Ann Rev Nutr* **2**: 323–341.
2) Sugiyama K, He P, Wada S, Saeki S. 1999. Teas and other beverages suppress D-galactosamine-induced liver injury in rats. *J Nutr* **129**: 1361–1367.
3) Kamagata-Kiyoura Y, Ohta M, Cheuk G, Yazdani M, Saltzman MJ, Nakamoto T. 1999. Combined effect of caffeine and prostaglandin E_2 on the proliferation of osteoblast-like cell. *J Periodontol* **70**: 283–288.
4) Sesso HD, Mgaziano J, Buring JE, Hennekens CH. 1999. Coffee and tea intake and risk of myocardial infarction. *Am J Epidemiol* **15**: 162–167.
5) Viani R. 1991. Instant coffee powder. *In*: Coffee (Documentation center, corporate affairs department, ed), p 39. Nestec Ltd., Switzerland.
6) Sakamoto W, Nishihira J, Fujie K, Handa H, Ozaki M, Yukawa S. 1998. Inhibition of macrophage migration inhibitory factor secretion from macrophages by vitamin E. *Biochim Biophys Acta* **1404**: 427–434.
7) Baptista T, Lacruz A, de Mendoza S, Mendoza Guillen JM, Silvera R, Angeles F, Mendoza MT, Hernandez L. 2000. Body weight gain after administration of antipsychotic drugs: correlation with leptin, insulin and reproductive hormones. *Pharmacopsychiatry* **33**: 81–88.
8) Cooper C, Atkinson J, Wahner HW, O'Fallon WM, Riggs BL, Judd HL, Melton III LJ. 1992. Is caffeine consumption a risk factor for osteoporosis? *J Bone Miner Res* **7**: 465–471.
9) Miyake Y, Kono S, Nishikawa M, Hamada H, Nishikawa H, Koga H, Sogawa R. 1999. Relationship of coffee consumption with serum lipids and lipoprotein in Japanese men. *Ann Epidemiol* **9**: 121–126.

10) Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Siaud P, Assenmacher I, Barbanel G. 1994. Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. *Am J Physiol* **267**: R167–R170.

11) Bradham CA, Plumpe J, Manns MP, Brenner DA, Trautwein C. 1998. Mechanism of hepatic toxity. TNF-induced liver injury. *Am J Physiol* **275**: G387–G392.

12) Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, Cerami A, Bucala R. 1995. MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* **377**: 68–71.

13) Han J, Lee JD, Tobias PS, Ulevitch RJ. 1993. Endotoxin induces rapid protein tyrosine phosphorylation in 70Z/3 cells expressing CD14. *J Biol Chem* **268**: 25009–25014.

14) Xiong Q, Hase K, Tezuka Y, Namba T, Kadota S. 1999. Acteoside inhibits apoptosis in D-galactosamine and lipopolysaccharide-induced liver injury. *Life Science* **65**: 421–430.