Capsiate, a non-pungent capsaicin analog, reduces body fat without weight rebound like swimming exercise in mice

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ABSTRACT

Enhancement of energy expenditure and reducing energy intake are crucial for weight control. Capsiate, a non-pungent capsaicin analog, is 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 suppressing body fat accumulation by capsiate administration is equal to exercise or not. The aim of this study is to compare the effects of repeated administration of capsiate and exercise and to investigate the weight rebound after repeated capsiate administration and/or exercise. In the present study, we report that 2 weeks treatment of capsiate and exercise increased energy metabolism and suppressed body fat accumulation during 4 more weeks of ad libitum feeding. The body weight in capsiate and exercise groups was significantly lower than that of control group. The oxygen consumption was significantly increased in capsiate and exercise groups than in the vehicle administered mice. In addition, the abdominal adipose tissue weight in capsiate and exercise groups was significantly lower than that of control group. These results indicate that suppressing body fat accumulation by capsiate intake is beneficial for maintaining an ideal body weight as exercise.

Obesity, particularly abdominal adiposity, increases the risk for some ailments such as type 2 diabetes mellitus and cardiovascular diseases (3). Therefore, reducing body fat is important for maintaining good health. There are two ways to reduce body fat: reduce energy intake or enhance energy expenditure. Hot red pepper is reported to increase energy expenditure and reduce energy intake (19–22). Hot red pepper contains capsaicin (15), an effective ingredient of hot red pepper, and capsaicin has been reported to enhance energy expenditure and reduce body fat accumulation in animal experiments (8, 9). Thus, hot red pepper has been considered useful food to reduce body fat.

Recently new analogs of capsaicin were identified from non-pungent type of red pepper, ‘CH-19 Sweet’, and named capsinoids (10, 11). The main capsinoids in CH-19 Sweet are capsiate, dihydrocapsiate, and nordihydrocapsiate, and the content ratio is 5 : 3 : 1, respectively (11). Capsiate enhances energy expenditure and raises core body temperature, and chronic capsiate administration suppresses body fat accumulation in mice as effectively as capsaicin (16, 17). Furthermore, acute CH-19 Sweet intake enhances a sympathetic nervous activity, thermogenesis, and energy expenditure as effectively as hot red pepper in humans (2, 17). Chronic intake of CH-19 Sweet re-
duces body fat and body weight in healthy people (7), and enhances energy expenditure and fat oxidation in obese people (6). Because CH-19 Sweet has little pungency in humans (11), it is possible that CH-19 Sweet (capsinoids) is more acceptable for many people than hot red pepper (capsaicin).

In our previous study (4), the weight reduction and the inhibition of body fat accumulation by capsiate were more ideal than that by dietary restriction because the body weight and body fat regain were smaller in the capsiate treatment group than in the dietary restriction group. However, it is poorly understood whether suppressing body fat accumulation by capsiate has the same effects as exercise. Understanding of the mechanism is important to utilize CH-19 Sweet (capsinoids) for anti-obesity food.

No published data test a side-by-side comparison of the effect of capsiate administration and exercise on body weight control. Our purpose was to compare the effects of repeated administration of capsiate and exercise in adult male mice and to investigate the weight rebound after repeated capsiate administration and/or exercise.

MATERIALS AND METHODS

Materials. Capsiate provided by the University of Shizuoka was synthesized as reported previously (10). Capsiate was dissolved in a 0.9% NaCl solution containing 3% ethanol and 10% Tween 80 immediately before the experiment, as described elsewhere (14, 15). 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 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 dairy photo period from 1800 to 0600. They 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 schematic representation for the experiment was shown in Fig. 1. In experiment 1, the mice were allowed to adapt to laboratory housing for stabilizing their metabolic condition. At 6 weeks of age, mice were divided into following three groups (n = 12): a vehicle-administered (Control) group; a capsiate-administered (Capsiate) group; and a vehicle-administered regular swimming exercise (Exercise) group. The mean body weight was adjusted among each group. Mice were orally administered with 5 mL/kg of body weight (10 mg/kg of body weight) of capsiate solution or 5 mL/kg of body weight of a vehicle every day for 2 weeks. During this period, commercial diet was provided to the mice in all of the groups every day, such that each mouse received same amount of food *ad libitum*. During this experimental period, the mice in exercise group were exercised in a pool 3 times a week, as described below. At 7–8 weeks of age, an indirect calorimetry was conducted to determine the whole body energy metabolism of each mouse. At 8–9 weeks of age, the mice in each group were assigned to 2 groups with equivalent average body weight, respectively. The half-number of mice (n = 6) in non-fasted condition were sacrificed after measuring their weights. Blood samples were collected and the serum for biochemical analysis was obtained after centrifugation of the blood (10000 × g, 4°C for 10 min). The muscle weights (gastrocnemius, quadriceps), fat pad weight (epididymal, perirenal fat), liver, left and right kidney, spleen and heart weights were determined. As the experiment 2, the other mice (n = 6) had free access to a commercial diet *ad libitum* for 4 weeks. During this period, the mice in exercise group were not exercised. The body weight in each mouse was monitored over the experimental period and determined the increased weights compared to that at 8 weeks of age, respectively. On the final day of the experiment, all mice were killed, and each serum sample and tissue was obtained as the experiment 1.

Swimming exercise. An adjustable-current water pool was used for mice to exercise. The details were described previously (13). Briefly, we used an acrylic plastic cage (90 cm long × 45 cm wide × 45 cm deep) filled with water to a depth of 38 cm. The
Capsiate reduces body fat like exercise

current in the pool was generated with a pump and the strength of the current was adjusted by opening and closing a valve. The current speed at the surface was measured with a digital current meter at the start of every swimming session, and we confirmed the current was at a constant speed. The water temperature was maintained at 34°C with electric heaters. In training session, the mice were made to swim for 60 min in the current with a flow rate of 6 L/min 3 times at 1-day interval in the week.

Respiratory gas analysis. Analysis of energy metabolism was carried out using the instruments equipped with 12 acrylic metabolic chambers, gas analyzers (model RL-600), and a switching system (model ANI6-A-S) to sample gas from each metabolic chamber. The details of methods were described in a previous report (16). Briefly, room air was pumped through the chambers and expired air was dried in a thin cotton column and then directed to a gas analyzer. The amount of fat and carbohydrate oxidized were calculated from the measured values of oxygen consumption and respiratory quotient using software for analysis, which is based on the Frayn’s equations (1). The data for each chamber were obtained every 13 min and stored on a spreadsheet. The instruments and software were obtained from Alco System, Chiba, Japan. Each mouse was placed into a metabolic chamber respectively and had free access to water and the same amount of food as in their home cage. To avoid the effects of a single capsiate administration, the measurement of respiratory gas was run 36 h after the last dose.

Statistical analysis. All values are presented as means ± SE. Comparisons of data were made by one-way ANOVA. When data were significant, each group was compared with the others by Fisher’s protected least significant difference test (Statview; SAS institute, Cary, NC). Results with values of $P < 0.05$ were considered statistically significant.

RESULTS

Body weight
The change in body weight of each group is shown in Fig. 2. The body weight of the capsiate and exercise groups was significantly lower than that of control group. The average of body-weight gain during 4 more weeks of ad libitum feeding was shown in Fig. 3. The body weight gain during 4 more weeks of ad libitum feeding in exercise group was significantly lower than that of the control and capsiate groups (Fig. 3).

Organ weights
The relative organ weight at 2 weeks and after 4 more weeks of ad libitum feeding was shown in Table 1. The relative weights of gastrocnemius and quadriceps muscles in the exercise group were significantly higher than those in the control and capsiate groups at both 2 weeks and after 4 more weeks of ad libitum feeding. There was no significant difference in the weight of liver, spleen and kidney among tree groups.

Adipose tissue weights
The relative weights of perirenal and epididymal ad-
The relative weights of perirenal and epididymal adipose tissues in the capsiate and exercise groups were significantly lower than those in the control group after 2 weeks (Fig. 4-A1, A2). The relative weights of perirenal adipose tissues in the capsiate and exercise groups were significantly lower than those in the control group (Fig. 4-B1), and the relative weights of epididymal adipose tissues in the exercise groups were significantly lower than those in the control group after 4 weeks of ad libitum feeding (Fig. 4-B2). There was no significant difference in the weight of epididymal adipose tissue weight between capsiate and exercise group, however, the suppression of epididymal fat accumulation by capsiate administration was similar to exercise group.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Capsiate</th>
<th>Exercise</th>
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</thead>
<tbody>
<tr>
<td><strong>2 Weeks feeding</strong></td>
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<tr>
<td>Gastrocnemius</td>
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<td>1.03 ± 0.03</td>
<td>1.16 ± 0.04*</td>
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<tr>
<td>Quadriceps</td>
<td>1.44 ± 0.04</td>
<td>1.44 ± 0.04</td>
<td>1.57 ± 0.03*</td>
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<tr>
<td>Liver</td>
<td>4.51 ± 0.15</td>
<td>4.83 ± 0.21</td>
<td>4.75 ± 0.11</td>
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<tr>
<td>Heart</td>
<td>0.41 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.42 ± 0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.32 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.43 ± 0.05</td>
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<td>0.41 ± 0.10</td>
</tr>
<tr>
<td><strong>After ad libitum feeding</strong></td>
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<tr>
<td>Gastrocnemius</td>
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<td>1.02 ± 0.03</td>
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<td>4.36 ± 0.05</td>
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<tr>
<td>Heart</td>
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<tr>
<td>Kidney</td>
<td>1.31 ± 0.03</td>
<td>1.38 ± 0.06</td>
<td>1.42 ± 0.10</td>
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Values are means SEM.
*Significantly different from the other groups (P < 0.05).

Fig. 4 Relative adipose tissue weight after the 2 weeks treatment and the feeding of unrestricted diet for more 4 weeks in the control, capsiate, and exercise groups. The organ weight percentages of perirenal fat (A1) and epididymal fat (A2) after the 2 weeks treatment, and perirenal fat (B1) and epididymal fat (B2) after the feeding of unrestricted diet for more 4 weeks in the control, capsiate, and exercise groups. Significantly different from the other groups, *P < 0.05, **P < 0.01.
Whole body energy metabolism

The whole body energy metabolism of each group after 2 weeks of feeding is shown in Fig. 5. Oxygen consumption, and fat and carbohydrate oxidation in the capsiate and exercise groups were significantly increased compared with control group. No significant difference in respiratory quotient was detected among the groups (data not shown).

DISCUSSION

In the present study, continuous administration of capsiate and swimming exercise suppressed body fat accumulation and raised oxygen consumption, an index of energy expenditure, suggesting that capsiate-treated and exercise mice burned more calories similarly. Capsiate-treated mice ingested the same amount of calories as control mice, so any increase in energy expenditure induced by capsiate may be attributable to the suppression of body fat accumulation.

We have shown previously that capsiate administered to mice promoted energy metabolism and suppressed body fat accumulation (16). We have also shown that capsiate up-regulates uncoupling proteins (UCPs) in skeletal muscle and brown adipose tissue, that are thought to play important roles in energy expenditure, the maintenance of body weight, and thermoregulation (12). In this study, capsiate suppressed body fat accumulation and raised oxygen consumption in the same as exercise. However, body weight gain was lower in the exercise group than that in the capsiate group. This result was thought to be caused by increment of muscles relative to body weight.

T3 and T4 are thyroid hormones that affect heat production. Continuous administration of T4 increases oxygen consumption and decreases body weight in ob/ob mice (18). However, we have demonstrated that administration of capsiate for 2 weeks did not increase serum thyroid hormone levels, suggesting that thyroid hormones are not involved in the increase in metabolic rate induced by capsiate (unpublished data). Himms-Hagen et al. (5) previously demonstrated that chronic treatment of beta3-adrenergic receptor agonists decreased serum free thyroid hormone levels but increased the content of UCP1 and 24-h energy expenditure. As described above, UCPs may contribute to the increase in metabolic rate induced by capsiate via the activation of the sympathetic nervous system, rather than via thyroid hormones. UCPs are upregulated in brown adipose tissue (BAT) by treatment of beta3-agonists (14). In a previous study, capsiate was shown to promote the secretion of adrenaline in mice (16), suggesting that capsiate activates the sympathetic nervous system.

In this study, the relative organ weight of perirenal and epididymal fat in capsiate group was significantly lower than that in control group. In addition, oxygen consumption in capsiate group was higher than that of control group. These results suggest that intake of capsiate upregulated energy metabolism.
and lowered body fat accumulation. In particular, fat oxidation in capsiate and exercise group was higher than that of the control group. This result was consistent with our previous report about the effect of capsiate on body fat accumulation and energy metabolism (12). However, it should be noted that the mice used in this study were normal closed colony Std-ddY mice, and it is possible that the different mouse strain, such as obese model mice, had different metabolic effects. In addition, it is possible that human had different metabolic effect by capsiate intake. Further study is needed to apply clinical use for preventing obesity and metabolic syndromes.

In summary, the present results revealed that increment of energy metabolism and the inhibition of body fat accumulation by capsiate are equal to exercise. In addition, the adipose tissue weight and oxygen consumption in exercise group were smaller to capsiate treatment group. These results suggest that capsiate is effective food ingredient to reduce body fat.

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REFERENCES