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Upregulation of uncoupling proteins by oral administration of capsiate, a nonpungent capsaicin analog

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Masuda, Yoriko, Satoshi Haramizu, Kasumi Oki, Koichiro Ohnuki, Tatsuo Watanabe, Susumu Yazawa, Teruo Kawada, Shu-ichi Hashizume, and Tohru Fushiki. Upregulation of uncoupling proteins by oral administration of capsiate, a nonpungent capsaicin analog. *J Appl Physiol* 95: 2408–2415, 2003. First published September 5, 2003; 10.1152/japplphysiol.00828.2002.—Capsiate is a nonpungent capsaicin analog, a recently identified principle of the nonpungent red pepper cultivar CH-19 Sweet. In the present study, we report that 2-wk treatment of capsiate increased metabolic rate and promoted fat oxidation at rest, suggesting that capsiate may prevent obesity. To explain these effects, at least in part, we examined uncoupling proteins (UCPs) and thyroid hormones. UCPs and thyroid hormones play important roles in energy expenditure, the maintenance of body weight, and thermoregulation. Two-week treatment of capsiate increased the levels of UCP1 protein and mRNA in brown adipose tissue and UCP2 mRNA in white adipose tissue. This dose of capsiate did not change serum triiodothyronine or thyroxine levels. A single dose of capsiate temporarily raised both UCP1 mRNA in brown adipose tissue and UCP3 mRNA in skeletal muscle. These results suggest that UCP1 and UCP2 may contribute to the promotion of energy metabolism by capsiate, but that thyroid hormones do not.

oxygen consumption; body fat; energy expenditure; CH-19 Sweet; thermogenesis

CAPSICUM FRUITS, red peppers, are used throughout the world as spices that stimulate gustation and make insipid foods more appetizing. The major pungent principle of red pepper is capsaicin (36), which has been reported to elevate body temperature (11), stimulate the secretion of catecholamines (52), promote energy expenditure (24), and suppress body-fat accumulation (23) in experimental animals. Consequently, capsaicin is thought to constitute a potential dietetic therapy for obesity and diabetes. However, capsaicin is strongly pungent and neurotoxic (22), which largely prohibits its administration to humans.

Yazawa et al. (55) reported that CH-19 Sweet, a nonpungent cultivar of *Capsicum annuum L.*, rarely contains pungent capsaicinoids but contains a large amount of two capsaicinoid-like substances. The chemical structures of these two substances have been identified and named capsiate and dihydrocapsiate by Kobata et al. (28). The acyl residue of capsiate is identical to that of capsaicin, although its aromatic portion is not vanillylamine, as it is in capsaicinoids, but vanillylalcohol.

Capsiate has no pungency to the human sense of taste (27). However, CH-19 Sweet, which contains a large amount of capsiate, as described above, has been reported to increase body temperature and oxygen consumption in humans (41). Our laboratory has previously demonstrated that continuous administration of capsiate suppresses body fat accumulation in mice, as does the administration of capsaicin (38, 40). Capsiate can be taken in large amounts without pain and should be as effective in humans as it is in experimental animals.

As shown above, our laboratory (38, 40) previously demonstrated that 2-wk treatment of capsiate suppresses body fat accumulation in mice. In the present study, to clarify the mechanism underlying this effect, we examined whether continuous administration of capsiate promotes energy expenditure. To this end, we measured respiratory gas by indirect calorimetry with mass spectrometry. This method has been used in experimental animals to detect changes in metabolism in response to the intake of foods or drugs. Furthermore, we also examined whether capsiate affects the uncoupling proteins (UCPs) in brown adipose tissue (BAT), white adipose tissue (WAT), and skeletal muscle. UCPs play important roles in energy expenditure, body weight maintenance, and thermoregulation. UCP1 is expressed exclusively in BAT (7, 32) and is a key molecule of thermogenesis in BAT, which is an important organ for cold- and diet-induced thermogenesis in rodents (32, 44). UCP2 was identified in 1997

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(13, 14) and ubiquitously expressed (13). Genetic studies have reported UCP2 linked to energy dissipation in humans (6, 14). UCP3 is an identified protein in skeletal muscle and is also expressed in BAT in rodents (5, 51). UCP3 in skeletal muscle is upregulated by fasting (2, 53). It is presently thought to act as a transporter protein of free fatty acid, rather than thermogenesis or energy balance (18, 45). Triiodothyronine (T_3) and thyroxine (T_4) are thyroid hormones that are potent modulators of adaptive thermogenesis by influencing several aspects of energy metabolism, such as substrate cycling, ion cycling, and mitochondrial proton leaks (19, 33). In the present study, we assayed the levels of UCPs and serum thyroid hormones after the administration of capsiate to clarify the mechanism underlying suppression of body fat accumulation in mice.

MATERIALS AND METHODS

Animals

Five-week-old male Std ddY mice (Japan Shizuoka Laboratory Center, Hamamatsu, Japan) were treated with a single dose or continuous treatment of capsiate. They were housed in standard cages ($33 \times 23 \times 12$ cm^3) under controlled temperature (22 ± 0.5°C), humidity (50%), and lighting (lights on from 1800 to 0600) conditions. Care and treatment of the experimental animals conformed to the Kyoto University guidelines for the ethical treatment of laboratory animals.

Chemicals

Capsiate was synthesized as reported previously (28). All other chemicals were from Nacalai Tesque (Kyoto, Japan) or Wako Chemicals (Osaka, Japan) and were of guaranteed reagent grade.

Experimental Procedures

Continuous treatment of capsiate. Mice were allowed to adapt to laboratory housing for at least 1 wk. Mice were housed in a windowless room with a 12:12-h light-dark cycle. Mice were randomly divided into two groups, so that the mean body weights of the groups were identical, and were administered vehicle (control) or capsiate (10 mg/kg body wt) via a stomach tube every day for 2 wk. Vehicle solution consisted of 0.85% NaCl solution, which contained 3% ethanol and 10% Tween 80, as described elsewhere (52). During this period, commercial diet (type MF; Oriental Yeast, Tokyo, Japan) was provided every day to the mice of each group, such that each mouse received the same amount of food. The mice were then killed, and the organs were removed and weighed.

Respiratory gas analysis. Twelve acrylic metabolic chambers, gas analyzers (model RL-600), and a switching system (model ANI6-A-S) to sample gas from each metabolic chamber were used to measure oxygen consumption and respiratory quotient in the mice. Each mouse was placed in a metabolic chamber designed to measure respiratory gas. These methods have been described in a previous report (21). 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 amounts of fat and carbohydrate oxidized were calculated from the values for oxygen consumption and respiratory quotient by using appropriate software. The data for each chamber were collected every 13 min and stored on a spreadsheet. The instruments and software were obtained from Alco System (Chiba, Japan). Two weeks after the start of treatment, we moved each animal in the control and capsiate groups to a metabolic chamber to measure, by indirect calorimetry, the 24-h oxygen consumption and the amounts of fat and carbohydrate oxidized. Oxygen consumption and the amounts of fat and carbohydrate oxidized were calculated as integrated values for the 12-h dark, the 12-h light, and the total 24-h periods. During the period in the metabolic chamber, each mouse had free access to water and the same amount of food as in their home cages. To avoid the effects of a single treatment, the measurement of respiratory gas was carried out at least 24 h after the last dose.

BAT UCP1 content analysis by Western blotting. Experimental methods were as reported previously (24). Briefly, BAT was removed from the mice, immediately frozen in liquid nitrogen, and stored at −80°C until analysis. BAT mitochondria were isolated as reported previously (8), and the total protein content in BAT was measured with a DC protein assay kit (Bio-Rad). The mitochondrial fraction (5 μg) isolated from the BAT of each mouse was subjected to reduced SDS-PAGE, transferred onto a nylon membrane, and reacted with anti-rat UCP1 serum (24). Its specificity has been shown as described elsewhere (16, 35, 54). UCP1 protein content was determined by Western blot analysis, as previously described (49). These membranes were then incubated with pig anti-rabbit IgG antibody conjugated with horseradish peroxidase (Dako Japan, Kyoto, Japan). UCP1 was thus quantified by densitometric and image analysis and expressed as a relative value for each group of mice.

Determination of serum thyroid hormone levels. Mice were killed by decapitation, and blood samples were collected on ice. Blood samples were immediately centrifuged at 8,000 *g* for 10 min, and serum samples were collected. Serum T_3 and T_4 levels were determined by using a T_3 Riabead kit and T_4 Riabead kit (Dinabot, Tokyo, Japan), respectively.

Single dose of capsiate. Mice were allowed to adapt to the laboratory housing for at least 1 wk before the experiment. During this period, mice were allowed free access to water and a stock diet (type MF). To avoid circadian variations in physical activity, experiments were performed between 1300 and 1700. Mice were prohibited access to food for 2 h before treatments to avoid any effects exerted by the components of the food on the animals' digestion or absorption. Capsiate was suspended in vehicle, and mice were administered capsiate solution (10 mg/kg body wt) via a stomach tube. As a control group, we used untreated mice. Mice were killed at either 0.5, 1, or 2 h after administration of capsiate, and the BAT and gastrocnemius muscle were then removed.

RNA isolation. BAT, epididymal fat, and gastrocnemius muscle were removed from the mice, immediately frozen in liquid nitrogen, and stored at −80°C until analysis for UCP mRNA expression. These organs were homogenized, and total RNA was isolated by using an SV Total RNA Isolation System (Promega).

RNA preparation and real-time fluorescence monitoring RT-PCR. Experimental methods have been reported previously (47). Briefly, using M-MLV reverse transcriptase (GIBCO-BRL), total RNA was reverse transcribed, according to the manufacturer's instructions, by using a thermal cycler (Takara PCR Thermal Cycler SP; Takara, Shiga, Japan). To quantify mRNA expression, PCR was performed by using a fluorescence temperature cycler (LightCycler System; Roche Diagnostics, Mannheim, Germany). Oligonucleotide primers for mouse UCP1, UCP2, UCP3, and GAPDH (used as an internal standard) cDNAs were designed (Table 1). Amplifi-

Table 1. *Sequences of PCR primers and GenBank accession numbers for UCPs and GAPDH*

Target Gene	GenBank No.	Primer Sequence
UCP1	U63419	F: ctcaggattggcctctacgactc
		R: ttggtgtacatggacatcgca
UCP2	U69135	F: ctggtcgccggcctgcagcgc
		R: gatcccttcctctcgtgcaat
UCP3	AF032902	F: tacccaaccttggctagacgc
		R: gcaacttctccttgatgtcgta
GAPDH	m32599	F: gaaggtcggtgtgaacggatt
		R: gaagacaccagtagactccacgacata

UCP, uncoupling protein; F, forward; R, reverse.

cation was performed according to a published protocol (43). Briefly, the reaction solution (10 μl final volume) contained 3 μM $MgCl_2$, 2 μl LightCycler DNA Master SYBR green I dye, and 5 μM each of the forward and reverse primers. The standard amplification program included 30 cycles, with a ramping rate of 20°C/s: denaturation at 95°C for 30 s, annealing at 55°C for 5 s, and extension at 72°C for 10 s. Fluorescence at 530 nm was recorded online during the extension phase. External standards for the PCR products were prepared for amplification from the mRNA of previous samples by the same method described above using the LightCycler. The external standards and samples were amplified simultaneously. The first cycle number indicated specific fluorescence against noise, and the logarithm of the concentration of the PCR product standard, the external standard curve, was calculated with LightCycler software version 3. To confirm the amplification of specific transcripts, melting-curve profiles were produced at the end of each run.

Statistical analysis. All data are expressed as means ± SE. Oxygen consumption and the amounts of carbohydrate and fat oxidized were analyzed by Dunnett's multiple-comparison test (Figs. 1 and 2). Relative organ weights were analyzed by an unpaired *t*-test (Table 2). UCP1 content data were analyzed by Tukey's multiple-comparison test (Fig. 3). UCP mRNA data were analyzed by an unpaired *t*-test (Fig. 4). UCP1 and UCP3 mRNA expression data were analyzed by Fisher's paired least significant difference multiple-comparison test (Fig. 5). Statistics were calculated with the StatView software package (Macintosh Version J 5.0, Abacus Concepts, Berkeley, CA), and differences were considered statistically significant at $P < 0.05$.

RESULTS

Effects of Continuous Treatment of Capsiate

Oxygen consumption was significantly higher in the capsiate group than in the control group for the 12-h dark, 12-h light, and total 24-h periods (Fig. 1). Carbohydrate oxidation was significantly higher in the capsiate group for the 12-h dark and total 24-h periods than in the control group (Fig. 2*A*). Fat oxidation was significantly higher in the capsiate group for the 12-h light and total 24-h periods than in the control group (Fig. 2*B*).

The mean body weight of mice in the capsiate group was significantly lower than that of mice in the control group (38.98 ± 0.82 vs. 42.06 ± 1.80 g; $P < 0.05$). Organ weights were divided by the body weight of each mouse and given as relative values. The relative weights of epididymal and perirenal fat in the capsiate group were significantly lower than those in the control group, and continuous administration of capsiate suppressed abdominal fat accumulation (Table 2). The relative weight of the gastrocnemius muscle of mice in the capsiate group was significantly higher than that of mice in the control group (Table 2). The weights of heart, liver, spleen, kidney, quadriceps muscle, and BAT did not differ between the groups (Table 2).

UCP1 content in the BAT mitochondrial fraction was significantly higher in the capsiate group than that in the control group (Fig. 3). In BAT, UCP1 mRNA levels were significantly higher in the capsiate group than in the control group, but UCP2 and UCP3 mRNA levels did not differ between the groups (Fig. 4*A*). In epididymal fat, UCP2 mRNA levels were significantly higher in the capsiate group than in the control group (Fig. 4*B*), and UCP1 mRNA levels were higher in the capsiate group than in the control group (Fig. 4*B*, $P = 0.052$). UCP3 mRNA levels in epididymal fat did not differ between groups (Fig. 4*B*). UCP1, UCP2, and UCP3 mRNA levels in gastrocnemius muscle did not differ between the groups (Fig. 4*C*).

Serum T_3 levels in the capsiate group did not differ from those in the control group (0.75 ± 0.03 vs. 0.73 ± 0.03 ng/ml). Serum T_4 levels in the capsiate group did not differ from those in the control group (4.56 ± 0.31 vs. 4.51 ± 0.31 μg/dl).

Levels of UCPs mRNA After a Single Dose of Capsiate

UCP1 mRNA levels in BAT in the capsiate group were significantly elevated ~2.2-fold, compared with levels in the control group, at 0.5 h after the administration of capsiate and returned to the sedentary levels at 1 h after treatment (Fig. 5*A*). UCP3 mRNA levels in gastrocnemius muscle in the capsiate group were elevated ~1.7-fold, compared with levels in the control

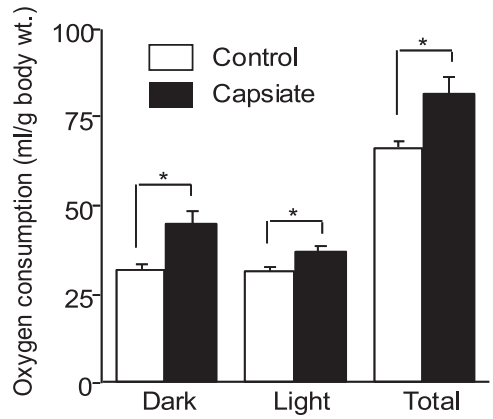

Fig. 1. Oxygen consumption in the 12-h light, 12-h dark, and total 24-h periods of mice administered vehicle (control) or capsiate (10 mg/kg wt) every day for 2 wk. Each mouse was placed in a metabolic chamber designed to measure respiratory gas individually and given free access to water and the same amount of food. Room air was pumped through the chambers, and the expired air was directed to a gas analyzer. Values are means ± SE; $n = 5$–6. *Significant difference between control and capsiate groups, by Dunnett's multiple comparison test ($P < 0.05$).

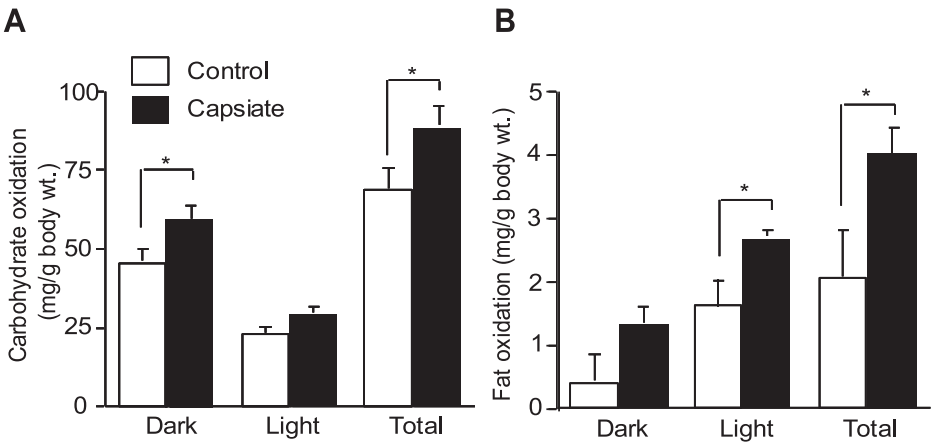

Fig. 2. The amount of carbohydrate (*A*) and fat (*B*) oxidized in the 12-h light, 12-h dark, and total 24-h periods in mice administered vehicle (control) or capsiate (10 mg/kg wt) every day for 2 wk. Each mouse was placed in a metabolic chamber designed to measure respiratory gas individually and was given free access to water and the same amount of food. Room air was pumped through the chambers, and expired air was directed to a gas analyzer. The amount of carbohydrate and fat oxidized was calculated from oxygen consumption and respiratory quotient. Values are means ± SE; n = 5–6. *Significant difference between control and capsiate groups, by Dunnett's multiple comparison test ($P < 0.05$).

group, at 0.5 h after the administration of capsiate (P = 0.061) and were significantly elevated ~1.9-fold at 2 h after treatment (Fig. 5*B*). The initial increases in UCP1 and UCP3 mRNAs did not differ statistically from one another. However, the levels of UCP1 mRNA decreased to the control levels almost immediately after treatment, whereas UCP3 mRNA tended to remain elevated in the capsiate group 0.5–2 h after treatment.

DISCUSSION

In the present study, continuous administration of capsiate raised oxygen consumption, an index of energy expenditure, suggesting that capsiate-treated mice burned more calories. Capsiate-treated mice ingested the same number of calories as control mice, so any increase in energy expenditure induced by capsiate may be attributable to the suppression of body fat accumulation. Continuous capsiate treatment promoted carbohydrate oxidation during the 12-h dark and total 24-h periods and fat oxidation during the 12-h light and total 24-h periods. Mice ingest more food during the dark period than during the light period and vegetate or sleep during the light period. These data suggest that capsiate-treated mice burn more dietary carbohydrate than vehicle-treated mice during the dark period and burn more body fat during the light period.

We have shown previously that capsiate administered to mice promoted energy metabolism and suppressed body fat accumulation (40). UCPs are thought to play important roles in energy expenditure, the maintenance of body weight, and thermoregulation. In the present study, we examined UCPs to explain the increase in energy expenditure induced by capsiate. A single dose of capsiate temporarily elevated UCP1 mRNA levels in BAT, and 2-wk treatment elevated the levels in the long term. We also showed that 2 wk of treatment with capsiate increased the UCP1 protein content in BAT. These results suggest that the increase in UCP1 protein content in BAT may result from temporary and/or chronic activation of *Ucp1* transcription. The increase in BAT UCP1 may contribute to the promotion of energy metabolism and the suppression of body fat accumulation induced by capsiate. It has been demonstrated that UCP1$^{-/-}$ mice consume less oxygen after treatment with a β_3-adrenergic receptor agonist, suggesting that BAT UCP1 may contribute to thermogenesis after the activation of the sympathetic nervous system (12). We showed upregulation of BAT UCP1 by continuous administration of capsiate, suggesting that capsiate may increase thermogenesis after the activation of the sympathetic nervous system. In previous studies, capsiate was shown to promote the secretion of adrenaline in mice (38), suggesting that capsiate activates the sympathetic nervous system. The increase in oxygen consumption after capsiate administration may contribute to the suppression of body fat by capsiate.

Stefl et al. (46) reported that aP2-*Ucp* mice, in that UCP1 overexpressed specifically in WAT, were resistant to obesity-induced high-fat diet. The mice tended to increase metabolic rate (46) and endogenous respiration of epididymal fat (29), suggesting that ectopic expression of UCP1 in WAT may play a role in energy expenditure. In the present study, continuous treatment of capsiate tended to increase UCP1 mRNA levels, suggesting that WAT UCP1 may contribute to the increase in energy expenditure by capsiate.

Unlike UCP1 or UCP3, UCP2 is expressed in many tissues (4, 13), and its function has been debated thoroughly. It has been reported that UCP2 has many physiological functions, namely, fat metabolism (13),

Table 2. *Relative organ weights of mice administered vehicle or capsiate*

	Control	Capsiate
Heart	0.37 ± 0.02	0.39 ± 0.01
Liver	4.46 ± 0.15	4.81 ± 0.10
Spleen	0.35 ± 0.01	0.34 ± 0.02
Kidney	1.23 ± 0.05	1.35 ± 0.02
Gastrocnemius muscle	0.90 ± 0.04	1.04 ± 0.02*
Quadriceps muscle	1.34 ± 0.06	1.43 ± 0.03
BAT	0.47 ± 0.04	0.41 ± 0.02
Epididymal fat	2.23 ± 0.38	1.28 ± 0.12*
Perirenal fat	1.16 ± 0.17	0.50 ± 0.01*

Values are means ± SE; n = 9–11. BAT, brown adipose tissue. *Significantly different from control, $P < 0.05$ by unpaired t-test.

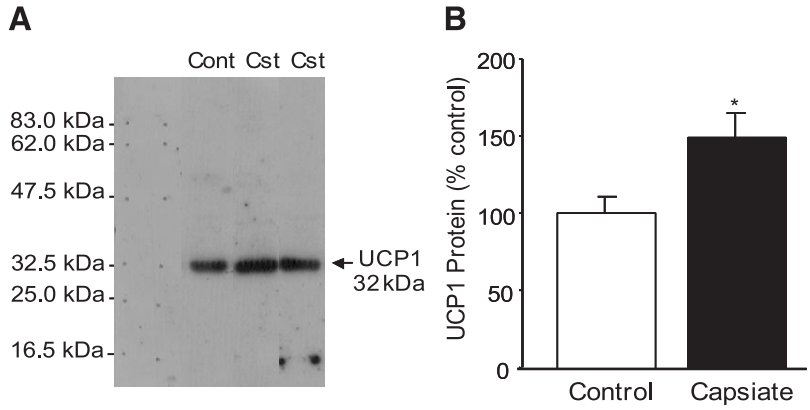

Fig. 3. *A*: Western blot analysis of uncoupling protein 1 (UCP1) in the interscapular brown adipose tissue (BAT) mitochondria of mice administered vehicle [control (Cont)] or capsiate (Cst) every day for 2 wk. *B*: UCP1 content was calculated as counts per the amount of total protein estimated from the data of Western blot analysis. Values are means ± SE; $n = 4$–5. *Significant difference between control and capsiate groups by Tukey's multiple-comparison test ($P < 0.05$).

energy dissipation (6), production of reactive oxygen species (1), insulin secretion (56), and nitric oxide production (26). UCP2 mRNA levels in many tissues were higher in UCP1$^{-/-}$ mice (12), and, conversely, UCP1 mRNA levels in WAT were higher in UCP2$^{-/-}$ mice (1). These data showed the possibility that UCP2 may have an alternative function for UCP1. We showed that capsiate increased UCP2 expressions in WAT, suggesting that WAT UCP2 may contribute to the increase in energy expenditure by capsiate.

T_3 and T_4 are thyroid hormones that affect heat production. Continuous administration of T_4 increases oxygen consumption and decreases body weight in *ob/ob* mice (50). However, we have demonstrated that administration of capsiate for 2 wk did not increase serum thyroid hormone levels, suggesting that thyroid hormones are not involved in the increase in metabolic rate induced by capsiate. Himms-Hagen et al. (17) previously demonstrated that chronic treatment of β_3-agonist 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 intercostal sympathetic nervous system, rather than via thyroid hormones.

UCP or UCP1 is upregulated in BAT by treatment of β_3-adrenergic receptor agonists (35). In a previous study, capsiate was shown to promote the secretion of adrenaline in mice (38), suggesting that capsiate activates the sympathetic nervous system. We examined whether capsiate activates the sympathetic nervous system in rats, according to the method of a previous report (40). A single dose of capsiate increased the sympathetic nervous system index in rats at 30 min after administration, compared with the control group (131.5 ± 17.0 and 85.5 ± 9.8% compared with before the treatment, respectively). In this study, the transcription of *Ucp1* induced by capsiate was activated in a comparatively rapid response, and UCP1 mRNA levels returned to sedentary levels at 1 h after the administration of capsiate. This transcriptional activation of *Ucp1* probably results from activation of the sympathetic nervous system. Capsiate increases free fatty acid levels in serum at 1 h after administration (38), probably as the result of the promotion of adrenaline

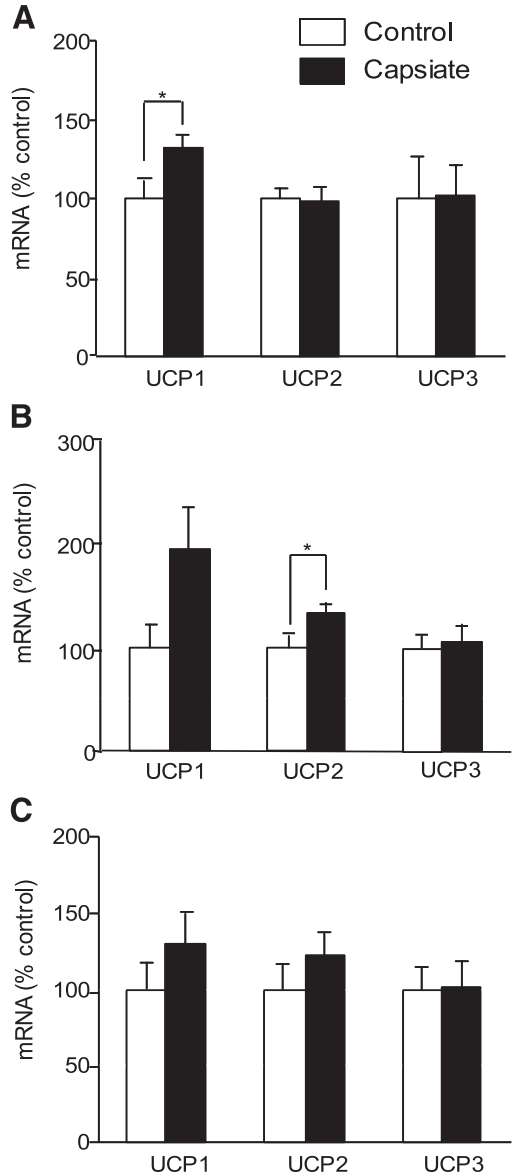

Fig. 4. UCP mRNA expressions in the interscapular BAT (*A*), epididymal fat (*B*), and gastrocnemius muscle (*C*) of mice administered vehicle (control) or capsiate (10 mg/kg wt) every day for 2 wk. UCP mRNA expressions were calculated as counts per GAPDH expression as internal standard. Values are means ± SE; $n = 9$–10. *Significant difference between 2 groups by unpaired *t*-test ($P < 0.05$).

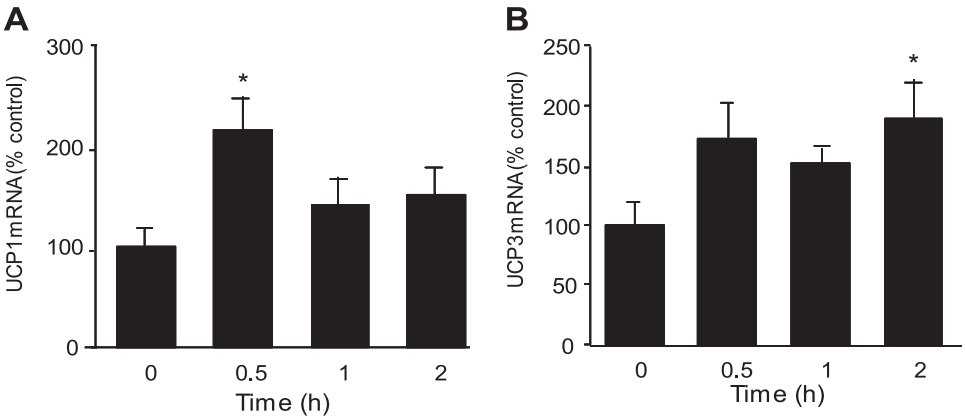

Fig. 5. UCP1 mRNA expressions in the interscapular BAT (*A*) and UCP3 mRNA expressions of mice in gastrocnemius muscle (*B*) administered capsiate. Mice were administered capsiate (10 mg/kg wt) and killed after 0.5, 1, or 2 h. Mice in the control group were untreated. UCP mRNA expression was calculated relative to the expression of GAPDH, which was used as an internal standard. Values are means ± SE; $n = 4$. *Significant differences between the 2 groups were established by Fisher's paired least significant difference test ($P < 0.05$).

secretion. Because a delay exists between the increase in free fatty acid levels and *Ucp1* transcriptional activation, sympathetic nervous stimulation, rather than free fatty acids, is believed to link capsiate to the increase in *Ucp1* transcription. This is supported by the fact that BAT is strongly innervated by the intercostal nerve (29) or capsaicin-sensitive nerve (42).

Continuous administration of capsiate singularly increased the relative weight of gastrocnemius muscle but did not increase that of quadriceps muscle, suggesting that capsiate probably has some effects on gastrocnemius muscle. We investigated the effect of capsiate on UCP3 in gastrocnemius muscle. UCP3 is expressed predominantly in skeletal muscle and is also expressed in BAT in rodents (5, 51). UCP3 mRNA in skeletal muscle is upregulated by fasting (2, 53) and exercise (14, 48). Kratky et al. (30) reported that the tissue-specific activity of lipoprotein lipase in skeletal muscle regulates the expression of UCP3 in transgenic mice. Fasting, exercise, or the overexpression of lipoprotein lipase induce high levels of fatty acids in serum or cells, suggesting that UCP3 in skeletal muscle may be upregulated by free fatty acids. In this study, we have shown that a single dose of capsiate induced a transient increase in UCP3 mRNA. Although the initial increases in UCP1 and UCP3 mRNAs were not significantly different, the capsiate-induced increase in UCP3 mRNA persisted for 2 h after administration, whereas the increase in UCP1 mRNA did not. Our laboratory (38) previously reported that capsiate elevated serum free fatty acid levels. This suggests that UCP3 transcription is activated by stimulation of the sympathetic nervous system at a relatively early time after capsiate treatment and that, at 2 h after treatment, elevated transcription is induced by rich free fatty acids in serum.

It has been reported that UCP3 is upregulated at 3 h after a single bout of exercise but returns to the sedentary level at 24 h (48). In contrast to this, Boss et al. (3) reported that endurance training downregulated UCP3. Moreover, UCP3 has been reported to be upregulated by fasting but return to sedentary level at 4 h after refeeding (20). Furthermore, it was suggested that a plausible physiological role for UCP3 is as a transporter protein in muscle and BAT mitochondria, not as a UCP (18, 45). It may be suggested that UCP3 is linked to the transport of fatty acids in the short term, rather than to thermogenesis and energy balance. We have shown that a single dose of capsiate temporarily increased UCP3 mRNA in skeletal muscle, but continuous treatment did not increase UCP3 mRNA. This suggests that UCP3 in skeletal muscle may not directly participate in the increase in energy expenditure induced by capsiate but may participate in fatty acid transport in the short term, leading to the suppression of body fat accumulation induced by capsiate. It may be supported that UCP1/UCP3 double-knockout mice were indistinguishable from UCP1 single-knockout mice (15).

In rodents, BAT plays a very important role in thermogenesis, whereas, in adult humans, BAT plays little part in thermogenesis (31). However, there are several reports linking UCP2 to energy expenditure in humans (6, 37). UCP2 may be involved in the regulation of the resting metabolic rate and the prevention of obesity in humans, which is comparable to the roles of UCP1 in rodents. We have shown that, not only UCP1, but also UCP2, was upregulated by capsiate in animals. Moreover, continuous treatment of capsiate tended to increased UCP1 levels in WAT. It is possible that capsiate should affect the transcription of UCPs in humans, leading to increase oxygen consumption. In fact, our laboratory (41) previously demonstrated that CH-19 Sweet, which contains a large amount of capsiate, increased oxygen consumption in humans.

It has been demonstrated that capsaicin induces an increase in UCP1 protein in BAT (24), in the concentration of serum adrenaline, and in oxygen consumption and induced a decrease in perirenal adipose tissue (24). Despite its lack of pungency, capsiate has effects similar to those of the more pungent capsaicin on UCPs, energy metabolism, adrenaline release, and body fat accumulation, suggesting that pungency itself is not involved in the various physiological effects of capsaicin. This may provide new insight into the action of capsaicin and its analogs in cells. Recently, data on the capsaicin receptor and its analogs have been accumulating. Caterina et al. (10) isolated a single cDNA encoding the capsaicin-gated channel and named this receptor vanilloid receptor (VR) subtype 1 (VR1). VR1-

expressing cells are activated not only by capsaicin but also by exposure to noxious heat, so VR1 is thought to be a nociceptor. These workers also isolated a VR-like protein subtype 1, as a subtype of VR, which is activated by noxious heat but not by capsaicin (7). In a previous report, our laboratory (39) demonstrated that increases in body temperature in mice induced by capsiate are inhibited by capsazepine, a capsaicin antagonist, suggesting that capsiate raises body temperature in mice via VR. Watanabe et al. (52) have reported that, even though capsaicin analogs with C14 to C20 alkyl side chains have no pungency, they stimulate the secretion of adrenaline as well as capsaicin does. Moreover, Kim et al. (25) reported that a chemically synthesized capsaicin analog with a C18 alkyl side chain showed no pungency but induced an acceleration of fat metabolism.

In summary, the pungency of capsaicin analogs may not contribute slightly to the physiological effects of these compounds, such as adrenaline release and increase in body temperature. We have shown that the metabolic effects by capsiate are akin to those by capsaicin in experimental animals. This study suggests that capsiate, a nonpungent capsaicin analog, may be an alternative to capsaicin for the therapeutic treatment of obesity in humans and implies the existence of a novel VR subtype distinct from nociceptors such as VR1, or gives a new perspective on the function of VR1.

DISCLOSURES

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