Regulation of the Body Fat Percentage in Developmental-Stage Rats by Methylxanthine Derivatives in a High-Fat Diet

Hiroko Inoue, Kazuo Kobayashi-Hattori, Yumi Horiiuchi, Yuichi Oishi, Souichi Arai, and Toshichika Takita

1Department of Nutritional Science, Faculty of Applied Bio-Science, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan
2Department of Food & Nutritional Science, College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai-City, Aichi 487-8501, Japan

Received September 14, 2005; Accepted December 21, 2005

We investigated the regulatory effects of structural differences among methylxanthine derivatives on the elevation of body fat percentage in developmental-stage rats. Caffeine, theophylline and theobromine were used as the methylxanthines. High-fat diets (20% lard) containing each methylxanthine (0.025%) were administered to male Sprague-Dawley rats for 12 weeks, with the result that the body fat percentage was generally reduced in each methylxanthine-fed group. The abdominal adipose tissue weight in the caffeine group was also significantly lower than that in the control group, the serum cholesterol and triglyceride levels in the caffeine group also being significantly lower than the levels in the control group. The study results suggest that caffeine could contribute most to preventing arteriosclerotic diseases.

Key words: high-fat diet; rat; methylxanthine derivative; body fat percentage; abdominal adipose tissue

The popularity of European and American-style diets in Japan has recently led to the ingestion of an excessive amount of animal fat. Changes in lifestyle have increased the incidence of visceral fat-type obesity and such resulting arteriosclerotic diseases as ischemic heart disease and cerebrovascular disorders. The rate of arteriosclerotic disease-related mortality is approximately 30%, this being comparable with that of cancer-related mortality. Lifestyle-related diseases such as hyperlipidemia, diabetes mellitus, and hypertension, which result from visceral fat-type obesity, are involved in the onset and deterioration of arteriosclerosis. These lifestyle-related diseases may readily accumulate, and are considered to reflect a multiple risk factor syndrome which is present prior to the onset of lifestyle-related diseases. It is important to prevent and improve visceral fat-type obesity which may eventually lead to the onset of arteriosclerosis. We have previously investigated this topic from the aspect of nutritional and functional food science, especially with respect to the possible anti-obesity functions of methylxanthine derivatives (MXDs) such as caffeine (CF) and theophylline (TP).

A number of experiments that are relevant to this have been carried out in vivo and in vitro. However, no information is available on the anti-obesity functions of low-dose MXDs administered over a long period to rats fed on a high-fat diet. We report here the results from feeding tests under these conditions, with special reference to the effect of the number and position of the methyl groups in MXDs.

Materials and Methods

Animals and diets. We used 4-week-old male Sprague-Dawley rats (CLEA Japan, Inc., Tokyo, Japan). The rats were housed in stainless steel apartment-type cages in an animal room controlled under the following conditions: temperature, 23 ± 1 ºC; relative humidity, 50 ± 1%; and lighting cycle, 12 hours (8:00–20:00). Each diet was provided from 17:00 to 9:00. The rats were acclimatized with an AIN-76 composition diet for 7 days and then divided into five groups (n = 6 each). This study was continued for 12 weeks. We established a control segment (CO) and experimental segments. The experimental segments involved four MXD dietary groups: XA, CF, TB (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan) and TP (Nakalai Tesque, Inc., Kyoto, Japan), the content of each MXD in the diet being set at 0.025%.

During the study period, a diet was prepared by replacing corn oil, as the source of...
Table 1. Composition of the Experimental Diets (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CO</th>
<th>XA</th>
<th>TP</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>DL-methionin</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>35.0</td>
<td>34.975</td>
<td>34.975</td>
<td>34.975</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lard</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Methylxanthine derivative</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Abbreviations: CO, control; XA, xanthine; CF, Caffeine; TP, Theophylline; TB, Theobromine
*AIN-76

Results

Effect of methylxanthines on the food intake, body weight and body fat

The food intake, final body weight, body fat percentage, and fat tissue weights of the abdomen and epididymis resulting from MXD administered to the high-fat diet rats are given in Fig. 1. There was no significant difference in the week-to-week variation in the food intake between the CO group and each MXD-administered group, although the food intake of the CO group tended to be higher than that of the MXD-administered groups. The addition of MXD to the feed at the 0.025% level had no effect on the food intake. The energy intake showed a similar trend to the food intake (data not shown). There was no significant difference in the final body weights between the CO group and each MXD-administered group. Among the MXD-administered groups, the TB group showed higher values than those of the CO and TP groups. Each MXD-administered group showed a significantly lower body fat percentage than the CO group. The fat tissue weight of the abdomen was significantly lower in the CF group than in the CO group, and there were respective downward and upward trends in the XA and TP groups and the TB group. There was no significant difference in the weight of fat tissues surrounding the epididymis between the CO group and each MXD-administered group, but there was a downward trend in the TP group. The respective presence of the xanthine moiety and other tissues that had been excised were returned to the dissected body. The body was weighed (A) and then placed into a polyethylene vessel. In addition, distilled water (20% of the weight of the dissected body) was 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 content was homogenized until it became a paste. This paste (10 g) was placed in a Petri dish and then lyophilized, weighed (C) and powdered. The fat in the powder was extracted with chloroform–methanol (2:1). Briefly, 1 g of the powder was put into 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 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 (%) = [(weight D) × (50/10) × (weight C/1) × (weight B/10)]/(weight A) × 100.

Statistical analysis. Each result was expressed as the mean ± standard error. Statistical processing was performed by Duncan’s multiple-comparison test,16) using SPSS 9.0 software (P < 0.05).

Measurement of organ/tissue weights and lipid levels in the serum and liver. After 7 hours of fasting, blood was collected by cardiocentesis under anesthesia with Nembutal. Blood samples were kept at room temperature for 30 minutes, and then centrifuged at 3,000 rpm for 15 minutes to isolate the sera. After blood collection, the heart, lung, liver, spleen, kidney, and adipose tissue (abdominal adipose tissue and adipose tissue around the epididymis) were each weighed. The serum levels of total lipid, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride (TG) were measured with a 7450 automatic analyzer (Hitachi Co., Ltd., Tokyo, Japan). To extract the hepatic lipid, the largest lobe of the liver (1 g) was homogenized in a mixture of chloroform–methanol (2:1) with a Polytron, and the homogenate was kept overnight in darkness. After filtering the suspension, the filtrate was used as an extract in the subsequent experiments. This extract was stored at −40°C until needed. The concentrations of total lipid, total cholesterol and triacylglyceride in the extract were respectively measured by a conventional gravimetric analysis, Determiner TC 555 (KYOWA MEDEX Co., Ltd., Tokyo, Japan) and Triglyceride E Test kit (Wako Pure Chemical Industries Co., Ltd.).

Measurement of the body fat percentage. The body fat percentage was measured by the method described previously.6,7) In brief, after removing the intestinal contents of the dissected rats with saline, the adipose oils and fats, with lard in accordance with the AIN-76 dietary composition,15) increasing the lard content from 5% to 20%, and decreasing the sucrose content accordingly (Table 1). The diets and water were given ad libitum. The body weight was measured twice a week, and the volume of residual food was measured every day. The animal experiments in the present study were performed according to the guidelines for the care and use of experimental animals established by the ethics committee of Tokyo University of Agriculture.
methyl groups at the 1, 3 and 7 sites may have been prerequisites for the inhibitory effects on the increase of body fat percentage and the accumulation of abdominal fat.

Effect of methylxanthines on the weight of organs

The organ weights are given in Table 2. There was no significant difference in the organ weights between the CO group and each MXD-administered group. Among the MXD-administered groups, the TP and XA groups showed lower spleen weights than the CF and TB groups, demonstrating that TP and XA administration tended to decrease the weight of the spleen, an organ that plays an important role in immune functions.

Effect of methylxanthines on the level of serum lipids

Variations in the serum lipid levels by MXD administration are given in Fig. 2. There was no significant difference in the total lipid levels between the CO group and each other group. Among the MXD-administered groups, the XA, CF and TP groups showed lower levels than the TB group, demonstrating an upward trend in the total lipid levels resulting from TB administration. The total cholesterol level was lower only in the CF group than in the CO group. Among the MXD-administered groups, the TB group showed higher a value than the other three groups, demonstrating an upward trend in the TC level resulting from TB administration. The HDL-cholesterol levels were lower in the XA, CF and TB groups than in the CO group, but not in the TP group. The CF group showed a downward trend in LDL-cholesterol level compared with each other group, although there was no significant difference among the groups. The triglyceride level was significantly lower in the CF group than the CO group, and there was a downward trend in the XA and TP groups. The reducing effects of serum cholesterol and triglyceride, both risk factors for atherosclerosis, were noted in CF with methyl groups at the 1, 3 and 7 sites.

Table 2. Organ Weights of Rats Fed on a High-Fat Diet with Methylxanthine Derivatives

<table>
<thead>
<tr>
<th>Groups</th>
<th>CO</th>
<th>XA</th>
<th>CF</th>
<th>TP</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.26 ± 0.07</td>
<td>1.20 ± 0.03</td>
<td>1.35 ± 0.10</td>
<td>1.20 ± 0.04</td>
<td>1.39 ± 0.03</td>
</tr>
<tr>
<td>Lung</td>
<td>1.72 ± 0.04</td>
<td>1.62 ± 0.04</td>
<td>1.72 ± 0.10</td>
<td>1.76 ± 0.02</td>
<td>1.80 ± 0.05</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.84 ± 0.03abc</td>
<td>0.77 ± 0.03abc</td>
<td>0.87 ± 0.05abc</td>
<td>0.75 ± 0.01abc</td>
<td>0.88 ± 0.03abc</td>
</tr>
<tr>
<td>Liver</td>
<td>15.9 ± 0.94</td>
<td>15.9 ± 0.40</td>
<td>16.4 ± 1.56</td>
<td>14.7 ± 0.76</td>
<td>17.7 ± 1.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.89 ± 0.16</td>
<td>2.80 ± 0.07</td>
<td>2.97 ± 0.18</td>
<td>3.02 ± 0.05</td>
<td>2.95 ± 0.17</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE (g), n = 6. Means in a row without a common letter significantly differ, p < 0.05.

Abbreviations: CO, control; XA, xanthine; CF, Caffeine; TP, Theophylline; TB, Theobromine
Effect of methylxanthines on the level of hepatic lipids

The lipid levels in the liver, when MXD had been administered, are given in Fig. 3. The total lipid level was higher in the TB group than the CO group, but there was no significant difference among the other MXD-administered groups. In respect of the triglyceride level, there was no significant difference between the CO group and each MXD-administered group, although there was an upward trend in the TB group. There was no significant difference in the total cholesterol level between the CO group and each MXD-administered group, although there was a downward trend in the XA and TP groups. Taken together, there was an upward trend in the lipid levels in the liver resulting from TB administration.

Discussion

We administered in this study a high-fat diet containing MXD to developmental-stage rats for 12 weeks, and investigated the influence of MXD on the body fat and serum lipid levels. MXDs generally inhibited the elevation of body fat percentage in the developmental-stage rats, suggesting that MXDs may prevent childhood obesity. Childhood obesity deteriorates to adulthood obesity which is the most important risk factor for lifestyle-related diseases.\(^{17-19}\) Adipose tissue is the largest endocrine tissue, and secretes adipocytokine with various physiological actions.\(^{20,21}\) The production and secretion of an excessive or insufficient amount of adipocytokine are apparent in the presence of obesity, and the imbalance closely contributes to the onset and deterioration of arteriosclerosis and diabetes mellitus.\(^{20,22-24}\) Previous studies have suggested that not only the absolute fat accumulation but also the accumulation of intraperitoneal visceral fat represented by mesenteric fat was closely involved in the onset of arteriosclerotic diseases associated with obesity.\(^{4,25-27}\) Therefore, body fat, and especially intraperitoneal visceral fat, should be reduced to prevent arteriosclerosis and diabetes mellitus. Among MXDs used in the present study, CF reduced visceral fat in the abdominal cavity. As CF promotes lipolysis in vitro,\(^{10-12,14}\) we speculate that lipolysis in the adipose tissue caused a decrease in the intraperitoneal adipose tissue weight in the CF group. These results are similar to those reported by Zheng \etal.\(^{28}\) who suggested that long-term administration of low-dose caffeine to mice markedly reduced their body fat. CF also suppressed the elevation of the serum TG level. A decrease in the serum TG level may regulate the supply of fatty acid to adipose tissue, resulting in body fat reduction.

Many studies have reported that the incidence of coronary heart disease (CHD) and its mortality rate increased with increasing serum cholesterol level.\(^{29-31}\) Recent studies have suggested that the risk of coronary arteriosclerosis increased with increasing serum TG level.\(^{32,33}\) The second and third most common causes of death in Japan are heart diseases such as angina and myocardial infarction, and strokes such as cerebral hemorrhage and cerebral infarction. These disorders are caused by arteriosclerosis. The risk factors for arterio-

![Fig. 2. Serum Lipid Concentrations in Rats Fed on a High-Fat Diet with Methylxanthine Derivatives.](image-url)

Each value is the mean ± SE, n = 6. Means without a common letter are significantly different (p < 0.05). Abbreviations: CO (□), control; XA (●), xanthine; CF (◆), caffeine; TP (□), theophylline; TB (■), theobromine.
sclerosis include hyperlipidemia, hypo-HDL-cholesterolemia, visceral fat accumulation, insulin resistance (impaired glucose tolerance, hyperinsulinemia), and hypertension. These five risk factors have been reported as a multiple risk factor syndrome. Therefore, to reduce the arteriosclerotic disease-related mortality rate, this multiple risk factor syndrome must be improved. CF decreased the body fat percentage, abdominal adipose tissue weight, and serum cholesterol and TG levels; i.e., CF improved the multiple risk factor syndrome. Therefore, CF may be a functional food component that could primarily prevent arteriosclerosis. However, the amount of CF that the rats ingested in this study was approximately 1,000 mg/day when converted to the intake by humans with a 60-kg body weight, a high level considering the report by Stavric et al. 34) Therefore, care must be taken in CF ingestion to alleviate the multiple risk factor syndrome.

In conclusion, we have shown that, among the administered MXDs, CF (1,3,7-trimethylxanthine) most regulated the levels of body fat percentage, adipose tissue weight, total cholesterol and TG in developmental-stage rats fed on a high-fat diet. CF could contribute to preventing arteriosclerotic diseases.

References


