Food Sci. Technol. Res., **15** (2), 147–152, 2009

Anti-Obesity and Hypotriglyceridemic Properties of Coffee Bean Extract in SD Rats

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Received September 22, 2008; Accepted December 2, 2008

Coffee bean extract (CBE) was prepared from raw green coffee beans and contained 10.0% caffeine and 27.0% chlorogenic acid. Male Sprague-Dawley rats were fed a diet containing 1% CBE for 4 weeks. Although there was no difference in food intake between rats fed the control diet without CBE and those fed the CBE-containing diet, body weight gain and white adipose tissue weight were significantly decreased in CBE-fed rats than in control rats. The CBE-fed group exerted a significant and extreme reduction in serum and liver triglyceride concentrations compared to the control group. Also, in the CBE-fed group, activities of fatty acid synthetic enzymes in the hepatic cytosol were significantly decreased, while that of fatty acid oxidative enzymes in the hepatic mitochondria was significantly increased. Our results suggest that CBE has potent anti-obesity and hypotriglyceridemic properties, and there is a possibility that these effects are exerted at least in part by the suppression of lipogenesis and the acceleration of lipolysis.

Keywords: coffee bean extract, anti-obesity, hypotriglyceridemic activity, lipogenesis, lipolysis

Introduction

Coffee is among the most widely consumed beverages in the world. Raw green coffee beans, which are materials of coffee, are rich in caffeine, chlorogenic acid, and its related components, such as quinic acid, caffeic acid, and *p*-coumaric acid. Caffeine has been reported to promote lipolysis in adipocytes of experimental animals and humans (Hasegawa and Mori, 2000; Zheng *et al.*, 2004; Lopez-Garcia *et al.*, 2006). Kobayashi-Hattori *et al.* (2005) has observed that caffeine reduced body fat mass and body fat percentage in a dose-dependent manner in rats fed high-fat diets. Chlorogenic acid, another main component of raw coffee beans, has been found to reduce cholesterol and triglyceride concentrations in serum and liver (Rodriguez de Sotillo and Hadley, 2002). Although there are several reports that coffee intake reduces body fat accumulation (Thom, 2007) and triglyceride levels (Carson *et al.*, 1994), moderate coffee intake does not seem to easily induce the suppression of obesity and the improvement of lipid profiles (Acheson *et al.*, 1980; Greenberg *et al.*, 2006). In general, a relatively large amount of caffeine is required to reduce body fat. While roasting raw coffee beans develops a mild and desirable aroma, it also reduces the caffeine and chlorogenic acid contents (del Castillo *et al.*, 2002). Therefore, to substantially enhance anti-obesity and lipid-lowering activities, a large intake of roasted coffee for a long period would be needed to reduce body fat and the hypolipidemic effect. On the other hand, coffee bean extract (CBE) is characterized to be rich in caffeine and chlorogenic acid. In the present study, we investigated the effects of CBE on body fat and lipid metabolism in rats.

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Materials and Methods

Preparation and determination of CBE CBE was obtained by extracting raw green coffee (*coffea canephora*) beans with 70% ethanol at 70°C for 2 h. The chemical composition of CBE preparation was determined. The crude protein and lipid contents, which were assayed by the Kjeldahl

method and Soxhlet method, were 29.2% and 0.3%, respectively. Moisture, which was determined as the loss in weight after drying 105°C for 24 h, was 2.2%. CBE contained 10.2% ash, as measured by the direct ignition method (540°C, overnight). Caffeine, chlorogenic acid, and its related components were assayed by HPLC with a Capcellpack C18 (4.6 × 250 mm, Shiseido, Tokyo, Japan) and a photodiode array detector (SPD-10 Avp Shimadzu, Kyoto, Japan), using anhydrous caffeine (Kishida Chemical Co., Ltd., Osaka, Japan) and chlorogenic acid (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) as standards. The solvents included either 2 mM H_3PO_4 (A) or CH_3CN (B), and a linear gradient of solvent A was changed to solvent B after 35 min. The flow rate was maintained at 1.0 mL/min. The amounts of caffeine and chlorogenic acid were 10.0% and 27.0%, respectively. The CBE included chlorogenic acid related components, 3-caffeoylquinic acid, a mixture of feruloylquinic acids, and 4,5-dicaffeoylquinic acid accounting for 5.5%, 16.0%, and 5.2%, respectively.

Animals and diets Male, 4-week-old Sprague-Dawley rats (Japan SLC, Inc., Hamamatsu, Japan) were housed individually in stainless-steel cages under a controlled atmosphere (temperature, 22 ± 1°C; humidity, 55 ± 5%; light cycle, 8:00-20:00). Rats were given a commercial pellet diet (Type CE-2, Clea, Tokyo, Japan) for 5 days and then divided into two groups of equal body weight. The control diet was prepared according to the formula recommended by the American Institute of Nutrition (Reeves *et al.*, 1993) (Table 1). Experimental diets contained 0.5% cholesterol and 0.125% sodium cholate and supplemented with 1% CBE, at the expense of cornstarch as in the control diets. Rats had free access to the diets and water for 4 weeks. Food intake and body weight of the rats were recorded daily. After the rats were fasted for 6 h, their blood was collected with decapitation, and perirenal and epididymal white adipose tissues and liver were immediately excised and weighed.

All animal studies were carried out under the guidelines for animal experiments at University of Nagasaki, Siebold (Nagasaki, Japan), and under Law No. 105 and Notification No. 6 of the Government of Japan.

Preparation of hepatic subcellular fractions A sample of freshly excised liver was homogenized in 6 volumes of 0.25 M sucrose solution containing 1 mM EDTA in a 10 mM Tris-HCl buffer (pH 7.4). After precipitating the nuclei fraction, the supernatant was centrifuged at 100,000 × *g* for 60 min to precipitate microsomes, with the remaining supernatant being used as the cytosol fraction. The mitochondrial and microsomal pellets were resuspended in the same 0.25 M sucrose solution.

Serum and liver lipid analyses Serum lipids were assayed enzymatically using commercial kits (Cholesterol E-Test, Triglyceride E-Test, Phospholipid C-Test, Wako Pure Chemical Industries, Osaka, Japan; and HDL-C, 2-Daiichi, Daiichi Chemicals, Tokyo, Japan). Lipid peroxide in serum was measured by a hemoglobin methylene blue assay with Determiner LPO kits (Kyowa Medex Co., Ltd., Tokyo, Japan). Liver lipids were extracted by the method of Folch *et al.* (1957). The concentrations of cholesterol, triglyceride, and phospholipid were measured by the methods of Sperry and Webb (1950), Fletcher (1968), and Rouser *et al.* (1966), respectively.

Measurement of hepatic enzyme activities The activities of cytosolic fatty acid synthase (FAS) (Kelly *et al.*, 1986), glucose 6-phosphate dehydrogenase (G6PDH) (Kessy and

Table 1. The composition of experimental diets.

	Control	Coffee bean extract
	g/kg of diet	
Casein (vitamin-free)	200	200
Corn oil	50	50
Mineral mixture (AIN-93G-MX)	35	35
Vitamin mixture (AIN-93-VX)	10	10
Cellulose powder	50	50
Cholinebitartrate	2	2
tert-Buthylhydroquinone	0.014	0.014
L-Cystine	3	3
Sucrose	100	100
α-Cornstarch	132	132
Cholesterol	5	5
Sodium cholate	1.25	1.25
Coffee bean extract	0	10
Cornstarch	to 1000	to 1000

Kletzien, 1984), malic enzyme (Ochoa, 1955), microsomal phosphatidic acid phosphohydrolase (PAP) (Walton and Possmayer, 1985), and mitochondrial carnitine palmitoyltransferase (CPT) (Markwell *et al.*, 1973) were determined in the liver. Protein was assayed by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Statistical analysis Data are reported as means ± SEM. Data were inspected by the Student's *t*-test. Values of $p < 0.05$ were considered statistically significant.

Results

Although there was no difference in food intake between rats fed the control diet and in those fed the CBE diet, body weight gain was significantly lower in CBE-fed rats than in control rats (Table 2). Relative liver weight was comparable between the two groups, but perirenal and epididymal white adipose tissue weights were significantly low in the CBE group.

The serum triglyceride level was extremely low in the CBE group, compared with the control group (Table 3). Feeding on the CBE diet induced the mild, but not significant, increase of the serum cholesterol concentration compared to feeding on the control diet, but it did not modulate the high density lipoprotein (HDL)-cholesterol concentration, consequently resulting in a lower HDL-cholesterol/total cholesterol ratio. The level of phospholipids in the serum was identical between both diets. The lipid peroxide concentration in the serum was significantly low in the CBE group.

Table 2. Effects of dietary coffee bean extract on growth parameters in rats.

	Control	Coffee bean extract
Body weight (g)		
Initial	143 ± 2	142 ± 3
Final	386 ± 10	303 ± 14*
Gain	244 ± 9	161 ± 12*
Food intake (g/day)	22.7 ± 0.5	21.5 ± 1.0
Tissue weight (g / 100g of body weight)		
Liver	6.33 ± 0.13	5.94 ± 0.28
White adipose tissue		
Perirenal	1.74 ± 0.20	0.65 ± 0.09*
Epididymal	1.28 ± 0.11	0.82 ± 0.06*
Perirenal + epididymal	3.03 ± 0.30	1.47 ± 0.13*

Each value is the mean ± SEM of 6 rats.

*Significantly different from the control group at $p < 0.05$.

Table 3. Effects of dietary coffee bean extract on serum and liver lipid concentrations in rats.

	Control	Coffee bean extract
Serum lipids		
Triglyceride (mmol/L)	2.04 ± 0.36	0.75 ± 0.13*
Total cholesterol (mmol/L)	4.35 ± 0.37	6.50 ± 0.51
HDL-cholesterol (mmol/L)	0.67 ± 0.07	0.62 ± 0.04
HDL-cholesterol/ Total cholesterol ratio (%)		
	15.9 ± 2.1	9.89 ± 1.50
Phospholipid (mmol/L)	2.36 ± 0.16	2.49 ± 0.11
Lipid peroxide (nmol/mL)	15.6 ± 1.0	10.5 ± 1.2*
Liver lipids (μmol/g)		
Triglyceride	85.4 ± 7.5	49.7 ± 3.6*
Cholesterol	179 ± 6	182 ± 17
Phospholipid	35.7 ± 0.7	40.2 ± 1.3*

Each value is the mean ± SEM of 6 rats.

*Significantly different from the control group at $p < 0.05$.

Table 4. Effects of dietary coffee bean extract on hepatic lipogenic and lipolytic enzyme activities in rats.

	Control	Coffee bean extract
	nmol/min/mg protein	
Lipogenic enzymes		
Cytosol		
Fatty acid synthase	5.29 ± 0.90	1.90 ± 0.78*
Glucose 6-phosphate dehydrogenase	17.2 ± 2.2	11.0 ± 1.0*
Malic enzyme	19.3 ± 0.84	13.8 ± 1.49*
Microsomes		
Phosphatidic acid phosphohydrolase	4.46 ± 0.19	5.29 ± 0.40
Lipolytic enzyme		
Mitochondria		
Carnitine palmitoyltransferase	3.93 ± 0.38	5.05 ± 0.31*

Each value is the mean ± SEM of 6 rats.

*Significantly different from the control group at $p < 0.05$.

The level of triglyceride in the liver was significantly lower in the CBE group than in the control group, whereas that of cholesterol was comparable between both groups (Table 3). Moreover, the hepatic phospholipid concentration increased in the CBE group compared to the control.

The activities of enzymes related to fatty acid synthesis, such as FAS, G6PDH, and malic enzyme, in the hepatic cytosol were significantly lower in the CBE group than in the control group (Table 4). There was no difference in the activity of PAP, the rate-limiting enzyme of triglyceride synthesis, in the hepatic microsomes between the control and CBE groups. The activity of hepatic mitochondrial CPT, the rate-limiting enzyme of mitochondrial β-oxidation, was significantly higher in rats fed the CBE diet than in those fed the control diet.

Discussion

Obesity is one of the risk factors for several lifestyle-related diseases, including coronary heart diseases, diabetes mellitus, hyperlipidemia, and is characterized by fat storage in the adipose tissues. The intake of certain beverages and functional foods has been shown to be effective at the suppression or reduction of body fat accumulation (Maki *et al.*, 2002; Nosaka *et al.*, 2003; Ikeda *et al.*, 2005). Rats who were fed CBE, which contains 10.0% caffeine and 27.0% chlorogenic acid as the principal constituents, showed suppressed body weight gain irrespective of food and energy intake compared to those fed the control diet not containing CBE (CBE group: 82.3 ± 3.6 kcal/day; control group: 86.8 ± 2.0 kcal/day). The perirenal and epididymal adipose tissue weights in CBE-fed rats were markedly lower than control rats, strongly suggesting that slower body weight gain in the CBE group is exerted by the suppression of visceral fat accumulation.

Caffeine has been demonstrated to reduce the weight of adipose tissues in experimental animals (Hasegawa and Mori, 2000; Zheng *et al.*, 2004). Kobayashi-Hattori *et al.* (2005) have shown that the intake of caffeine elevated the serum level of catecholamine in rats fed a high fat diet, and they presumed that the enhancement of the production of catecholamine accelerated lipolysis. Caffeine of CBE is thought to encourage the degradation of fat in adipose tissues by stimulating catecholamine secretion. The portion of fatty acids that released from adipose tissues is transferred to the liver and is then oxidized. Therefore, the decreasing deposition of visceral fat may be in part related to the enhanced oxidation of fatty acids in the liver. In the present study, CBE increased the activity of mitochondrial CPT in the liver. This enhanced activity is considered to be responsible for the reduction of adipose tissue weight and the suppression of body weight gain.

Another reason for the anti-obesity activity of CBE may be the suppression of postprandial hypertriglyceridemia. Han *et al.* (1999, 2001) have pointed out that slower absorption of dietary fat decreased the deposition of visceral fat. Shimoda *et al.* (2006) have shown that CBE and caffeine, but not chlorogenic acid, suppress the elevation of the serum triglyceride level after oral oil administration to mice. Thus, the caffeine in CBE might suppress body fat accumulation via suppressing postprandial hypertriglyceridemia. Since we did not measure fecal fat excretion, it is unclear whether CBE suppressed dietary fat absorption in the intestine. However, CBE intake effectively decreased both liver and serum triglyceride concentrations. If CBE induces the inhibition of intestinal fat absorption, the activities of hepatic lipogenic enzymes may increase to compensate for the reduction in the

triglyceride level in the body. In the present study, these enzyme activities were significantly suppressed by CBE intake, indicating that dietary fat absorption in the intestine was not suppressed. More detailed experiments are necessary to clarify this effect.

CBE effectively lowered serum and hepatic triglyceride concentrations. The activities of cytosolic FAS, malic enzyme, and G6PDH in the liver were decreased, whereas that of mitochondrial CPT in the liver was increased in CBE-fed rats. The reduction in the serum and hepatic triglyceride levels in CBE-fed rats is thought to be induced by both the suppression of fatty acid synthesis in hepatic cytosol and the acceleration of fatty acid oxidation in hepatic mitochondria. Chlorogenic acid has been shown to inhibit FAS activity (Li *et al.*, 2006), while there are few reports that caffeine affects the activities of fatty acid synthetic enzymes. Therefore, chlorogenic acid in CBE may be responsible for the suppression of fatty acid synthesis in the liver. Shimoda *et al.* (2006) have observed that caffeine and chlorogenic acid alone have no effect on CPT activity in the liver mitochondria of mice. The combination of caffeine and chlorogenic acid or other components of CBE may induce the enhancement of CPT activity. Kobayashi-Hattori *et al.* (2005) has reported that caffeine intake elevates the activity of acyl-CoA oxidase in the liver. Caffeine in CBE may therefore accelerate hepatic lipolysis by increasing acyl-CoA oxidase activity but not CPT activity.

The serum lipid peroxide level in the CBE group was two-thirds of that in the control group. Since chlorogenic acid has an antioxidant property (Rodriguez de Sotillo *et al.*, 2002), it is presumed to contribute to the reduction in the lipid peroxide level. The antioxidant activity of CBE is expected to reduce the risk of cardiovascular diseases by suppressing oxidation of low-density lipoprotein cholesterol and total cholesterol.

Raw green coffee bean contains cafestol, which is a diterpene, and potently increases serum cholesterol level in humans and experimental animals (Urgert and Katan, 1997; Post *et al.*, 2000). CBE prepared from raw green coffee beans contains cafestol. However, CBE-fed rats showed no significant increases in the serum cholesterol concentration compared to a control diet. Also, the level in the liver was the same between the control and CBE groups. Since CBE contains a relatively large amount of chlorogenic acid, which decreases low density lipoprotein cholesterol and total cholesterol concentrations (Rodriguez de Sotillo and Hadley, 2002), it might not increase the serum cholesterol level.

In conclusion, CBE appears to effectively suppress body fat and serum triglyceride levels through at least in part the decrease in fatty acid synthesis and the acceleration of fatty acid oxidation, showing that CBE may be a novel functional food material for suppressing fat deposition.

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