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Effects of CH-19 Sweet, a Non-Pungent Cultivar of Red Pepper, in Decreasing the Body Weight and Suppressing Body Fat Accumulation by Sympathetic Nerve Activation in Humans

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'CH-19 Sweet' is a non-pungent red pepper and enhances the energy expenditure in humans in like manner to the pungent red pepper. We investigated in this study the effects of a repeated intake of CH-19 Sweet for two weeks on the body weight and body fat in humans. Changes in the autonomic nervous activity after ingesting CH-19 Sweet were also measured by a power spectral analysis. We established a new protocol which allows the precise detection of weight change in humans by using fewer subjects. These methods were used to show that the repeated intake of CH-19 Sweet reduced the body weight and suppressed body fat accumulation. Furthermore, the body weight loss due to the repeated intake of CH-19 Sweet was significantly correlated with the sympathetic nervous response after its ingestion. We propose that the repeated intake of CH-19 Sweet reduced the body weight and suppressed body fat accumulation by sympathetic nervous activation in humans.

Key words: CH-19 Sweet; capsiate; sympathetic nervous activity; weight loss; human

Growing fat tissue secretes certain adipocytokines which worsen the multiple risk-factor syndrome.[1)] It is therefore important to control the accumulation of body fat in order to maintain good health. High energy expenditure has been advocated as a potential way of preventing the accumulation of body fat.

The administration of capsaicin, which is the main ingredient of pungent red pepper, is known to markedly stimulate energy expenditure through activation of the sympathetic nervous system.[2,3)] The results of animal experiments have shown that capsaicin stimulated sympathetic nervous activity, promoted the secretion of catecholamine and increased the expression of uncoupling protein (UCP) in brown adipose tissue.[2,4,5)] Consequently, capsaicin raised the oxygen consumption and core temperature.[6)]

Although the physiological action of capsaicin has been clear for some time, it has not yet been put into practical use due to the difficulties involved in using such a pungent condiment. It is very difficult to ingest the pungent red pepper in sufficient quantity to control excessive weight.

Yazawa *et al.* have recently reported their success in breeding a new non-pungent type of red pepper.[7)] CH-19 Sweet, originally named by Yazawa, contains only a very small amount of capsaicin. Instead, capsiate, a non-pungent member of the family of capsaicin, is included in a large amount.[8)] The similarity between the structures of capsiate and capsaicin led us to expect that it might have a similar physiological effect on raising the energy expenditure. The results of our previous study based on this idea show that capsiate activated energy expenditure in like manner to capsaicin,[9,10)] and the experimental results clearly indicated that the intake of capsiate would promote energy expenditure in both rodents and humans.

The purpose of the present study was to determine whether the increase in energy expenditure induced by a capsiate intake would be sufficient to suppress the accumulation of body fat in humans. In general, a large-scale design with many subjects is adopted out of necessity in research on food functions in humans because it is difficult to repeat the experiments. How-

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Abbreviations: RMR, resting metabolic rate; CT, computed tomography; RQ, respiratory quotient; LFC, low-frequency component; HFC, high-frequency component; DW, dry weight; SNS, sympathetic nervous system; UCP, uncoupling protein; BMI, body mass index

ever, since all human experiments are based on animal experiments, a large-scale experiment would be risky because of big gaps between large-scale human experiments and animal experiments. We therefore developed a new experimental protocol which allows for small scale and high accuracy to fill the gaps. This experimental protocol was required to be capable of detecting a slight change in body weight or body fat in humans. In a large-scale experiment, the individual nature of each basal metabolism would be canceled out by using a large number of subjects, even with all subjects taking the same number of calories. However, since a small-scale experiment cannot cancel out such individuality when taking the same number of calories, it is necessary to an appropriate diet to maintain a constant weight and ensure little individuality.

The present method made it possible to determine the fixed energy requirement of each subject to maintain a constant body weight. This requirement was measured in advance and the diets were optimized to minimize the change in body weight in the control group. In addition, we relieved the stress of prolonged dietary control, particularly when habitually taken items such as coffee and gum are prohibited.

Materials and Methods

Subjects. We excluded female subjects from the present study because of their menstrual cycle, people who take medication regularly and those with abnormal bowel habits. People who routinely perform excessive exercise were also excluded, as were extremely lean people with a body mass index (BMI) of less than 17 or obese people with BMI of greater than 30 and those who reported themselves to be sick, suffering from such ailments as diabetes and hyperlipemia. We recruited volunteers with a wide range of ages who had a living activity strength of 2 (a Japanese term, equivalent to a physical activity level of 1.5), and who lived near or worked at our university, because all measurements were carried out at the university and we wanted to be able to stringently control the volunteers. As a result, 12 males were selected for our study and assigned to two groups: the CH-19 Sweet group ($n = 7$; age, 32.29 mean $\pm$ 11.84 SD years) and the control group ($n = 5$; age, 27.80 mean $\pm$ 7.66 SD years). There were no smokers in either group. All subjects were treated by following the guidelines established by the Helsinki Declaration. Each subject provided his informed consent after being informed of the objectives and methods of the experiment. The Medical Ethics Committee of the Japanese Society of Nutrition and Food Science approved this study.

Protocol. We selected a treatment period of two weeks for CH-19 Sweet intake, based on our previous results from animal experiments.[9,11] This short intake period was also selected to minimize the stress on the subjects as much as possible. Nevertheless, we thought it highly likely that statistically significant differences would appear within this test period for the following reasons: First, in a previous study, we found that capsiate administration for two weeks clearly decreased the body fat in mice.[9,11] Second, we also found that the 24-hr oxygen consumption in mice was increased by 25% with capsiate administration (10 mg/kg wt.) for two weeks,[11] suggesting that the resting metabolic rate (RMR) may also be increased by repeated capsiate administration. Our previous studies had shown that the metabolic rate was increased 10% by a single capsiate administration (10 mg/kg wt.) in mice[9] as well as by a single CH-19 Sweet administration (0.1 g/kg wt.) in humans,[12] so we speculated that the total energy expenditure would increase by repeated intake of CH-19 Sweet for two weeks in humans. We estimated that the total metabolic rate would increase by an average of at least 10% per day throughout the treatment period, although this speculation was not based on any experimental data. The rough value for the total metabolic rate of a 30-year-old Japanese male living an average life is 2250 kcal,[13] so we speculated that the increase in energy expenditure due to the CH-19 Sweet intake would be 200–300 kcal per day. The simple sum of the energy expenditure increase for two weeks was thus approximately 3500 kcal. Since 1 g of fat tissue is equivalent to about 7 kcal energy, the decrease in fat tissue was hypothesized to be approximately 500 g in two weeks. Supposing that the increase in energy expenditure was not due only to fat oxidation but also to carbohydrate oxidation, this meaning glycogen reduction because 1 g of glycogen is 4 kcal and is hydrated with approximately 3 g water, the body weight loss per calorie consumption would be greater. We speculated that the total decrease in body weight due to the repeated intake of CH-19 Sweet for two weeks would exceed 1000 g. To detect this change, we decided to very precisely analyze a relatively small number of subjects for two weeks, this enabling us to suppress the variation of body weight and fat weight due to various factors. Furthermore, the required sample size to detect significant differences with the unpaired *t*-test was 7.7, which was calculated by using 70 kg as the average body weight, 1 kg as the weight loss, 0.7 kg as the SD, which is equivalent to 1% of the coefficient of variation, and 80 as the statistical power. Accordingly, we believed we would be able to detect the weight loss due to the CH-19 Sweet intake if we suppressed the weight fluctuation within a coefficient of variation of 1%, and aimed at having the range of weight fluctuation fall within 1%.

Based on these considerations, we set an adjustment period of one week to fix the total energy intake that would suppress weight fluctuation, this being followed by a treatment period of two weeks during which the subjects were administered CH-19 Sweet.

During the experimental period, the subjects were

Table 1. Typical Daily Menu (Subject I)

	Menu	Energy (kcal)	P (g)	F (g)	C (g)
		2254	88.72	63.67	345.43
breakfast	roll of bread, 2 pieces (32 g × 2)	210	6.00	6.00	32.00
	corn cream soup	72	1.30	2.10	12.00
	ham (loin roll) (50 g)	58	17.20	2.70	5.60
	cherry tomato (50 g)	15	0.55	0.05	3.50
	cucumber (100 g)	14	1.00	0.10	3.00
	mayonnaise (12 g)	88	0.20	8.80	0.50
	yogurt (90 g)	81	3.50	1.89	12.60
lunch	rice (300 g)	453	6.90	1.80	102.00
	"stir-fried vegetables & seafood" frozen pack (190 g)	120	5.90	7.80	5.70
	milk (200 ml)	133	6.60	7.60	9.50
	fresh cabbage (75 g)	17	0.98	0.15	3.90
	non-oil dressing (15 g)	15	0.50	0.02	3.03
supper	rice (300 g)	453	6.90	1.80	102.00
	instant miso soup with brown seaweed	28	1.60	0.70	3.70
	hamburger steak, 1 piece (26 g)	69	3.17	4.83	3.17
	hamburger steak with tofu, 1 piece (26 g)	33	2.50	1.25	3.00
	cutlet of beef and lotus root, 1 piece (25 g)	61	2.50	3.25	5.50
	fried chicken, 1 piece (15 g)	48	3.00	1.65	5.40
	meat ball, 2 pieces (16.6 g × 2)	57	3.40	2.90	4.20
	potsticker (steam-baked meat pie), 2 pieces (20 g × 2)	77	3.00	3.66	8.00
	boiled chicken with seaweed, 1 piece (27 g)	35	2.60	1.70	2.30
	stir-fried burdock root and carrot, 1 piece (25 g)	38	4.50	0.90	3.20
	savory steamed egg custard, 1 piece (80 g) (egg cooked in the form of bean curd)	33	2.90	1.80	1.20
	cherry tomato (50 g)	15	0.55	0.05	3.50
	fresh cabbage (75 g)	17	0.98	0.15	3.90
	non-oil dressing (15 g)	15	0.50	0.02	3.03
green tea 500 ml/day					

P, protein; F, fat; C, carbohydrate

required to eat the same breakfast, lunch and supper every day, because changing the meal contents for each subject would have been complicated and we wanted to eliminate the influence of differences in meal ingredients on the energy metabolism (Table 1). Additionally, the subjects were instructed to abstain from drinking alcohol, performing irregular exercise and eating any food other than that designated, but were allowed to drink water freely.

To reduce stress during the experimental period, the menu had a variety of foods so that the subjects would not get bored and would be satisfied (Table 1). Fresh vegetables were supplied every day. The subjects could also ingest specific amounts of their favorite foods and beverages which they selected every day: coffee, café au lait, green tea, additional milk, diet coke, gum, isotonic drinks and ice cream (Table 2). In addition, if any individual disliked certain foods, a part of his menu was modified. Although the designed menus were largely satisfactory, some changes were necessary: the menu for subject H excluded mayonnaise, and that for subject L excluded milk and replaced cucumber with cabbage (Table 2). Although subject K tended to be constipated because of the change in his dietary habits, we were able to improve his situation by increasing the amount of cabbage from 75 g to 150 g.

Adjustment of the total energy intake. We adjusted the total energy intake per day for each subject so that each was able to keep his body weight nearly stable. The total energy intake for each subject at the start of the adjustment period was determined by subtracting the energy of self-selected foods and beverages from the required energy intake calculated from the "Recommended Dietary Allowances for the Japanese, 6th Revision."[13] For example, men 18–29 years old, whose average weight is 64.7 kg, require 2300 kcal at living activity strength 2. Although each total energy intake was calculated according to the average of a cluster, this provided only a rough indication for each subject. Therefore, it was not necessary to adjust the total energy intake with some subjects, but adjustment was necessary with others. We adjusted the amount of rice or bread to adjust the total energy intake. During the adjustment period, if a subject's weight increased or decreased for 2 successive days, his total energy intake was adjusted in consideration of his defecation or not. As a result of our adjustments, the range of each weight fluctuation was within 0.5% of the average weight during the last 3 days of the adjustment period, and the mean of its coefficient of variation was 0.202%, suggesting that we were able to successfully detect the weight loss due to CH-19 Sweet with our small number of subjects.

Table 2. Individual Menu

Subject	Intake energy	Self-selected favorite menu	Changed menu
A	1847 kcal		
B	2002 kcal	café au lait (200 ml)	
		green tea (500 ml)	
C	2991 kcal	coffee (500 ml)	
		milk (400 ml)	
		green tea (200 ml)	
D	2254 kcal	diet coke (200 ml)	
E	2199 kcal	coffee (300 ml)	
		fresh cream, 3 pieces	
F	2819 kcal	stick gum, 5 pieces	
		coffee (300 ml)	
		milk (500 ml)	
		green tea (500 ml)	
G	2494 kcal	isotonic drink (500 ml)	
H	2593 kcal	coffee (600 ml)	deleted mayonnaise at breakfast
I	2254 kcal		
J	2580 kcal	green tea (500 ml)	
		ice cream, 1 piece	
K	2696 kcal		cabbage 75 g → 150 g at breakfast and dinner
L	2145 kcal	tablet gum, 3 pieces	cucumber → cabbage at breakfast deleted milk at lunch

CH-19 Sweet group, A–G; control group, H–L

The subjects were given meals with minimal caloric fluctuation by appropriately mixing standardized commercial frozen foods, retort pouch food, mayonnaise and non-oil dressing adjusted for single usage, packaged corn cream and single servings of miso soup. The usage of standardized commercial foods was ideal for adjusting the caloric intake of each individual because the serving sizes of such foods are strictly controlled.

CH-19 Sweet and capsiate content. During the CH-19 Sweet intake period (treatment period), the total CH-19 Sweet intake was 0.4 g per kg standard weight per day, this being administered at one-third of the total intake before each meal. CH-19 Sweet was provided by Ajinomoto Co. and stored in a freezer. The subjects ingested it uncooked and frozen. In the present study, three lots of CH-19 Sweet were used sequentially: lot A on days 1–3, lot B on days 4–6 and lot C on days 7–14. Since CH-19 Sweet provides little energy (4 kcal/100 g), it was speculated that this energy input would not affect the weight loss. Therefore, the control group took nothing instead of CH-19 Sweet and was given the same meals as the CH-19 Sweet group.

The capsiate content of CH-19 Sweet was analyzed by HPLC, details of this HPLC procedure having been fully described by Maeda *et al.*[14] Briefly, fresh fruits of CH-19 Sweet were freeze-dried, homogenized after removing the seeds and calyces, and extracted with EtOAc for 5 min after removing the neutral lipids with acetone. The extract was analyzed by HPLC under the following conditions: column, μBondapak C18 (Waters, Massachusetts, USA; 10 μm, 3.9 × 150 mm); solvent, 70% methanol; flow rate, 1 ml/min; UV detection, 280 nm.

Physical condition monitoring. We monitored each subject's physical condition and activity every day, and recorded the stool frequency and all information about illness, physical injury, sleep and defecation. This information was gathered by interview.

Body weight. Body weight was measured while wearing underwear with a digital balance (FW100KAI digital weight scale; A&D Company, Tokyo, Japan; 0.01 kg weighing accuracy) after the subjects had avoided water for an hour and voided their bladders. Measurement was carried out every day before each meal, and the mean of the three daily body weights is defined as the body weight of the day.

Body composition and body fat distribution. The body composition and body fat distribution were measured during the adjustment period and on the last day of the treatment period. The body composition was evaluated by the relative fat percentage as measured by an air displacement plethysmograph, (BOD POD Body Composition System; Life Measurement Instruments, Concord, CA, USA). Details of this measurement procedure have been described previously by McCrory *et al.* and by Miyatake *et al.*[15,16] The percentage fat was automatically calculated by a computer, body density was calculated as the mass/body volume, and percentage body fat was calculated by using Brozek's formula.

The body fat distribution was determined by a computed tomography (CT) scan according to the procedure described by Tokunaga *et al.*,[17,18] in which the total cross-sectional area, subcutaneous fat areas and visceral fat area were measured at the level of the

umbilicus. All CT scans were performed in the supine position with a Somatom AR.SP CT scanner (Siemens, Erlangen, Germany). The intraperitoneal area with the same density as the subcutaneous fat layer is defined as the visceral fat area.

Respiratory gas analysis. RMR and the respiratory quotient (RQ) were measured breath-by-breath in the recumbent position before breakfast with a mass spectrometer (ARCO 2000; Arco System, Chiba, Japan), once during the adjustment period and then after one and two weeks of treatment. Measurement was carried out for 20 minutes after the subject had been sitting quietly in a chair for 30 minutes in the experimental room. Data for the first 5 minutes were not used in order to eliminate the influence of posture change.

Blood pressure. Blood pressure was measured by an HEM-757 sphygmomanometer (Omron Healthcare Co., Kyoto, Japan). Measurement was performed before breakfast.

Autonomic nervous response. To evaluate the response to CH-19 Sweet, we measured each subject's autonomic nervous activity at lunch time with or without the intake of CH-19 Sweet during the treatment period. First, the autonomic nervous activity before the meal was measured for 15 minutes. Then, after eating lunch with or without CH-19 Sweet for 15 minutes, the autonomic nervous activity was measured again for 60 minutes.

In order to evaluate the autonomic nervous activity, we used a power spectral analysis of the temporal intervals between each heart beat (R–R intervals). Details of the power spectral analysis procedure have been fully described by Moritani *et al.*[19,20] A power spectral analysis by fast Fourier transformation was performed on consecutive 256-s time series of the R–R interval data obtained during the test.

To evaluate the autonomic nervous system activity of each subject in the present study, we analyzed both the low-frequency component (LFC, 0.035–0.15 Hz) and high-frequency component (HFC, 0.15–0.5 Hz), the latter being the respiration-linked component. In general, HFC is associated almost entirely with vagal nerve activity, while LFC might be mediated mainly by sympathetic nerve activity and slightly by vagal nerve activity.

All subjects breathed in synchrony with a metronome at 15 times/min (0.25 Hz) to ensure that respiratory-linked variations in the heart rate did not overlap with low-frequency heart rate fluctuations (below 0.15 Hz) from other sources.

Since the basal autonomic nervous activity differs among individuals, the mean value for the autonomic nervous activity before the meal was standardized to 100%, and the relative values after eating lunch with or without CH-19 Sweet were then compared.

Data analysis. Each data value is expressed as the mean ± SEM. The effects of time, treatment, and time × treatment were evaluated by two-way repeated-measures ANOVA. A paired *t*-test was used to compare each group with the baseline. Statistics were calculated by using the StatView software package (Windows version J 5.0; Abacus Concepts, Berkeley, CA, USA). A probability level of <0.05 was considered to be statistically significant.

Results

Energy intake

The strict adjustment of the total energy intake per day for each subject enabled the range of weight fluctuation for each subject to be maintained within ±0.5% of the average weight in the final 3 days of the adjustment period, and the mean total energy intake per day to 2372 ± 419 (SD) kcal and 2454 ± 239 (SD) kcal in the CH-19 Sweet and control groups, respectively. No significant difference was apparent between the two groups. The mean energy intake per day of CH-19 Sweet was 26.6 ± 4.0 (SD) kcal.

Body weight

The body weight in the control group was nearly stable and decreased by only 0.4% during the treatment period, suggesting that the total energy intake selected for the adjustment period was almost equal to the total energy expenditure. On the other hand, the body weight in the CH-19 Sweet group started to decrease immediately after initiating the treatment period and attained statistical significance compared to the control group on day 3 of the treatment period (Fig. 1).

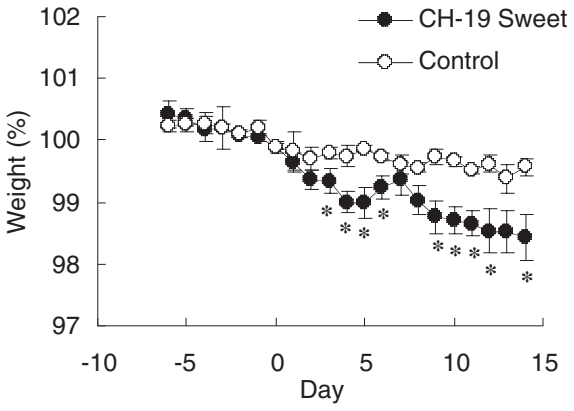

Fig. 1. Time-Course Characteristics of Body Weight in the CH-19 Sweet (n = 7) and Control (n = 5) Groups.

Body weight was measured every day before each meal, and the mean of the three measurements is defined as the body weight of the day. The mean value for body weight in the last 3 days of the adjustment period was standardized to 100%, and all values are expressed by relative values. Body weight decreased more significantly with time in the CH-19 Sweet group as compared to the control group. Each value is the mean ± SEM. $^{*}P < 0.05$ by the unpaired t-test.

Table 3. Characteristics of Subjects in the CH-19 Sweet (n = 7) and Control (n = 5) Groups When Measurements of Body Fat by BODPOD and CT Scan Were Carried Out during the Adjustment Period for Each Amount of Food Ingested and after 2 Weeks of Treatment

		Adjustment period		Week 2	
		CH-19 Sweet	Control	CH-19 Sweet	Control
body weight	(kg)	69.90 ± 4.29	69.10 ± 5.08	68.13* ± 4.14	68.65 ± 5.11
BMI	(kg/m^2)	23.77 ± 1.12	22.82 ± 1.83	23.17* ± 1.07	22.66 ± 1.81
body fat percentage	(%)	20.91 ± 3.46	17.56 ± 3.14	20.66 ± 3.48	18.76* ± 3.06
fat mass	(kg)	15.10 ± 2.97	12.51 ± 2.89	14.49 ± 2.84	13.28 ± 2.94
fat-free mass	(kg)	54.79 ± 2.74	56.59 ± 2.98	53.64* ± 2.86	55.38* ± 2.78
total fat area	(cm^2)	189.00 ± 43.09	151.18 ± 37.62	173.23* ± 38.71	149.14 ± 36.82
visceral fat area	(cm^2)	72.44 ± 23.07	36.08 ± 11.48	60.74 ± 15.53	38.36 ± 10.31
subcutaneous fat area	(cm^2)	116.56 ± 26.85	115.10 ± 26.61	112.49 ± 25.27	110.78 ± 26.72

Each value is the mean ± SEM. *$P < 0.05$ vs. baseline by paired t-test.

Physical condition

There was no indication of any physical abnormality during the study, nor was there any indication of illness or of extremely poor sleep during the experimental period. One subject (I) in the control group suffered a knife cut to his finger. The stool frequency did not differ between the groups, but did decrease slightly in both groups during the experiment, although there was no significant difference between the adjustment and treatment periods (data not shown). It is thought that the number of times for defecation served as an index of excrement weight, even though it does not express correct excrement weight. Thus, an increase in excrement weight is not believed to have contributed to the weight loss observed in the CH-19 Sweet group.

We confirmed on the measurement day that subjects' lifestyle had been stable and that the subjects had not been performing any irregular exercise. We also set the total energy intake per day for each subject to keep the body weight nearly stable by taking physical activity into account. Since some subjects remarked that the amount of exercise during the experiment was less than usual, we think that physical activity contributed little to the weight loss associated with the CH-19 Sweet intake.

All subjects consulted with a doctor on day-1, 7 and 13, and all were able to participate throughout the full experimental period without any deterioration in their physical condition. In spite of providing the same meal every day, the procedures that carried out resulted in dissatisfaction with the meals not being serious, and we were able to complete our experiment with very little stress on the subjects.

Effects of caffeine and dietary fiber

We allowed the subjects in the present study to freely ingest a specified amount of certain favorite foods. Two subjects in the control group and four in the CH-19 Sweet group choose to take additional caffeine by drinking coffee, café au lait, and additional green tea. Since we had determined the amount of foods which would not change the body weight during the adjustment period, it is considered that the effect of the caffeine contained in these favorite foods could be excluded. Moreover, the amount of caffeine intake did not correlate with the rate of weight loss of the four people in the CH-19 Sweet group (data not shown). Thus, we believe that the weight loss in the CH-19 Sweet group did not depend on caffeine, although it has been reported that caffeine accelerated energy expenditure in humans.[21–23)]

The amount of dietary fiber intake by the CH-19 Sweet group was more (2.85 ± 0.43 g/day) than that by the control group, because of the dietary fiber content of the fruits of CH-19 Sweet. Howarth *et al.* have studied a series of reported values for the weight loss effect of a dietary fiber intake, arriving at an average daily intake of 10 g/day, with an average intake period of 2.9 months, and an average weight reduction of 1.3 kg.[24)] Obese people were the subjects in this series of studies. Moreover, Howarth *et al.* have also reported that the degree of weight loss from dietary fiber was greater with obese people than with non-obese people.[24)] Thus, the weight loss effect has been assumed to be weaker with non-obese people. We therefore believe that the increase in dietary fiber (2.85 g/day) ingested for two weeks did not affect the weight loss experienced by the CH-19 Sweet group.

Body composition and body fat distribution

There was no difference in any of the parameters of the body composition and body fat distribution between the CH-19 Sweet group and the control group during both the adjustment period and the treatment period (Table 3). Although the body weight and BMI changes in the control group were minimal after the treatment period, the body fat percentage and fat mass increased, and the fat-free mass decreased (Table 3). On the other hand, in the CH-19 Sweet group, although the body fat percentage was changed very little by the treatment, the body weight, BMI, fat mass and fat-free mass were found to have decreased (Table 3). Moreover, the total fat area of the umbilicus in the CH-19 Sweet group was significantly less after the intake of CH-19 Sweet for two weeks (Table 3). The changes in each index from the adjustment period are shown in Figs. 2 and 3. Significant differences between the two groups were apparent

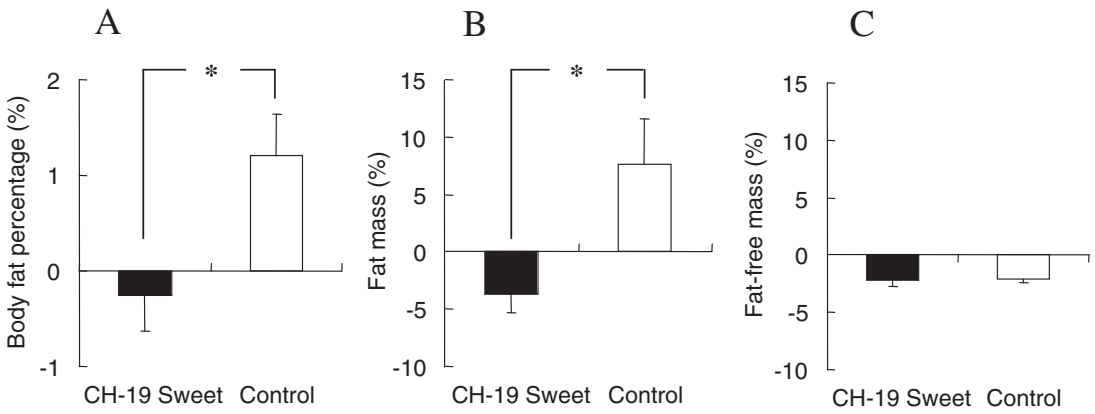

Fig. 2. Changes in Body Fat Percentage (A), Fat Mass (B) and Fat-Free Mass (C) Expressed as Relative Values during the Two-Week Treatment Period.

The body fat percentage and fat mass were slightly decreased in CH-19 Sweet group, but increased in the control group, resulting in significant differences between both groups. Each value is the mean ± SEM. $^{*}P < 0.05$ by unpaired t-test.

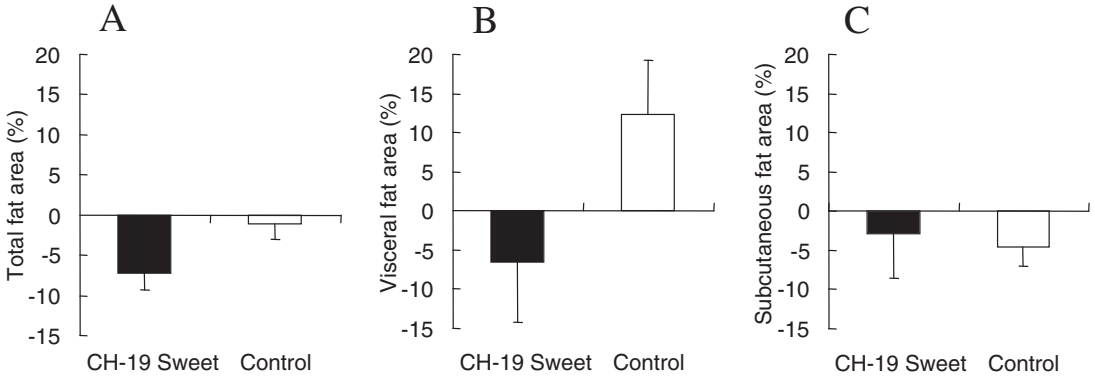

Fig. 3. Changes in Total Fat Area (A), Visceral Fat Area (B) and Subcutaneous Fat Area (C) Expressed as Relative Values for the Two-Week Treatment Period.

The variation in total fat area and visceral fat area were different between the CH-19 Sweet and control groups, but the variation in subcutaneous fat area was not different. Each value is the mean ± SEM.

in the change of body fat percentage and percentage change of fat mass (Fig. 2). The decrease in the total fat area of the umbilicus in the CH-19 Sweet group was greater than that in the control group (Fig. 3). The percentage of visceral fat area also tended to decrease in the CH-19 Sweet group, but did not change in the control group; no difference was apparent in the change of fat-free mass between groups.

Respiratory gas analysis

None of the parameters of respiratory gas differed between the CH-19 Sweet and control groups during either the adjustment period or the treatment period (Table 4). No difference was apparent in the parameters of respiratory gas between the adjustment and treatment periods in the control group (Table 4). While the resting oxygen consumption, carbohydrate oxidation and RMR of the CH-19 Sweet group did not differ between the adjustment period and the treatment period, RQ was significantly decreased after the CH-19 Sweet intake for 1 and 2 weeks, and there was a tendency toward increased fat oxidation ($P < 0.1$).

Blood pressure

The systolic and diastolic blood pressure increased slightly in both the CH-19 Sweet and control groups, although the difference was not significant, nor were there any significant differences between the two groups (Fig. 4).

Autonomic nervous response

LFC, which indicates the sympathetic nervous activity, tended to increase 26 and 34 minutes after the intake during lunch of CH-19 Sweet compared with the control group, while HFC, which indicates the parasympathetic nervous activity, did not differ between the two groups (data not shown). We tested the correlation between the weight loss and autonomic nervous response after the intake of CH-19 Sweet at lunch (Fig. 5). The autonomic nervous response was represented by a change in the

Table 4. Analysis of Expiration Gas in the CH-19 Sweet (n = 7) and Control (n = 5) Groups during the Adjustment Period for Each Amount of Food Ingested and after 1 and 2 Weeks of CH-19 Sweet Ingestion

		Adjustment period		Week 1		Week 2	
		CH-19 Sweet	Control	CH-19 Sweet	Control	CH-19 Sweet	Control
VO_2	(ml/min)	203.72 ± 25.66	188.58 ± 17.41	185.38 ± 29.65	182.46 ± 17.40	197.67 ± 21.34	185.06 ± 17.64
RQ		0.917 ± 0.027	0.886 ± 0.022	0.859* ± 0.019	0.895 ± 0.022	0.889* ± 0.021	0.875 ± 0.016
C	(mg/min)	751.80 ± 121.06	756.19 ± 87.71	641.99 ± 66.33	737.27 ± 82.50	796.64 ± 82.18	728.96 ± 73.70
F	(mg/min)	24.14 ± 7.04	33.88 ± 4.21	39.12 ± 9.10	30.66 ± 4.67	38.43 ± 9.87	38.09 ± 4.91
RMR	(cal/min)	957.36 ± 140.31	926.65 ± 90.36	794.80 ± 100.96	898.41 ± 88.66	969.53 ± 123.94	905.83 ± 87.31

Each value is the mean ± SEM. VO_2, oxygen consumption; RQ, respiratory quotient; C, carbohydrate oxidation; F, fat oxidation; RMR, resting metabolic rate
*$P < 0.05$ vs. baseline by paired *t*-test.

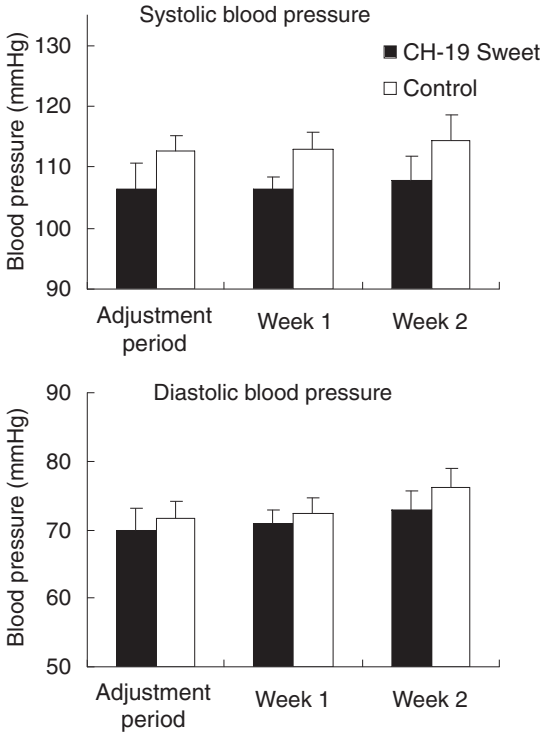

Fig. 4. Systolic and Diastolic Blood Pressure in the CH-19 Sweet (n = 7) and Control (n = 5) Groups for the Adjustment and Treatment Periods.

The data given are the mean blood pressure values for one week. Neither blood pressure value changed throughout the experimental period. Each value is the mean ± SEM.

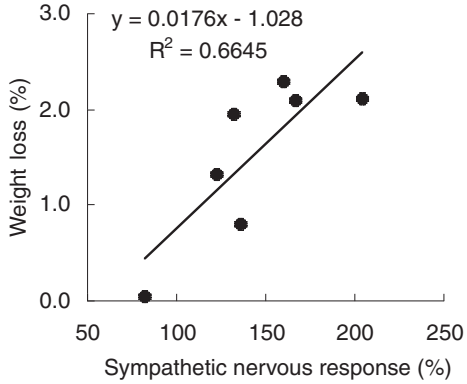

Fig. 5. Correlation between the Weight Loss in Two Weeks and the Sympathetic Nervous Response after the Intake of CH-19 Sweet.

The sympathetic nervous response is defined as the mean value of the low-frequency component (%) after the intake of CH-19 Sweet. Sympathetic nervous response was significantly correlated with the weight loss ($P < 0.05$).

percentage of autonomic nervous activity after the intake of CH-19 Sweet at lunch. The weight loss was significantly correlated with the sympathetic nervous response.

Capsiate content

The weight loss effect caused by CH-19 Sweet stabilized between day 4 and day 6 of the treatment period (Fig. 1). Since the weight loss effect was found to change, we measured the capsiate content of each lot of CH-19 Sweet, finding that the capsiate content of the lot used from day 4 to day 6 of the treatment period (lot B, 2.12 ± 0.13 (SEM) mg/g dry weight (DW)) was less than that of the lot used from day 1 to day 3 (lot A, 2.35 ± 0.13 (SEM) mg/g DW). We immediately discontinued using lot B and changed to a lot whose capsiate content was equivalent to that of lot A (lot C, 2.57 ± 0.12 (SEM) mg/g DW). The capsiate content tended to be greater in lot C than in lot B ($P < 0.1$ by Tukey's *post hoc* test). We believe that the differences in the amount of capsiate in the three lots of CH-19 Sweet were due to differences in the origin, *etc.* Since lot B had relative less capsiate, the weight loss effect might have been weaker from day 4 to day 6 of the treatment period.

Discussion

The present experimental design enabled us to reduce the stress in our subjects and raise the accuracy of the experiment as much as possible by precisely controlling the test conditions for each subject. We established similar conditions for our two groups by strictly managing each subject so that statistically significant differences would appear even when it was a small-scale experimental design. In this context, the present paper also reports a small-scale and precise preliminary

experimental design using humans for detecting the effects of food ingredients while showing the suppression of body fat accumulation by CH-19 Sweet.

Although the control subjects continued to eat the same meals after the adjustment period, there was little weight change during the treatment period. We therefore believe that the conditions used for the adjustment period were appropriate. Since individual differences in RMR were canceled by this method, it is highly significant to be able to adjust the amount of energy medication by using the weight change as an index. The body fat percentage in the control group had significantly increased by the end of the experiment when compared with the initial values. Thus, in order to minimize changes in the body composition in the control group, it may be necessary to adjust meal ingredients more than was done in the present study. The precise design of the present experiment enabled us to sensitively detect the weight loss due to the CH-19 Sweet intake and identify apparent differences with a small number of subjects for a comparatively short experimental period. This type of experimental protocol is useful as a pre-stage experiment before the large-scale experiment that is generally used with many subjects over a long period of time.

The results of the present study reveal that a single administration of CH-19 Sweet activated sympathetic nervous system (SNS) activity. Sympathomimetic compounds such as capsaicin have been assumed to correct the decreased SNS activity proposed as a possible factor leading to obesity.[25] Consequently, CH-19 Sweet may have the potential to improve the decreased SNS activity, which is an etiological feature of obesity. In addition, the amount of weight loss due to the repeated intake of CH-19 Sweet for two weeks was positively correlated with the degree of SNS activation by a single administration of CH-19 Sweet. Matsumoto *et al.* have reported that the SNS activity in obese young women was significantly less than that in non-obese women when the subjects ingested food containing capsaicin or a mixed food.[26,27] These reports suggest that there were large individual differences in the response of the autonomic nervous system to food ingredients. The degree of SNS activation caused by a single administration of CH-19 Sweet may serve as a potent marker to indicate whether a subject will respond to the repeated intake of CH-19 Sweet. By evaluating the autonomic nervous system response in advance, the validity of the repeated intake of CH-19 Sweet may become more marked.

The respiratory gas analysis and measurement of the body fat distribution in the present study suggest that CH-19 Sweet enhanced fat utilization. However, we were unable to detect any marked increase in energy expenditure by the respiratory gas analysis conducted in the early morning after fasting. The repeated administration of capsiate for two weeks to mice increased oxygen consumption, this increase being greater during periods of darkness, when locomotion and food behavior were increased, than during periods of light.[11] Moreover, our laboratory has revealed that the increase in fat oxidation was greater in mice that had been forced to run on a treadmill after capsiate administration than in mice that had rested after capsiate administration,[28] suggesting the possibility of generating a synergistic effect by combining capsiate intake and exercise. Thus, in the present study, the energy expenditure might also have been increased by CH-19 Sweet intake during daylight when the subject's activity increased.

We found that the CH-19 Sweet intake tended to reduce visceral fat rather than subcutaneous fat. Since the expression of β-adrenoreceptors is higher in visceral fat than in subcutaneous fat, lipolysis by catecholamine is strongly induced in visceral fat.[29] Additionally, because a single administration of capsiate has increased catecholamine secretion in mice,[9] lipolysis in visceral fat might have occurred more strongly than in subcutaneous fat as a result of the increase in catecholamine secretion caused by the CH-19 Sweet intake.

The repeated administration of capsiate to mice for two weeks has increased the UCP1 expression in brown adipose tissue, and a single administration of capsiate has increased the expression of *UCP1* mRNA in brown adipose tissue, *UCP2* mRNA in white adipose tissue, and *UCP3* mRNA in muscle.[11] Thus, the hyperexpression of UCPs may contribute to the suppression of body fat accumulation caused by the intake of CH-19 Sweet. Further studies are needed to clarify whether the CH-19 Sweet intake indeed induced hyperexpression of UCPs in humans.

A capsaicin receptor gene was cloned in 1997 and named vanilloid receptor 1 (VR1);[30] it is now called TRPV1 as a member of the TRP super-family.[31] Capsiate also activates TRPV1 as well as capsaicin, which has been investigated by patch clamp experiments.[32] Although capsiate administration has increased the body temperature of mice, this increase was suppressed by capsazepine, which is an antagonist of TRPV1.[10] These results suggest that capsiate would be an agonist for TRPV1 as well as capsaicin. The present study has shown that, because CH-19 Sweet induced various physiological effects, the capsiate contained in CH-19 Sweet is thought to have been accepted by TRPV1. We have shown that capsiate activated energy expenditure as well as capsaicin in mice.[9]

Although capsiate did not induce any of the averse responses that were induced by capsaicin when applying to the oral cavity of mice, capsiate has induced nociceptive responses with similar dose dependence to capsaicin by injecting subcutaneously into the hindpaws.[32] Thus, it has been assumed that capsiate had the potential to activate TRPV1 with similar potency to that of capsaicin, but did not activate TRPV1 in the oral cavity.[32] Capsiate has high lipophilicity and is easily broken down under normal aqueous conditions, so it has been assumed that capsiate cannot penetrate the epithe-

lium and reach TRPV1 in the oral cavity.[32] We have also found that capsaicin could be detected in circulating blood after an intragastric administration, while capsiate could not be detected in mice (our unpublished data). Although capsaicin may act in the oral cavity, in the gastrointestinal tract, and through circulating blood after its absorption, capsiate may act only in the gastrointestinal tract. To elucidate why non-pungent capsiate increased the energy expenditure as well as capsaicin, it remains to be investigated in which organs capsiate and capsaicin act. It has been suggested that hot pepper inhibits the fat intake for a short time by enhancing SNS activity, and that the main site for this action of hot pepper is not in the oral cavity.[33] Thus, the increased energy expenditure *via* SNS activation by both capsiate and capsaicin may be induced independently of pungency. In addition to capsiate, some non-pungent capsaicin analogues have also increased catecholamine secretion.[34] The present observation that the repeated intake of CH-19 Sweet for two weeks induced weight loss by SNS activation reconfirms that sufficient physiological effects can be induced without pungency.

Lejeune *et al.* have reported that the intake of capsaicin for 12 weeks did not affect the body weight but increased fat oxidation.[35] Belza *et al.* have reported that the intake of a combination of capsaicin, caffeine, catechins, L-tyrosine, and calcium for seven days increased the energy expenditure by about 40 kcal/day.[36] In the present study, the body weight was decreased by 1.7 kg by the intake of CH-19 Sweet, the calculated increase in energy expenditure by the degree of weight loss being 350–500 kcal/day. The effect of CH-19 Sweet was greater than that in these previous reports. The weight loss effect due to CH-19 Sweet may have been clearly demonstrated by the precise experimental design, or capsiate may have been more effective than capsaicin. Nagao *et al.* have reported that the ingestion of tea rich in catechins for four weeks reduced body fat by 0.5 kg,[37] and St-Onge *et al.* have reported that the ingestion of medium-chain triacylglycerols for four weeks reduced body fat by 0.8 kg.[38] Since the ingestion of CH-19 Sweet for two weeks reduced body fat by 0.6 kg, CH-19 Sweet is suggested to be as effective for reducing body fat as such compounds as catechins and medium-chain triacylglycerols.

In summary, the present results suggest that the repeated intake of CH-19 Sweet for two weeks suppressed body fat accumulation, especially visceral fat accumulation, and reduced body weight by SNS activation. Although the suppression of body fat accumulation due to capsaicin and pungent hot pepper have been reported,[39] the intake of a physiologically effective dosage was difficult because of the pungency. It has been suggested that CH-19 Sweet and capsiate, an effective ingredient of CH-19 Sweet, were useful for the suppression of body fat accumulation because they were not pungent, unlike capsaicin, but had the same physiological effects.[9–12,32] Indeed, in the present study, we found that the repeated intake of CH-19 Sweet for two weeks reduced body weight and suppressed body fat accumulation in humans. The anti-obese effects of CH-19 Sweet or capsiate should be confirmed by a large-scale future experiment. We finally emphasize that the method used in the present study was useful for precisely detecting weight change and was less stressful for subjects.

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