Note

Effect of Caffeine on the Body Fat and Lipid Metabolism of Rats Fed on a High-Fat Diet

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The intake of caffeine (CF) at 0.025, 0.05 or 0.1% for 21 days progressively reduced the body fat mass and body fat percentage in Sprague-Dawley (SD) rats fed on a high-fat diet with increasing administration level. Moreover, CF increased the serum concentrations of catecholamines and free fatty acids in SD rats orally administered with CF (5 mg/kg). These results suggest that the intake of CF reduced body fat by lipolysis via catecholamines. CF has potential as a functional food ingredient with an anti-obesity action.

Key words: caffeine; body fat; lipid metabolism; catecholamine; obesity

Obesity, a state in which fat aberrantly accumulates in adipose tissues, has become a public health concern due to the primary factor of lifestyle-related diseases such as hypertension, diabetes mellitus and hyperlipidemia. The prevalence of preobesity (a body mass index of 25–29.9 kg/m2) and obesity (≥30 kg/m2) in Japan has respectively been reported to be 24.5% and 2.3% in males and 17.8% and 3.4% in females aged 20 years and over.1) Obesity is also one of the risk factors for coronary heart diseases and cardiovascular diseases. Hence, the prevention and improvement of obesity will lead to prophylaxis of such diseases.

Studies on obesity in the field of food science have focused on the search for functional food ingredients that suppress the accumulation of body fat. Caffeine (CF), which is consumed worldwide in the form of drugs or beverages such as coffee and tea, has been reported to break down fat at the level of adipose cells.2) CF has also been demonstrated to reduce the weight of adipose tissues in experimental animals fed with a normal diet. For instance, Bukowiecki et al.3) have reported that the intake of 0.057% and 0.2% of CF for 9 weeks both reduced the weight of parametrical white adipose tissues in rats. Chen et al.4) have shown that a CF intake (4 mg/day) for 4 weeks decreased the weight of body fat in genetically obese mice, and Michna et al.5) have reported that the ingestion of 0.04% CF for 15 weeks decreased the weight of the parametrical fat pad by 43% in mice. Zheng et al.6) have shown that a 0.05% CF intake for 16 weeks reduced the weight of intraperitoneal adipose tissues in mice. However, to our knowledge, little information is available on the relationship between the CF dose and its effect on body fat under the influence of a high-fat diet. In particular, the effect of the dose of CF on the body fat percentage remains to be elucidated. The present-day intake of fat by the Japanese is four times higher than that in 1958.7) From the viewpoint of such a nutritional background, studying the effect of CF on body fat under the influence of a high-fat diet has social significance. The purpose of the present study was to clarify the relationship between the CF dose and its effect on body fat under the influence of a high-fat diet. We investigated the body fat mass, body fat percentage, serum and hepatic lipid parameters and the excretion of fecal lipid in rats pair-fed with a high-fat diet containing CF (at 0, 0.025, 0.05 or 0.1%) for 3 weeks (Experiment 1). In addition, to examine the correlation between body fat and catecholamines, we measured the time-course characteristics of the serum levels of catecholamines and free fatty acids in rats orally administered with CF (Experiment 2).

Experiment 1. Male SD rats (4 weeks old) purchased from Clea Japan (Tokyo, Japan) were individually housed in cages in a room with controlled temperature (22 ± 1°C), humidity (60 ± 5%) and lighting (12-h light:dark cycle). They were allowed free access to tap water and a standard diet prepared according to AIN-76 for 7 days for acclimatization. After this acclimatization, the rats were assigned to 4 groups (n = 8/group) and pair-fed with an experimental diet (AIN-76) containing CF (at 0, 0.025%, 0.05% or 0.1%) and lard (20%) for 21 days. After this feeding period, blood, adipose tissues...
and the liver were collected from each anesthetized rat that had been deprived of food for 7 h to measure the serum and hepatic lipid parameters and the total weight of the adipose tissues. Feces were collected for 3 days from the beginning of CF ingestion and before the end of the experiment. The body fat percentage was measured by the method described previously.\(^5\) Briefly, after removing the intestinal content of a dissected rat by flushing with saline, the adipose tissues that had been excised were returned to the dissected body, which was weighed (A) and then placed in a polyethylene vessel. Distilled water (20% of the weight of the dissected body) was also added to the vessel. After being autoclaved at 120°C for 2.5 h, the contents of the vessel were allowed to cool naturally and then weighed (B). Next, the contents were homogenized into a paste. This paste (10 g) was placed in a Petri dish, before being lyophilized, weighed (C) and powdered. The fat in the powder was extracted with chloroform–methanol (2:1). Briefly, 1 g of the powder was placed in the chloroform–methanol solution (20 ml). The suspension was incubated overnight at 40°C to extract the fat. The suspension was then filtered, and the resulting filtrate was adjusted to 50 ml with the chloroform–methanol solution. The weight of fat (D) in the filtrate (10 ml) was measured by a conventional gravimetric analysis. The body fat percentage was calculated with the following equation: body fat percentage (%) = \(\frac{\text{weight C} - \text{weight B}}{\text{weight A}} \times 100\). The serum concentrations of total cholesterol (TC), cholesterol-ester (CE), high-density lipoprotein-cholesterol (HDL-C), total lipids (TL), triglycerides (TG) and free fatty acids (FFA) were measured with an automatic analyzer (Hitachi Ltd., Tokyo, Japan), using a diagnostic kit for each. The concentrations of hepatic TG and TC were determined with a Triglyceride G test kit (Wako Pure Chemical Industries Ltd., Osaka, Japan) and the Zak–Henly method.\(^9,10\) The concentration of fecal TL was determined by a conventional gravimetric analysis.

**Experiment 2.** Male SD rats (6 weeks old) were individually housed as described for Experiment 1. They were allowed free access to tap water and a standard diet for 7 days for acclimatization and then divided into 2 groups (n = 6/group). After being deprived of food for 7 h, distilled water and CF (5 mg/kg) were orally administered to the control group and test group, respectively. At 0, 30, 60, 120 and 180 min after the administration, blood was collected from each anesthetized rat. The serum concentrations of catecholamines (epinephrine, norepinephrine and dopamine) were determined by the HPLC method. The serum concentration of FFA was measured with an automatic analyzer (Hitachi). The animal experiments in the present study (Experiments 1 and 2) were performed according to the guidelines for the care and use of experimental animals established by the ethics committee of the Tokyo University of Agriculture.

Table 1 shows the effect of CF intake on the body weight gain, body fat mass and body fat percentage of rats fed on a high-fat diet (Experiment 1). Although no significant difference was apparent in the body weight gain between the control group and the CF-administered groups, there were significant differences in the levels between the 0.05% CF- and other CF-administered groups. In terms of body fat, CF significantly decreased the body fat mass with increasing administration level. The body fat percentage was also reduced by supplementing CF in a dose-dependent manner. These results show that the CF intake suppressed the accumulation of body fat. There have been several reports on the anti-obesity action of CF alone under the condition of a normal diet by Zheng et al.,\(^6\) who showed that a 0.05% CF intake reduced the weight of intraperitoneal adipose tissues in mice, and by Bukowiecki et al.,\(^3\) who reported that 0.057% CF decreased the weight and triglyceride content of parametrical white adipose tissues in rats. The results of the present study show that even 0.025% CF, which corresponds to the daily intake of CF that is contained in tea and coffee, had an anti-obesity effect under the influence of a high-fat diet. The effect at the lower administration level is favorable from the viewpoint of normal daily CF ingestion. In addition, we have demonstrated that CF ingestion decreased the body fat percentage of rats in a dose-dependent manner. This decrease in body fat percentage accompanied by the reduction in body fat mass strongly supports the anti-obesity action of CF.

To elucidate the mechanism for the anti-obesity effect, we measured the serum levels of catecholamines and FFA in rats orally given CF once (Experiment 2). Figure 1 shows the time-course characteristics of the serum levels of catecholamines (epinephrine, norepinephrine and dopamine) and FFA. Regarding epinephrine (Fig. 1A), norepinephrine (Fig. 1B) and dopamine (Fig. 1C), CF significantly increased their levels within 30 min after its administration, before the levels in the CF-administered group gradually dropped to those in the control group. These results indicate that the CF administration elevated the serum levels of epinephrine, norepinephrine and dopamine, suggesting that CF stimulated the sympathetic nervous system. CF has also inhibited the action of CAMP phosphodiesterase, which increases cellular cAMP and can induce prolongation of

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight gain (g)</th>
<th>Total body fat mass (g/rat)</th>
<th>Body fat percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>224 ± 7(^b)</td>
<td>36.3 ± 2.8(^a)</td>
<td>13.4 ± 1.1(^a)</td>
</tr>
<tr>
<td>0.025% CF</td>
<td>233 ± 5(^b)</td>
<td>29.1 ± 1.9(^b)</td>
<td>10.6 ± 0.7(^b)</td>
</tr>
<tr>
<td>0.05% CF</td>
<td>210 ± 7(^b)</td>
<td>26.0 ± 1.5(^b)</td>
<td>10.3 ± 0.8(^b)</td>
</tr>
<tr>
<td>0.1% CF</td>
<td>240 ± 6(^a)</td>
<td>25.1 ± 1.8(^a)</td>
<td>8.9 ± 0.6(^a)</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± SE (n = 8/group). The statistical analysis was performed by Duncan’s multiple-range test. Means in the same column not sharing a common superscript letter are significantly different (p < 0.05).

Table 1. Effect of Caffeine on the Body Weight Gain, Body Fat Mass and Body Fat Percentage of Rats Fed on a High-Fat Diet
sympathetic stimulation. In respect of the level of FFA (Fig. 1D), the CF-administered group showed no change by 30 min after its administration, compared to the control group, but 60 and 120 min after the administration, the CF-administered group indicated a significantly higher FFA level than the control. This result shows that CF induced lipolysis. Thus, the anti-obesity effect of CF on body fat presumably results from the enhancement of lipolysis via catecholamine release by prolonging the sympathetic stimulation by CF. CF has also been reported to enhance the production of catecholamine and FFA in humans. Robertson et al. have reported that a single oral administration of CF elevated the levels of plasma epinephrine and norepinephrine. The level of plasma FFA has also been increased by an oral CF administration. However, to our knowledge, little information is available concerning the effect of CF on the body fat mass and body fat percentage in humans. In view of the studies on humans and our results for rats, the present study suggests that the CF intake may reduce the body fat mass and body fat percentage in humans. Apart from the production of catecholamine and FFA by CF, there is an interesting report on the change in level of uncoupling protein (UCP)-1, which contributes to thermogenesis, after a CF administration. Kogure et al. have shown that the subcutaneous administration of CF increased the mRNA expression of UCP-1 in the brown adipose tissues of obese mice. Since the expression of UCP-1 is controlled by the sympathetic nervous system, CF may also enhance thermogenesis in brown adipose tissues through the up-regulation of UCP-1 expression via activation of the sympathetic nervous system. A further study concerning the anti-obesity mechanism for CF is now in progress at the molecular level.

Table 2 shows the effects of the CF intake on the serum, hepatic and fecal lipid parameters in rats fed on the high-fat diet (Experiment 1). All the CF-administered groups showed lower levels of serum TC and CE than the control group. No significant difference in the level of serum HDL-C was apparent between the control and the CF-administered groups, although there was a significant difference in the levels between the 0.025% CF- and other CF-administered groups. No significant difference in the levels of serum TL and TG was apparent between the control and 0.025% CF-fed groups, but the two parameters were significantly decreased by the supplementation of 0.05% and 0.1% CF. The level of serum FFA was significantly reduced with increasing CF administration level. No significant difference in the level of hepatic TC was apparent between the control and the CF-administered groups. Although there was no significant difference in the level of hepatic TG between the control and the 0.025% CF-fed groups, the levels in 0.05% and 0.1% CF-fed groups were significantly lower than that in the control group. In terms of the excretion of fecal lipid, there was no significant difference across all groups for 3 days from the beginning of CF ingestion. The excretion of fecal lipid for 3 days before the end of the experiment was also not affected by supplementation with 0.025% and 0.5% CF. However, the intake of 0.1% CF significantly increased the excretion of fecal lipid. Taken together,

![Fig. 1. Time-Course Characteristics of the Serum Levels of Epinephrine, Norepinephrine, Dopamine and Free Fatty Acids in Rats Orally Administered with Caffeine.](image-url)

Each value is expressed as the mean ± SE (n = 6/group). The statistical analysis was performed by Student’s t-test on data treated with a variance analysis. An asterisk indicates significant difference (p < 0.05). ○, control group; ●, caffeine-administered group.
these results show that CF had not only a serum TC-lowering effect, but also serum and hepatic TG-lowering effects. A risky relationship between serum lipids and cardiovascular disease has been reported,18,19 so lowering the serum cholesterol and triglyceride levels is important for preventing high-mortality lifestyle-related cardiovascular diseases. CF can therefore be expected to help to prevent such diseases. In our preliminary study, the CF intake elevated the activity of hepatic acetyl-CoA oxidase in rats fed on a high-fat diet (unpublished data). The decreases in serum and hepatic TG are possibly due to the increased hepatic β-oxidation activity by CF. Other investigators have also reported that CF reduced the levels of serum TG20–22 and hepatic TG23 in rats, although our results for serum cholesterol and fecal lipid levels are inconsistent with other reported results. Some studies20,21,24 have shown that CF increased the serum TC level in experimental animals. Hostmark et al.22,25 showed that coffee drinking or CF ingestion reduced the fecal excretion of neutral sterols and cholesterol by rats. Although differences between their studies and ours are unknown at present, the promotion of fecal lipid excretion by CF may be partly involved in the cholesterol- and triglyceride-lowering effects.

In conclusion, we have shown that CF had such anti-obesity effects as reducing the body fat mass and body fat percentage in a dose-dependent manner in rats fed with a high-fat diet, presumably due to increased lipolysis via catecholamines. The novelty of this study is in that we clarified the relationship between the level of CF intake and its effect on body fat, in particular on the body fat percentage, under the influence of a high-fat diet. In addition, CF showed cholesterol- and triglyceride-lowering effects with the same diet. In the light of such beneficial effects, CF has potential use as a functional food ingredient that would prevent lifestyle-related diseases in humans who tend to ingest a diet containing a high level of fat.

References


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