Capsinoids, Non-Pungent Capsaicin Analogs, Reduce Body Fat Accumulation without Weight Rebound unlike Dietary Restriction in Mice

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Enhancing energy expenditure and reducing energy intake are both crucial for weight control. Capsinoids, which are non-pungent capsaicin analogs, are known to suppress body fat accumulation and reduce body weight by enhancing energy expenditure in both mice and humans. However, it is poorly understood whether the suppression of body fat accumulation by capsinoids has an advantage over dietary restriction. This study shows that the oxygen consumption was increased in mice administered with capsinoids but not in dietary-restricted mice, although there was a similar suppression of body fat accumulation in both groups. The weight rebound was more notable in the dietary-restricted mice than in the mice administered with capsinoids. These results indicate that suppressing body fat accumulation by capsinoids was more beneficial than a restricted diet for maintaining body weight.

Key words: capsaicin; capsinoid; CH-19 Sweet

Obesity, particularly abdominal adiposity, increase the risk for such ailments as type 2 diabetes mellitus and cardiovascular disease.1) Reducing body fat is therefore important for maintaining good health. There are two ways to reduce body fat: reduce the energy intake and enhance the energy expenditure.

Hot red pepper is a typical traditional food that can increase energy expenditure and reduce energy intake.2–5) Hot red pepper contains capsaicin,6) the effective ingredient of hot red pepper, and capsaicin has been reported to enhance energy expenditure and reduce body fat accumulation in animal experiments.7,8) Although hot red pepper is therefore considered a useful food to reduce body fat, capsaicin has strong pungency and is a noiceptive stimulant, so that not all people can eat much of it and avoid an upset stomach.

New analogs of capsaicin have recently been identified from the non-pungent type of red pepper, CH-19 Sweet, and named capsinoids.9,10) The main capsinoids in CH-19 Sweet are capsiate, dihydrocapsiate, and nordihydrocapsiate, the respective content ratio being 5:3:1.9) Capsiate enhances energy expenditure and raises the core body temperature, and chronic capsiate administration has suppressed body fat accumulation in mice as effectively as capsaicin.1,12) Furthermore, an acute CH-19 Sweet intake in humans has enhanced sympathetic nervous activity, thermogenesis, and energy expenditure as effectively as hot red pepper,12,13) a chronic CH-19 Sweet intake has reduced the body fat and body weight in healthy people,14) and a chronic capsinoid intake has enhanced the energy expenditure and fat oxidation in obese people.15) Since CH-19 Sweet (capsinoids) has little pungency for humans,9) it is possible that it may be more acceptable for many people than hot red pepper (capsaicin).

However, it is poorly understood whether suppressing body fat accumulation by capsinoids has an advantage over that by dietary restriction. Understanding the mechanism for this is important to utilize CH-19 Sweet (capsinoids) as an anti-obesity food.

No published data have tested a side-by-side comparison of the effect of capsiate administration and dietary restriction on body weight control. Our purpose was to investigate the effects of repeated administration of capsiate and dietary restriction on adult male mice and to investigate the weight rebound after repeated capsiate administration and dietary restriction.

Materials and Methods

Materials. Capsiate provided by the University of Shizuoka was synthesized as previously reported.10) Capsiate was dissolved in a 0.9% NaCl solution containing 3% ethanol and 10% Tween 80 immediately before the experiment, as described elsewhere.11) This diluent also served as a control. The capsiate solution (6.48 mmol/L) was administered orally to each mouse.

Animals and diets. Five-week-old male Std ddY mice obtained from Japan Shizuoka Laboratory Center (Hamamatsu, Japan) were housed in standard cages (33 × 23 × 12 cm) under controlled conditions of temperature (22 ± 0.5°C), humidity (50%), and a daily photo period from 6:00 to 18:00. The animals were given free access to water and a commercial diet (type MF; Oriental Yeast, Tokyo, Japan). The care and treatment of the experimental animals conformed to...
Kyoto University guidelines for the ethical treatment of laboratory animals.

**Experimental design.** The mice were adapted to laboratory housing for a week to stabilize their metabolic condition. The mice were assigned at 6 weeks of age to one of the following groups (n = 12): the vehicle-administered (control) group; the capsiate-administered (capsiate) group; the vehicle-administered 10% dietary restriction (10% DR) group; and the vehicle-administered 30% dietary restriction (30% DR) group. The mean body weight was adjusted among each group. The mice were orally administered with 5 ml/kg of body weight (10 mg/kg of body weight) of either the capsiate solution or a vehicle once daily for 2 weeks. During this period, the mice in the control and capsiate groups were fed with a commercial diet such that each mouse received the same amount of food ad libitum. The mice in the 10% DR and 30% DR groups were respectively fed with 90% and 70% of a control group diet. After 2 weeks of feeding, indirect calorimetry was conducted to determine the whole body energy metabolism of each mouse. Following this determination, the mice in each diet group were respectively assigned to two groups such that the body weight average of the two groups was equal. Half of each group of mice were then sacrificed. Blood samples were collected, and the serum for a biochemical analysis was obtained after centrifuging the blood at 10,000 g for 4 °C for 10 min. The muscles (gastrocnemius and quadriceps), fat pads (epididymal, perirenal, and inguinal), interscapular brown adipose tissue (IBAT), liver, left and right kidneys, spleen and heart were each weighed. The remaining half of the mice had access to a commercial diet ad libitum without any dietary restriction for four more weeks. The body weight of each mouse was monitored over the experimental period. The body weight gain, defined as the increase of body weight for the last 4 weeks of the ad libitum feeding period, was calculated by subtracting the weight at the end of all experiments from that at the end of the administration period. In addition, the fat pads (epididymal, perirenal, and inguinal) were each weighed. The schematic representation of the experiment is shown in Fig. 1.

**Respiratory gas analysis.** An analysis of the energy metabolism was carried out using instruments equipped with 12 acrylic metabolic chambers, gas analyzers (model RL-600), and a switching system (model ANI6-A-S) to sample the gas from each metabolic chamber. The respiratory gas was measured for 36 h after the last dose to avoid the effect of a single capsiate administration.

**Biochemical analysis.** Serum was stored at –20 °C until needed for measurement. The plasma glucose, triglyceride, and non-esterified fatty acid (NEFA) concentrations were measured by using Triglyceride G-test, NEFA C-test, and Glucose CII-test assay kits (Wako Pure Chemical Industries, Kyoto, Japan) according to the manufacturer’s instructions.

**Fig. 1.** Experimental Scheme for the Study.

**Results**

**Body weight and organ weight**

The change in body weight of each group is shown in Fig. 2. The effect of each treatment appeared statistically significant after 7 d or later in the 10% DR and 30% DR groups, and after 12 d in the capsiate group. The average body weights of the capsiate, 10% DR, and 30% DR groups were respectively 3.2, 4.5, and 9.2 g less than that of the control group. As was the case for the body-weight gain, both capsiate and dietary restriction reduced the body-fat accumulation (Table 1). The relative weights of perirenal fat and epididymal fat in the capsiate, 10% DR, and 30% DR groups were respectively 3.2, 4.5, and 9.2 g less than that of the control group. In addition, the inhibition of body-fat gain in the 30% DR group was significantly greater than those in the capsiate and 10% DR groups. The relative spleen weight in the 30% DR group was significantly lower than those in the other groups, although there were no differences for muscle, liver, heart, kidney, inguinal fat, and IBAT among the groups.

**Serum components and hepatic triglyceride**

The serum components and hepatic triglyceride of each group under sedentary conditions are shown in Fig. 3. Plasma glucose levels in the capsiate, 10% DR, and 30% DR groups were significantly lower than that in the control group. Plasma NEFA and triglyceride were...
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The hepatic triglyceride content in the 30% DR group was the lowest decreased by the consumption of capsiate. The hepatic triglyceride content in the 30% DR group was the lowest among all the groups, the figures in the capsiate and 10% DR groups being significantly lower than that in the control group.

Whole body energy metabolism
The whole body energy metabolism of each group after 2 weeks of feeding is shown in Fig. 4. The oxygen consumption in the capsiate group was significantly higher than that in the other groups, whereas the figures in the 10% DR and 30% DR groups did not differ from the control group. Fat oxidation in the capsiate group was significantly higher than those in the control and 30% DR groups, whereas the 10% DR and 30% DR groups did not differ from the control group. Carbohydrate oxidation in the 30% DR group was significantly lower than those in the control and capsiate groups. No significant difference in RQ was detected among the groups.

### Table 1. Body Weight and Relative Organ Weight after 2 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Capsiate</th>
<th>10%DR</th>
<th>30%DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>42.05 ± 1.64a</td>
<td>38.83 ± 1.12ab</td>
<td>37.51 ± 1.29b</td>
<td>32.83 ± 0.92c</td>
</tr>
<tr>
<td>M. gastro</td>
<td>1.00 ± 0.12</td>
<td>1.02 ± 0.03</td>
<td>1.02 ± 0.03</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>M. quad</td>
<td>1.03 ± 0.08</td>
<td>1.18 ± 0.13</td>
<td>1.07 ± 0.04</td>
<td>1.04 ± 0.04</td>
</tr>
<tr>
<td>Liver</td>
<td>4.84 ± 0.30</td>
<td>5.02 ± 0.24</td>
<td>5.31 ± 0.12</td>
<td>4.74 ± 0.17</td>
</tr>
<tr>
<td>Heart</td>
<td>0.36 ± 0.02</td>
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<td>0.37 ± 0.01</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.34 ± 0.03a</td>
<td>0.31 ± 0.03a</td>
<td>0.31 ± 0.03a</td>
<td>0.23 ± 0.02b</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.31 ± 0.04</td>
<td>1.28 ± 0.09</td>
<td>1.37 ± 0.09</td>
<td>1.26 ± 0.06</td>
</tr>
<tr>
<td>Perirenal fat</td>
<td>0.91 ± 0.10a</td>
<td>0.63 ± 0.08b</td>
<td>0.64 ± 0.08b</td>
<td>0.29 ± 0.06c</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>2.01 ± 0.20a</td>
<td>1.48 ± 0.11b</td>
<td>1.52 ± 0.15b</td>
<td>0.79 ± 0.16c</td>
</tr>
<tr>
<td>Inguinal fat</td>
<td>0.96 ± 0.19</td>
<td>0.70 ± 0.07</td>
<td>0.84 ± 0.08</td>
<td>0.62 ± 0.12</td>
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±SEM (n = 6)
Body weight gain and fat weight after feeding an unrestricted diet for four more weeks

The average body-weight gain during four more weeks feeding of the capsiate group was 1.32 g less than that of the control group, which was not significant, whereas that of the 30% DR group was 2.79 g greater than that of the control group, which was significantly higher than that of the other groups (Fig. 5). The relative perirenal fat weight of the capsiate group was significantly lower than those of the other groups, whereas that of the 30% DR group was significantly higher than that of the control group. Similarly to the perirenal fat results, the relative epididymal fat weight of the capsiate group was significantly lower than that of the control group, whereas that of the 30% DR group was significantly higher than that of the control group. The relative inguinal fat weights of the capsiate, 10% DR, and 30% DR groups were significantly lower than that of the control group (Fig. 5).

Discussion

The repeated administration of capsiate for 2 weeks reduced the body weight gain and body fat accumulation in the mice. These results are consistent with those in the previous report,11) showing that the present experimental conditions were appropriate. Moreover, the repeated administration of capsiate enhanced the oxygen consumption and fat oxidation. These reductions in the serum components and hepatic triglyceride are thought to have been caused by the enhanced basal energy expenditure and fat oxidation. The body weight gain and body fat accumulation in the mice was reduced in the 10% DR and 30% DR groups. The amounts of oxygen consumption and fat oxidation in the dietary-restriction groups were no different from those in the control group, unlike the capsiate group. The amount of carbohydrate oxidation in the 30% DR group was significantly lower than that in the control group. This reduction is thought to have been a compensatory effect to reduce the energy expenditure. These results are consistent with those in the previous report.16) The concentrations of blood glucose, blood NEFA, blood triglyceride, and hepatic triglyceride were reduced in the capsiate group under sedentary conditions. These reductions in the serum components and hepatic triglyceride are thought to have been responsible for the enhanced basal energy expenditure and fat oxidation by the repeated administration of capsiate. It is possible that the reduction of leptin concentration by capsiate was responsible for the compensation to inhibit the effect of the enhanced energy expenditure by capsiate.

The body weight gain of the capsiate group was lower than that of the energy-restricted groups. The relative organ weights of perirenal, epididymal and inguinal fat at 10 weeks of age were also significantly lower than those of the control group. In addition, the oxygen consumption of the capsiate group was higher than that of the other groups. These results suggest that the intake of capsiate up-regulated the energy metabolism and reduced the weight gain. In particular, the fat oxidation of the capsiate group was higher than that of the other groups, this result also being consistent with that in our previous report.16) It should be noted that the mice used in this study were young growing mice, and it is possible that a different age such as using older mice had a different metabolic effect. It is not clear that the effect of capsaicinoids continued after the administration, because we only measured the respiratory gas for 2 weeks and not for 6 weeks. In addition, it is possible that a capsiate intake would have a different metabolic effect on humans. Further studies are needed to apply to clinical use for preventing obesity and metabolic syndromes.

In summary, the present results reveal that the weight rebound and inhibition of body fat accumulation by capsiate were both better than those by dietary restriction, because the body weight and body fat regain were
lower in the capsiate treatment group than in the dietary restriction group. These results suggest that capsiate could be an effective food ingredient to maintain appropriate levels of body weight and body fat.

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References