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Enhanced Energy Expenditure and Fat Oxidation in Humans with High BMI Scores by the Ingestion of Novel and Non-Pungent Capsaicin Analogues (Capsinoids)

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The biochemical and physiological indices were monitored in 44 subjects after 4-week capsinoids (capsaicin analogues with low pungency) intake. The subjects were randomly assigned to 3 groups: CSNs3 (3 mg/kg of capsinoids), CSNs10 (10 mg/kg of capsinoids) and the control (placebo). Measurements were performed in the morning on overnight-fasted subjects. The oxygen consumption (VO_2), resting energy expenditure (REE) and fat oxidation increased slightly compared to pre-administration values without any adverse effects, although the increase was not significant. The increase in fat oxidation was positively and significantly correlated with the body mass index (BMI). A meta-analysis was therefore conducted on a subgroup consisting of subjects with BMI ≥ 25 ($n = 28$). As a result, not only VO_2 increased significantly ($p < 0.05$) in the CSNs10 group, but also REE in the CSNs10 group and fat oxidation in the CSNs3 and CSNs10 groups tended to increase ($p < 0.1$). Consequently, a capsinoids intake would be able to enhance the energy expenditure and fat burning in humans, particularly those with high BMI.

Key words: 'capsiate'; capsinoid; resting energy expenditure; fat oxidation; body mass index

It is of utmost importance to improve obesity for health maintenance in life. Obesity is the result of the energy intake exceeding the energy expenditure.[1] It is thus necessary to either reduce the energy intake or enhance the energy expenditure to resolve the problem of obesity. However, it is not easy to attain the weight loss only by reducing the energy intake because of an increasing sense of hunger or a risk of attenuation in the basal metabolic rate (BMR). The latter often causes one of the reasons for weight regain.[2] Therefore, it is advantageous to include certain strategies to enhance the energy expenditure in a weight-loss program together with reducing the energy intake ("dieting").

According to the National Nutritional Survey in Japan between 1988 and 2004,[3] the mean body weight of the Japanese populace was continuously increasing in those years, although the dietary intake tended to decrease and habitual physical activities remained unchanged. This continual increase in body weight could be attributable to the attenuation of BMR with age. BMR constitutes a major share (60–70%) of the daily energy expenditure and has been documented to decrease with age[4–10] and with a high body mass index (BMI).[11,12]

Capsaicinoids have been well documented as substances for increasing BMR. For example, in a human trial where capsaicin at a relatively high-dose was ingested for 3 months, increased fat oxidation was reported to be sustained together with sustained elevation of the resting energy expenditure (REE).[13] However, capsaicin has such strong pungency and nociceptive stimulus that not all people can eat much and avoid a stomach ache. Capsinoids including 'capsiate' are non-pungent capsaicinoid analogues and are derived from a non-pungent cultivar of *Capsicum annuum L.* ('CH-19 sweet'). The non-pungent capsinoid known as 'capsiate' is known to have similar physiological effects to those of capsaicin: a single oral administration of 'capsiate' has been reported to increase the oxygen consumption (VO_2)[14,15] and body temperature[16] in mice like capsaicin, reflecting increased energy expenditure. A chronic (2-week) administration of 'capsiate' to mice has increased the 24-hr total VO_2, fat tissue uncoupling protein (UCP-1) and its mRNA associated with a reduction in the fat tissue weight and body weight.[17] As for human studies, there is a report describing enhanced VO_2 and body temperature in volunteers immediately after a single intake of 'CH-19 sweet' fruit.[18] This present study was designed to assess the

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Abbreviations: CSNs3, group ingesting 3 mg/day of capsinoids; CSNs10, group ingesting 10 mg/day of capsinoids; BMI, body mass index; VO_2, oxygen consumption; REE, resting energy expenditure; RQ, respiratory quotient; BMR, basal metabolic rate; FFM, fat free mass; BAT, brown adipose tissue; UCP, uncoupling protein; SNS, sympathetic nervous system; TG, triglyceride; FFA, free fatty acid; MCT, medium-chain triglyceride

change in energy metabolism after a long-term intake of capsinoids. To avoid any acute capsinoid effects, various parameters were monitored approximately 24 hours after the last ingestion of capsinoids during the course of a 4-week ingestion study. In addition, the extracted oil of 'CH-19 sweet' fruit was prescribed in this study.

Obese or obesity-prone subjects with BMI $>$ 23 were recruited (the Japan Society for the Study of Obesity has defined BMI $\geq$ 25 as obese and BMI = 22.2 as standard weight). REE has been reported to be somewhat dependent on the BMI value; a decrease in REE adjusted for the fat-free mass (FFM) was accompanied with a BMI increase,[12] and an increase in the attenuation rate of sleep energy expenditure was accompanied with a BMI increase.[19] Thus, we investigated not only the effect on resting energy expenditure by a long-term intake of capsinoids, but also whether the effects of capsinoids depended on the BMI values.

Materials and Methods

Subjects. Men and postmenopausal women (30–65 years old) with a BMI score higher than 23 kg/m^2 were recruited from the Soiken Clinic databank in Osaka. Those who had abnormal clinical laboratory test values, hepatopathy, nephropathy, cardiovascular disturbance, breathing disorder, endocrinopathy, defective metabolism, gastric ulcers, allergic symptoms to red pepper, or under medical treatment for obesity and hyperlipemia were excluded. Men with waist $<$ 85 cm/height $>$ 170 cm, and those with waist $<$ 80 cm/height $<$ 170 cm were excluded. The remaining 48 subjects (39 males and 9 females) were randomly assigned to the CSNs3 group receiving daily 3 mg/day of capsinoids, CSNs10 group receiving 10 mg/day of capsinoids or the placebo (control) group. Four subjects withdrew for personal reasons, leaving 44 eligible subjects: CSNs3 (n = 14), CSNs10 (n = 15) and controls (n = 15). The subjects were briefed on the purpose and the outline of this study, and signed a consent form before the study. This study was approved by the Institutional Review Board for studies of food in humans by Ajinomoto Co., Inc. (Tokyo, Japan) and by the Joint Institutional Review Board of Soiken, Inc. and Soiken Clinic (Osaka, Japan), in accordance with the concept of the Helsinki Declaration.

Test sample. The sample was oil extracted from 'CH-19 sweet' fruit containing capsinoids. Dried fruits from 'CH-19 sweet' were extracted with hexane and treated with medium-chain triglyceride (MCT) before refining by evaporation and condensation. The extracted oil diluted with canola oil was sealed in marigold-colored soft capsules containing 1 or 2 mg of capsinoids. Capsules not containing the extracted oil were used as a placebo.

The capsinoids consisted of three different forms: 'capsiate', dihydrocapsiate and nordihydrocapsiate.[20,21] For a comparison of their potency, three synthetic compounds were assessed for their potency toward calcium uptake, adrenalin secretion with i.v. administration and change in VO_2 with intragastric administration. Each capsinoid elicited virtually similar effects (unpublished data), so the content of capsinoids was expressed as the total amount of capsinoids.

Apart from capsinoids, 'CH-19 sweet' extracted oil contained small amounts of MCT (0.019 or 0.064 g due to different batches) and capsaicin (0.0020 or 0.0067 mg). Qualitatively, these contents would affect the energy metabolism,[13,22–25] but the amounts used in the present study seem to have been too small to exert any biological effect based on the effective amount reported in published documents.

Protocol. The three groups were compared in a randomized double-blind study over a 4-week period. Each group ingested 5 capsules; for CSNs3, there were three capsules each containing 1 mg of capsinoids and two capsules of the placebo. The dose was temporarily set as "normal dose (3 mg)" or "high dose (10 mg)" based on a previous observation that 2.1–7.0 mg/day of capsiate significantly increased VO_2 in human subjects.[18] The respiratory gas, body weight, height, diastolic and systolic blood pressure and pulse rate were monitored at 0 (before ingestion), 2 and 4 weeks post-treatment. Blood samples were also taken for hematological and blood biochemical analyses. All the subjects underwent the study as a single sample population at the Soiken Clinic with analyses conducted under the supervision/management of the designated clinician.

Three days before the measurement day, the subjects were instructed to refrain from snacks and alcoholic beverages. On the day before the measurement day, the subjects took menu-designated meals 3 times a day and were asked to take supper by 21:00 hr (no eating nor drinking thereafter), so that the subjects fasted overnight for more than 12 hr. Details of the meals are as follows: heat energy was 500, 735 and 765 kcal for breakfast, lunch and supper, respectively; protein, fat, carbohydrate and salt were 25, 10, 70 and 3.5 g for breakfast, 35, 22, 90 and 3.5 g for lunch, and 40, 23, 90 and 3.5 g for supper, respectively. On the day for monitoring the data already mentioned, the subjects were asked not to take any meal, smoke or do physical exercise after being awaken but up to 350 ml of drinking water was allowed until 2 hr before monitoring. In addition to the test-meal intake, the subjects were asked not to change their normal eating, smoking and physical exercise habits.

Respiratory gas analysis. The resting energy expenditure (REE) was measured by the breath-by-breath method, using a respiratory gas analyzer (AE-300S; Minato Medical Science, Osaka, Japan). The measurements were carried out for 20 min after overnight fasting. Each subject sat quietly for 30 min and then for 10 min with a face mask before measurement in the

sitting position. The data obtained during the last 10 min were analyzed. The resting energy expenditure (REE), respiratory quotient (RQ), glucose oxidation and fat oxidation were calculated by the VO_2 and carbon dioxide production.

Body weight, height, BMI, blood pressure, pulse rate and medical examination. All the subjects underwent measurements of body weight, standing height, blood pressure, and pulse rate, and a medical examination at the Soiken Clinic. The subjects were requested to visit the clinic without taking the test sample capsules. The blood pressure and pulse rate were measured in a sitting position after a 10-min rest. All medical examinations were performed by the designated medical doctor. BMI was calculated as the body weight (kg) divided by the square of the height (m^2).

Blood test. Blood samples were taken in the clinic from all the subjects after overnight fasting, and the following measurements were taken using peripheral blood samples: blood properties (leucocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count), aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyltranspeptidase, total bilirubin, total protein, albumin, alkaline phosphatase, urea nitrogen, uric acid, creatinine, electrolytes (Na^+, K^+, Cl^-, Ca^{2+}, and Mg^{2+}), blood glucose, insulin, hemoglobin A_{1c}, total ketone body, 3-hydroxybutyric acid, acetoacetic acid, total cholesterol, HDL-cholesterol, LDL-cholesterol, RLP-cholesterol, TG, free fatty acid (FFA) and phospholipids.

Blood sampling was performed after a 10 min rest in a sitting position. All measurements were taken by SRL Co., Ltd., and Osaka Serum Microbiology Laboratories Co., Ltd., according to appropriate methods.

Recording the daily diet and number of walking steps. The subjects were asked to record either the dietary contents to confirm they had kept to their normal eating routines or to utilize a record for nutritional analysis. Dieticians calculated the nutritional ingredient intake (energy, protein, fat, carbohydrate, cholesterol, and dietary fiber) based on the 5th Revision of the Japan Standard Food Ingredient Table. The numbers of daily walking steps were recorded with a pedometer before the day when the subject visited the clinic at 0, 2 and 4 weeks post-treatment.

Statistical analyses. Each value is expressed as the mean $\pm$ standard error. The respiratory gas analysis, body weight, BMI, diastolic and systolic blood pressure, pulse rate, nutrient intake and number of walking steps were compared between the groups by the Dunnett multiple-comparison test. In addition, comparisons in each group among the data at 0, 2 and 4 weeks post-treatment were made with the Bonferroni multiple-comparison test. In respect of the hematological and biochemical data, comparisons were made between the groups with the Dunnett multiple-comparison test. All the values obtained at 0 week were compared with the corresponding values obtained at 4 weeks with Student's *t*-test. Statistical software (SPSS Ver. 11.5, SPSS Co., Ltd.) was employed in the respective comparisons, where a risk factor of $p < 0.05$ in the paired testing was considered statistically significant.

Results

Respiratory gas analysis

The results of the respiratory gas analysis are shown in Table 1 and Fig. 1. No significant inter-group differences were observed in any parameters (Table 1). However, changes in VO_2, REE, RQ, glucose oxidation and fat oxidation were slightly higher in the capsinoids-treated groups (CSNs3 and CSNs10), though the increases were not significant. Furthermore, CSNs3 and CSNs10 showed a lower mean value for the glucose oxidation level together with a higher mean value for fat oxidation level than the controls (Fig. 1), although the differences were not significant.

To discover a more responsive subgroup by a meta-analysis, we inspected the correlations between every above-mentioned parameter and the BMI value at 0 week. The BMI value tended to be correlated with the fat oxidation increase of either CSNs3 or CSNs10 ($R = 0.424$, $p = 0.131$ or $R = 0.447$, $p = 0.109$), and was correlated well with the combined fat oxidation increases in both CSNs3 and CSNs10 ($R = 0.434$, $p = 0.018$) (Fig. 2), suggesting that the higher-BMI subjects would respond more to capsinoids by enhancing fat oxidation. We then tentatively introduced the BMI $\geq 25\,kg/m^2$ criterion for making a subgroup that would presumably more responsive. BMI $\geq 25\,kg/m^2$ is a standard criterion for obesity described by the Japan Society for the Study of Obesity. The meta-analysis on the population (a subgroup) with BMI $\geq 25\,kg/m^2$ gave a significant VO_2 increase between 2 weeks and 0 week ($p < 0.05$) in CSNs3, with an increasing tendency ($p < 0.1$) for REE (Table 2). Fat oxidation at 0 week tended to be lower in CSNs3 than in the controls ($p < 0.1$). Moreover, VO_2 was significantly higher ($p < 0.05$) in CSNs10, with an increasing tendency of REE and fat oxidation ($p < 0.1$), when compared with the controls (Fig. 3). Fat oxidation in CSNs3 also tended to be higher than that in the controls ($p < 0.1$). There were no significant changes in VO_2, REE, RQ, glucose oxidation and fat oxidation in the control group throughout the measurements.

Body weight, BMI, blood pressure, pulse rate and medical examination

Data on the body weight, BMI, diastolic and systolic blood pressure, and pulse rate are summarized in

Table 1. VO_2, REE, RQ, Fat Oxidation and Glucose Oxidation Values before and after the Intake of Capsinoids

		week		
		0	2	4
VO_2 (ml/min/kg)	CSNs10	3.12 ± 0.09	3.18 ± 0.09	3.15 ± 0.07
	CSNs3	3.13 ± 0.06	3.21 ± 0.08	3.17 ± 0.08
	controls	3.21 ± 0.07	3.23 ± 0.09	3.21 ± 0.08
REE (kcal/day/kg)	CSNs10	21.4 ± 0.59	21.8 ± 0.62	21.6 ± 0.52
	CSNs3	21.5 ± 0.41	22.0 ± 0.55	21.8 ± 0.58
	controls	22.0 ± 0.49	22.2 ± 0.58	22.1 ± 0.52
RQ	CSNs10	0.78 ± 0.01	0.79 ± 0.01	0.78 ± 0.01
	CSNs3	0.79 ± 0.01	0.79 ± 0.01	0.79 ± 0.01
	controls	0.78 ± 0.01	0.80 ± 0.01	0.79 ± 0.01
Fat oxidation (mg/min)	CSNs10	80.6 ± 4.7	82.4 ± 5.9	83.4 ± 5.1
	CSNs3	78.5 ± 3.5	82.2 ± 4.5	81.0 ± 5.2
	controls	87.4 ± 5.2	81.9 ± 5.8	80.7 ± 3.6
Glucose oxidation (mg/min)	CSNs10	71.3 ± 13.0	77.6 ± 12.9	71.3 ± 11.6
	CSNs3	80.6 ± 10.4	77.2 ± 8.2	77.2 ± 10.1
	controls	71.2 ± 9.1	85.8 ± 10.8	86.4 ± 10.3

Each values is the mean ± SE. CSNs10, n = 15; CSNs3, n = 14; controls, n = 15.

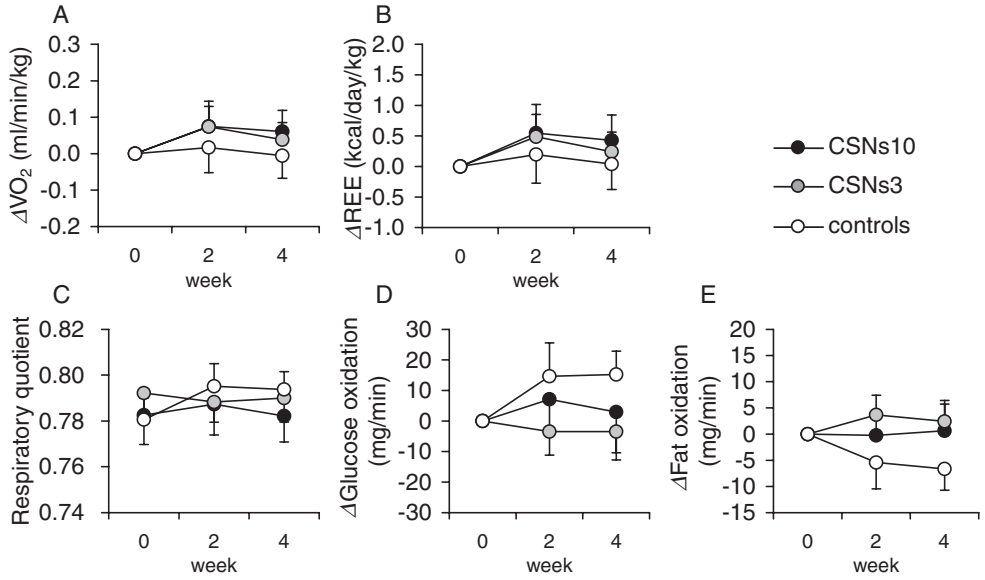

Fig. 1. Changes in (A) Oxygen Consumption (VO_2), (B) Resting Energy Expenditure (REE), (C) Respiratory Quotient (RQ), (D) Glucose Oxidation and (E) Fat Oxidation from the Initiation of Sample Ingestion.

Respiratory gas analyses were performed on overnight-fasted subjects without taking the test sample in the morning. Each value is expressed as the mean ± SE. (CSNs3, n = 14; CSNs10, n = 15; controls, n = 15).

Table 3. The body weight and BMI of CSNs3 and CSNs10 were tended to decrease during the 2 to 4 week period but significant differences were not observed between any two of the three groups. Significant differences in the diastolic and systolic blood pressure were not apparent between any two of the three groups. An increasing trend was observed in the pulse rates of CSNs3 and CSNs10 through the 2 to 4 week period, although the increases were within the normal range. In addition, the supervising physician did not extract any causal effect of capsinoids ingestion from individual interviews.

Although a significant weight loss could be expected in the subgroup with BMI $\geq 25\,kg/m^2$, that was not the case (data not shown). The systolic blood pressure had increased significantly by week 4 in CSNs10 (data not shown), although the increase value was still within the normal range.

Blood examination

The blood parameters related to the carbohydrate and fat metabolism demonstrated no significant inter-group differences. Significantly higher in insulin ($p < 0.05$) and a tendency to higher blood glucose ($p < 0.1$) and

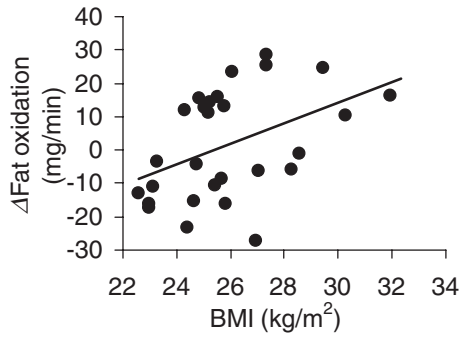

Fig. 2. Combined Correlations between the Increases in Fat Oxidation at 4 Weeks Post-Treatment and BMI at 0 Week Post-Treatment (n = 29) in CSNs3 (3 mg/kg of capsinoids) and CSNs10 (10 mg/day of capsinoids).

Fat oxidation is expressed as the increase from 0 to 4 weeks post-treatment. The correlation coefficient is 0.4363 ($p = 0.018$).

FFA levels ($p < 0.1$) were observed in CSNs10 between weeks 0 and 4. A tendency to higher phospholipids ($p < 0.1$) in CSNs3 and a significant increase in blood glucose level ($p < 0.01$) in the controls were observed between weeks 0 and 4.

The HDL-cholesterol level had a gender difference, so the data were analyzed separately. However, no difference was apparent between any two of the three groups. When focused on the subgroup with BMI $\geq 25\,kg/m^2$, the insulin level in CSNs3 tended to be lower than the control level at 4 weeks ($p < 0.1$; data not shown). The total ketone body and acetoacetic acid levels in CSNs3 tended to be higher than the control values at 4 weeks ($p < 0.1$). The control values at 4 weeks indicated a decreasing tendency for the total ketone body level ($p < 0.1$) and a significantly decreasing 3-hydoxybutyric acid level ($p < 0.05$; data not shown).

The hematological and biochemical data only yielded significant differences in the lactodehydrogenase level between CSNs10 and the control at 0 and 4 weeks. As this difference existed before the treatment started, there would not have been any clinical implications. No significant inter-group differences were apparent in the other parameters. There were some significant inter-period differences throughout the three groups in such parameters as hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, alanine aminotransferase, γ-glutamyltranspeptidase, total bilirubin, albumin, alkaline phosphatase, urea nitrogen, uric acid, K^+, Cl^-, Ca^{2+}, and Mg^{2+}. All differences, however, were within the normal range. No marked differences, indicating abnormalities in hepatic and renal functions, were not apparent.

A gender-differentiated analysis and analysis of the subgroup with BMI $\geq 25\,kg/m^2$ were also carried out. Although there were significant inter-group or inter-period differences in some parameters (data not shown), all differences were small in absolute terms and within the normal range, suggesting they did not pose any clinical problems.

Daily diets and number of walking steps

The daily nutrient intake and number of walking steps were examined before the day when the subjects underwent monitoring at the clinic (Table 4). Any significant inter-group differences were observed. While the protein intake tended to increase in CSNs10 between 0 and 4 weeks, the variation was within the normal range, implying that the influence would be negligible. In the subgroup with BMI $\geq 25\,kg/m^2$, the intake of cholesterol was significantly less in CSNs10 than in the control ($p < 0.05$), although the variation was within the normal range (data not shown).

Table 2. VO_2, REE, RQ, Fat Oxidation and Glucose Oxidation Values before and after the Intake of Capsinoids in Subjects with BMI $\geq 25\,kg/m^2$

		week		
		0	2	4
VO_2 (ml/min/kg)	CSNs10	3.09 ± 0.09	3.23 ± 0.10	3.17 ± 0.08
	CSNs3	3.10 ± 0.06	3.22 ± 0.08 #	3.16 ± 0.09
	controls	3.21 ± 0.09	3.19 ± 0.08	3.15 ± 0.08
REE (kcal/day/kg)	CSNs10	21.2 ± 0.64	22.1 ± 0.63	21.8 ± 0.57
	CSNs3	21.3 ± 0.44	22.1 ± 0.57 +	21.7 ± 0.65
	controls	22.0 ± 0.57	21.9 ± 0.51	21.7 ± 0.51
RQ	CSNs10	0.80 ± 0.01	0.79 ± 0.01	0.78 ± 0.01
	CSNs3	0.80 ± 0.01	0.79 ± 0.01	0.79 ± 0.01
	controls	0.78 ± 0.01	0.80 ± 0.01	0.80 ± 0.01
Fat oxidation (mg/min)	CSNs10	80.0 ± 3.3	91.2 ± 5.5	91.3 ± 4.0
	CSNs3	78.9 ± 3.2]†	85.6 ± 3.1 +	84.5 ± 4.6
	controls	92.6 ± 4.3]†	86.4 ± 5.6	84.1 ± 3.7
Glucose oxidation (mg/min)	CSNs10	92.8 ± 12.9	86.1 ± 13.9	80.8 ± 12.6
	CSNs3	89.5 ± 9.1	82.0 ± 8.0	79.9 ± 9.0
	controls	80.6 ± 8.5	92.4 ± 13.5	95.3 ± 11.9

Each value is the mean ± SE. CSNs10, n = 8; CSNs3, n = 11; controls, n = 9. +, #: $p < 0.1$, 0.05 *vs.* baseline by the Bonferroni test. †: $p < 0.1$ between 2 groups by the Dunnett test.

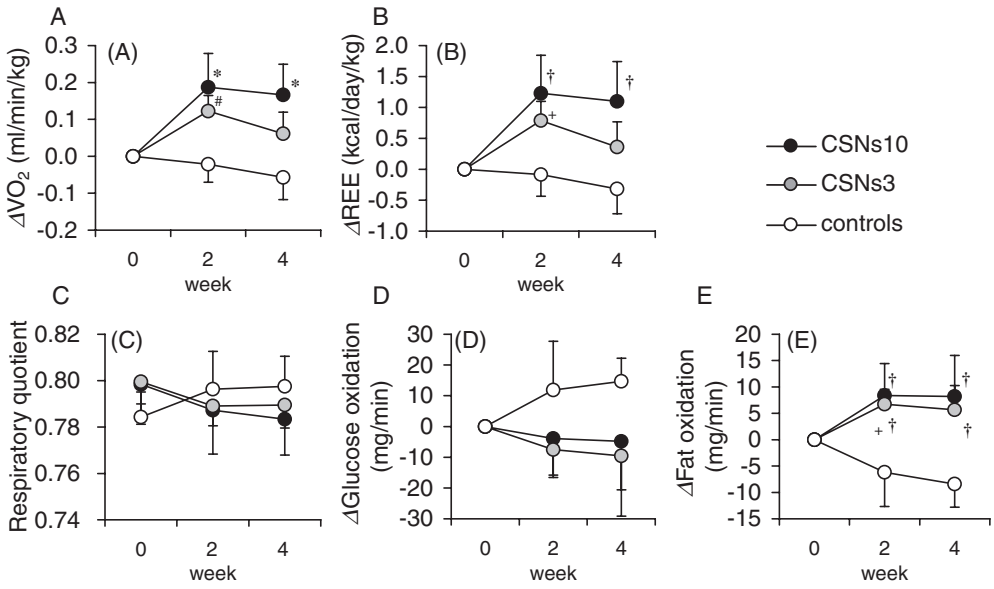

Fig. 3. Change in (A) Oxygen Consumption (VO_2), (B) Resting Energy Expenditure (REE), (C) Respiratory Quotient (RQ), (D) Glucose Oxidation and (E) Fat Oxidation from the Initiation of Sample Ingestion in Those Subjects with BMI $\geq 25\,kg/m^2$.

Respiratory gas analyses were performed on overnight-fasted subjects without taking the sample in the morning. Each value is expressed as the mean ± SE. Subjects with BMI $\geq 25\,kg/m^2$ were assigned to 3 groups: CSNs3 (3 mg/kg of capsinoids, n = 11), CSNs10 (10 mg/day of capsinoids, n = 8) and controls (placebo, n = 9). Differences (*, †) where $p < 0.05$ or 0.1 were compared with the controls, while those ($^\#$, $^+$) where $p < 0.05$ or 0.1 were compared with the 0 week post-treatment values.

Table 3. Changes in Body Weight, BMI, SBP, DBP and Pulse Rate before and after Intake of Capsinoids

		week		
		0	2	4
Weight (kg)	CSNs10	73.22 ± 3.44	73.01 ± 3.44	72.76 ± 3.48 +
	CSNs3	72.39 ± 3.15	72.05 ± 3.15 +	72.10 ± 3.16
	controls	73.44 ± 3.19	73.35 ± 3.10	73.42 ± 3.08
BMI (kg/m^2)	CSNs10	26.18 ± 0.76	26.10 ± 0.76	26.01 ± 0.78 +
	CSNs3	26.02 ± 0.64	25.90 ± 0.65 +	25.92 ± 0.65
	controls	26.10 ± 0.87	26.07 ± 0.81	26.09 ± 0.81
SBP (mmHg)	CSNs10	110.67 ± 2.58	113.07 ± 3.65	113.33 ± 3.58
	CSNs3	114.43 ± 3.45	108.29 ± 2.34	111.00 ± 3.42
	controls	112.40 ± 2.32	114.27 ± 2.60	114.00 ± 2.17
DBP (mmHg)	CSNs10	73.73 ± 2.49	73.07 ± 2.72	74.80 ± 2.55
	CSNs3	77.43 ± 2.62	74.86 ± 2.31	71.29 ± 2.24
	controls	77.20 ± 2.63	76.27 ± 2.31	74.27 ± 1.55
Pulse rate (bpm)	CSNs10	66.53 ± 2.40	69.07 ± 1.69	71.47 ± 2.13 +
	CSNs3	66.29 ± 1.88	70.57 ± 2.07 +	67.14 ± 1.70
	controls	64.80 ± 1.52	68.00 ± 1.62	69.33 ± 1.55

Each value is the mean ± SE. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. CSNs10, n = 15; CSNs3, n = 14; controls, n = 15. +: $p < 0.1$ *vs.* baseline by the Bonferroni test.

Discussion

The novel and much less pungent capsaicin analogues, capsinoids, have an ester bond that replaces the amide bond linking the vanilloid group of capsaicin with a fatty acid. The patch-clamp method has shown that 'capsiate' (one of the capsinoids) binds with the capsaicin receptor TRPV1.[26] In addition, 'capsiate' has been reported to increase the body temperature, enhance VO_2 and decrease body fat.[15,16] The increase in body temperature is inhibited by the TRPV1 antagonist, capsazepine.[16] These results will indicate that capsinoids and capsaicin work *via* the TRPV1 receptor. TRPV1 is found on the surface of the oral cavity and digestive tract, so capsinoids may activate without being absorbed into the peripheral circulation (unpublished data).

In the present study, capsinoids were extracted from 'CH-19 sweet' fruit and orally given at a dose of 3 or 10 mg/day for 4 consecutive weeks to investigate their

Table 4. Average Daily Intake of Energy, Protein, Fat and Carbohydrate, Cholesterol, Fiber from Meals and Average Daily Number of Walking Steps before the Measurement Days

		week			
		0	2	4	
Energy (kcal/day)	CSNs10	1863.5 ± 76.1	1874.9 ± 69.2	1872.1 ± 54.5	
	CSNs3	1877.6 ± 52.4	1893.8 ± 64.6	1867.1 ± 49.4	
	controls	1908.6 ± 31.9	1874.5 ± 64.8	1902.1 ± 82.9	
Protein (g/day)	CSNs10	70.8 ± 2.9	75.8 ± 3.0	75.1 ± 2.4	+
	CSNs3	75.5 ± 3.5	76.8 ± 2.8	77.8 ± 2.4	
	controls	77.2 ± 2.7	75.6 ± 2.8	78.5 ± 3.8	
Fat (g/day)	CSNs10	54.4 ± 3.6	56.6 ± 3.1	55.6 ± 3.2	
	CSNs3	59.9 ± 3.7	54.2 ± 3.2	57.1 ± 2.8	
	controls	61.4 ± 1.7	57.4 ± 2.8	61.9 ± 3.2	
Carbohydrate (g/day)	CSNs10	261.6 ± 10.4	253.7 ± 9.1	256.6 ± 7.8	
	CSNs3	249.3 ± 8.8	264.5 ± 10.8	250.0 ± 9.8	
	controls	252.7 ± 7.5	253.4 ± 11.8	247.7 ± 13.0	
Cholesterol (mg/day)	CSNs10	350.0 ± 19.4	382.1 ± 19.8	398.4 ± 26.7	
	CSNs3	401.6 ± 19.7	391.9 ± 22.5	423.1 ± 26.3	
	controls	412.5 ± 27.3	443.7 ± 34.0	430.2 ± 33.6	
Fiber (g/day)	CSNs10	11.8 ± 0.6	11.4 ± 0.6	11.5 ± 0.6	
	CSNs3	13.0 ± 1.1	13.5 ± 1.2	13.1 ± 1.1	
	controls	13.4 ± 0.7	12.3 ± 0.7	12.6 ± 0.9	
Steps (steps/day)	CSNs10	11771.2 ± 1322.2	11499.8 ± 1294.5	11390.5 ± 1044.2	
	CSNs3	11019.9 ± 1320.6	11149.6 ± 1106.4	11436.5 ± 1295.2	
	controls	12050.7 ± 1367.9	11678.4 ± 1452.8	11597.0 ± 1209.9	

Each value is the mean ± SE. CSNs10, n = 15; CSNs3, n = 14; controls, n = 15. +: $p < 0.1$ *vs.* baseline by the Bonferroni test.

effect on energy metabolism in humans. The results disclosed a significant correlation between the increase in fat oxidation and BMI. The increase in VO_2 was found to be significant, if a meta-analysis was made on the subjects with BMI $\geq 25\,kg/m^2$. The increase in VO_2 could likely be attributable to the enhancement (either significant or insignificant) of REE and fat oxidation, which were dose-dependently observable in this study.

In a previous animal study, a single oral administration of 'capsiate' increased VO_2 following a 3-hr period.[15] A similar trend has been observed in previous humans study using 'CH-19 sweet' fruits, where increased VO_2 was maintained for at least 1 hr after oral administration.[18] Contrary to the foregoing two studies, the respiratory gas was analyzed more than 24 hr after the last intake of capsinoids in the present study in order to monitor the baseline elevation rather than the acute response. This protocol was based on the previous observation of mice treated with 'capsiate' for a 2-week period (10 mg/kg) displaying increased VO_2 and fat oxidation for 24 hr even when measurements were performed 24 hr after the final administration.[17] The results of this study show that baseline elevation in VO_2 seems less evident than acute one in human subjects. However, the increase in VO_2 and tendency for increasing REE and fat oxidation by the capsinoid intake for 4 weeks strongly suggest that a long-term treatment with capsinoids would enhance BMR.

In the animal experiment just referred to,[17] increases in UCP1 protein and UCP1 mRNA of BAT as well as UCP2 of white adipose tissue were demonstrated. Moreover, a single oral dose of 'capsiate' was shown to elevate UCP1 mRNA of BAT and UCP3 mRNA of skeletal muscle.[17] Although BAT itself has been found only in human neonates, UCP1 mRNA has been isolated from human white adipose tissue that evidently contained islets of brown adipocytes.[27,28] As UCP2 expression in fat tissue[29] and UCP3 expression in skeletal muscles[30] have been reported in humans, the UCP-inducing activity of capsinoids may, at least in part, have contributed to enhancing the energy metabolism in this study.

Human BMR is generally measured immediately after waking up in the morning, with the subject resting in the supine position in a thermally comfortable environment without having eaten breakfast. The contents of the REE measured in the present study include not only basal metabolism, but unavoidable 'metabolism due to other factors.' To standardize the 'metabolism due to other factors' among the subjects, they were asked to maintain their routine dietary habits, exercise, and dietary composition of meals. An increase in resting metabolism observed in the present study could mainly have been due to an increase in BMR.

Fat oxidation tended to be higher in CSNs3 and CSNs10 than in the controls. Although there was no significant change in either the RQ, plasma TG or ketone body levels, RQ in both CSNs3 and CSNs10 tended to be lower. Enhanced fat oxidation may contribute to increased energy expenditure. The increasing trend of

FFA level in CSNs10 is compatible with this notion, because if fat oxidation in CSNs10 would have been enhanced, lipolysis should have occurred beforehand which would induce an increase in the peripheral FFA level. Consistent with this notion, Masuda *et al.* have reported previously that a 2-week continuous administration of 'capsiate' to mice enhanced fat oxidation in the light phase (the resting period for mice) and induced a VO_2 increase.[17)]

A single oral administration of 'capsiate' to mice has been shown to increase the catecholamine, FFA and blood glucose levels concomitantly with a decrease in TG level,[15)] and enhanced fat oxidation during running exercise was augmented.[31)] These results suggest that an intake of capsinoids would influence fat metabolism such as lipolysis in fat tissues *via* the sympathetic nervous system (SNS). Cumulative studies about the long-term effect of capsinoids on fat metabolism are elucidating that enhanced metabolism may not be solely attributable to the elevation of UCP expression.

While BMI $= 22\,kg/m^2$ is designated as the lowest criterion for the incidence of disease complications in Japan, BMI $\geq 25\,kg/m^2$ is categorized as obese. BMI has positive correlations with body fat, leptin level and PAI-1, and negative correlations with the adiponectin and ghrelin levels.[32–34)] BMI is also correlated positively with a decreasing rate of sleep energy expenditure.[19)] These indices correlated with BMI are all interrelated with the status of energy metabolism.[35–38)] Thus, a change in energy metabolism by capsinoids intake may most likely be dependent on BMI. In fact, increases in REE and fat oxidation by a long-term intake of capsinoids were more evident in the subjects with BMI $\geq 25\,kg/m^2$. In obese subjects, the SNS activity is known to be depressed,[39,40)] and the response of SNS activity to curry (conditional capsaicin stimulation) and sugar have been reported to be weak.[39,41)] This lowered SNS activity will at least in part contribute to establishing a positive energy balance resulting in obesity or a high BMI status. Based on the meta-analysis in this study, we presume that that effects of capsinoids were more effectively manifest in the subjects with higher BMI. If this is valid, repeated SNS stimulation *via* the TRPV1 receptor might facilitate the efficiency of the SNS reflex arcs in obese subjects to respond to stimuli other than capsinoids. Obviously, more studies are needed to confirm this hypothesis.

Inter-group differences in the body weight and BMI were not significant, although a decreasing trend was observed in CSNs3 and CSNs10. The subjects had been instructed not to alter this exercise routine and dietary habits, and no obvious alterations were in fact observed. Thus, the weight loss, if any, must have been associated mainly with an increase in REE and (although not measured in this study) a suspected acute increase in energy expenditure immediately after ingesting the capsinoids. A much longer intake period may be required to induce a significant weight loss in humans.

No significant changes in either diastolic and systolic blood pressure or pulse rate were apparent during the test period. In addition, although significant changes were observed in certain hematological and biochemical data, and blood-overall examination items, these changes were within the normal range, and would not pose any significant clinical concern to a subject. Any adverse effects related to causal relationships of the sample were not extracted in this study.

In summary, a continuous 4-week intake of capsinoids enhanced VO_2 and tended to enhance REE and fat oxidation in subjects with obesity-categorized BMI scores ($\geq 25\,kg/m^2$; according to the Japanese standard). This implies that a capsinoid-induced change is an important factor leading to possible weight reduction. Since the pungency of the doses used in this study was slight and the dose easy to be ingested, capsinoids are thought to be potentially useful and applicable to many people.

References

1) Stunkard, A. J., Current views on obesity. *Am. J. Med.*, **100**, 230–236 (1996).
2) Fricker, J., Rozen, R., Melchior, J. C., and Apfelbaum, M., Energy-metabolism adaptation in obese adults on a very-low-calorie diet. *Am. J. Clin. Nutr.*, **53**, 826–830 (1991).
3) Ministry of Health Labour and Welfare of Japan, The National Health and Nutrition Survey in Japan, 2003, Daiichi-Shuppan, Tokyo (2006).
4) Das, S. K., Moriguti, J. C., Mccrory, M. A., Saltzman, E., Mosunic, C., Greenberg, A. S., and Roberts, S. B., An underfeeding study in healthy men and women provides further evidence of impaired regulation of energy expenditure in old age. *J. Nutr.*, **131**, 1833–1838 (2001).
5) Fukagawa, N. K., Bandini, L. G., and Young, J. B., Effect of age on body composition and resting metabolic rate. *Am. J. Physiol.*, **259**, E233–238 (1990).
6) Klausen, B., Toubro, S., and Astrup, A., Age and sex effects on energy expenditure. *Am. J. Clin. Nutr.*, **65**, 895–907 (1997).
7) Pannemans, D. L., and Westerterp, K. R., Energy expenditure, physical activity and basal metabolic rate of elderly subjects. *Br. J. Nutr.*, **73**, 571–581 (1995).
8) Roberts, S. B., Fuss, P., Heyman, M. B., and Young, V. R., Influence of age on energy requirements. *Am. J. Clin. Nutr.*, **62**, 1053S–1058S (1995).
9) Vaughan, L., Zurlo, F., and Ravussin, E., Aging and energy expenditure. *Am. J. Clin. Nutr.*, **53**, 821–825 (1991).
10) Visser, M., Deurenberg, P., Van Staveren, W. A., and Hautvast, J. G., Resting metabolic rate and diet-induced thermogenesis in young and elderly subjects: relationship with body composition, fat distribution, and physical activity level. *Am. J. Clin. Nutr.*, **61**, 772–778 (1995).
11) Astrup, A., Gotzsche, P. C., Van De Werken, K., Ranneries, C., Toubro, S., Raben, A., and Buemann, B., Meta-analysis of resting metabolic rate in formerly obese subjects. *Am. J. Clin. Nutr.*, **69**, 1117–1122 (1999).

12) De Luis, D. A., Aller, R., Izaola, O., Gonzalez Sagrado, M., and Conde, R., Resting energy expenditure, cardiovascular risk factors and insulin resistance in obese patients. *Ann. Nutr. Metab.*, **49**, 381–385 (2005).
13) Lejeune, M. P., Kovacs, E. M., and Westerterp-Plantenga, M. S., Effect of capsaicin on substrate oxidation and weight maintenance after modest bodyweight loss in human subjects. *Br. J. Nutr.*, **90**, 651–659 (2003).
14) Kawada, T., Watanabe, T., Takaishi, T., Tanaka, T., and Iwai, K., Capsaicin-induced beta-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. *Proc. Soc. Exp. Biol. Med.*, **183**, 250–256 (1986).
15) Ohnuki, K., Haramizu, S., Oki, K., Watanabe, T., Yazawa, S., and Fushiki, T., Administration of capsiate, a non-pungent capsaicin analog, promotes energy metabolism and suppresses body fat accumulation in mice. *Biosci. Biotechnol. Biochem.*, **65**, 2735–2740 (2001).
16) Ohnuki, K., Haramizu, S., Watanabe, T., Yazawa, S., and Fushiki, T., CH-19 sweet, nonpungent cultivar of red pepper, increased body temperature in mice with vanilloid receptors stimulation by capsiate. *J. Nutr. Sci. Vitaminol.* (Tokyo), **47**, 295–298 (2001).
17) Masuda, Y., Haramizu, S., Oki, K., Ohnuki, K., Watanabe, T., Yazawa, S., Kawada, T., Hashizume, S., and Fushiki, T., Upregulation of uncoupling proteins by oral administration of capsiate, a nonpungent capsaicin analog. *J. Appl. Physiol.*, **95**, 2408–2415 (2003).
18) Ohnuki, K., Niwa, S., Maeda, S., Inoue, N., Yazawa, S., and Fushiki, T., CH-19 sweet, a non-pungent cultivar of red pepper, increased body temperature and oxygen consumption in humans. *Biosci. Biotechnol. Biochem.*, **65**, 2033–2036 (2001).
19) Zhang, K., Sun, M., Werner, P., Kovera, A. J., Albu, J., Pi-Sunyer, F. X., and Boozer, C. N., Sleeping metabolic rate in relation to body mass index and body composition. *Int. J. Obes. Relat. Metab. Disord.*, **26**, 376–383 (2002).
20) Kobata, K., Sutoh, K., Todo, T., Yazawa, S., Iwai, K., and Watanabe, T., Nordihydrocapsiate, a new capsinoid from the fruits of a nonpungent pepper, capsicum annuum. *J. Nat. Prod.*, **62**, 335–336 (1999).
21) Kobata, K., Todo, T., Yazawa, S., Iwai, K., and Watanabe, T., Novel capsaicinoid-like substances, capsiate and dihydrocapsiate, from the fruits of a nonpungent cultivar, CH-19 Sweet, of pepper (*Capsicum annuum L.*). *J. Agric. Food Chem.*, **46**, 1695–1697 (1998).
22) Kovacs, E. M., and Mela, D. J., Metabolically active functional food ingredients for weight control. *Obes. Rev.*, **7**, 59–78 (2006).
23) Matsuo, T., Matsuo, M., Taguchi, N., and Takeuchi, H., The thermic effect is greater for structured medium- and long-chain triacylglycerols versus long-chain triacylglycerols in healthy young women. *Metabolism*, **50**, 125–130 (2001).
24) St-Onge, M. P., Bourque, C., Jones, P. J., Ross, R., and Parsons, W. E., Medium- versus long-chain triglycerides for 27 days increases fat oxidation and energy expenditure without resulting in changes in body composition in overweight women. *Int. J. Obes. Relat. Metab. Disord.*, **27**, 95–102 (2003).
25) St-Onge, M. P., and Jones, P. J., Greater rise in fat oxidation with medium-chain triglyceride consumption relative to long-chain triglyceride is associated with lower initial body weight and greater loss of subcutaneous adipose tissue. *Int. J. Obes. Relat. Metab. Disord.*, **27**, 1565–1571 (2003).
26) Iida, T., Moriyama, T., Kobata, K., Morita, A., Murayama, N., Hashizume, S., Fushiki, T., Yazawa, S., Watanabe, T., and Tominaga, M., TRPV1 activation and induction of nociceptive response by a non-pungent capsaicin-like compound, capsiate. *Neuropharmacology*, **44**, 958–967 (2003).
27) Cannon, B., and Nedergaard, J., Brown adipose tissue: function and physiological significance. *Physiol. Rev.*, **84**, 277–359 (2004).
28) Garruti, G., and Ricquier, D., Analysis of uncoupling protein and its mRNA in adipose tissue deposits of adult humans. *Int. J. Obes. Relat. Metab. Disord.*, **16**, 383–390 (1992).
29) Pecqueur, C., Alves-Guerra, M. C., Gelly, C., Levi-Meyrueis, C., Couplan, E., Collins, S., Ricquier, D., Bouillaud, F., and Miroux, B., Uncoupling protein 2, *in vivo* distribution, induction upon oxidative stress, and evidence for translational regulation. *J. Biol. Chem.*, **276**, 8705–8712 (2001).
30) Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J. P., Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett.*, **408**, 39–42 (1997).
31) Haramizu, S., Mizunoya, W., Masuda, Y., Ohnuki, K., Watanabe, T., Yazawa, S., and Fushiki, T., Capsiate, a nonpungent capsaicin analog, increases endurance swimming capacity of mice by stimulation of vanilloid receptors. *Biosci. Biotechnol. Biochem.*, **70**, 774–781 (2006).
32) Matsubara, M., Maruoka, S., and Katayose, S., Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur. J. Endocrinol.*, **147**, 173–180 (2002).
33) Oh, K. W., Lee, W. Y., Rhee, E. J., Baek, K. H., Yoon, K. H., Kang, M. I., Yun, E. J., Park, C. Y., Ihm, S. H., Choi, M. G., Yoo, H. J., and Park, S. W., The relationship between serum resistin, leptin, adiponectin, ghrelin levels and bone mineral density in middle-aged men. *Clin. Endocrinol.* (Oxf.), **63**, 131–138 (2005).
34) Shiiya, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M. S., Tanaka, M., Nozoe, S., Hosoda, H., Kangawa, K., and Matsukura, S., Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J. Clin. Endocrinol. Metab.*, **87**, 240–244 (2002).
35) Mantzoros, C. S., The role of leptin in human obesity and disease: a review of current evidence. *Ann. Intern. Med.*, **130**, 671–680 (1999).
36) Minokoshi, Y., and Kahn, B. B., Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. *Biochem. Soc. Trans.*, **31**, 196–201 (2003).
37) Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Muller, C., Carling, D., and Kahn, B. B., Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, **415**, 339–343 (2002).

38) Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S., Ueki, K., Eto, K., Akanuma, Y., Froguel, P., Foufelle, F., Ferre, P., Carling, D., Kimura, S., Nagai, R., Kahn, B. B., and Kadowaki, T., Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat. Med.*, **8**, 1288–1295 (2002).

39) Matsumoto, T., Miyawaki, C., Ue, H., Yuasa, T., Miyatsuji, A., and Moritani, T., Effects of capsaicin-containing yellow curry sauce on sympathetic nervous system activity and diet-induced thermogenesis in lean and obese young women. *J. Nutr. Sci. Vitaminol.* (Tokyo), **46**, 309–315 (2000).

40) Nagai, N., and Moritani, T., Effect of physical activity on autonomic nervous system function in lean and obese children. *Int. J. Obes. Relat. Metab. Disord.*, **28**, 27–33 (2004).

41) Paolisso, G., Manzella, D., Ferrara, N., Gambardella, A., Abete, P., Tagliamonte, M. R., De Lucia, D., Furgi, G., Picone, C., Gentile, S., Rengo, F., and Varricchio, M., Glucose ingestion affects cardiac ANS in healthy subjects with different amounts of body fat. *Am. J. Physiol.*, **273**, E471–478 (1997).