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TRPV1 Agonist Monoacylglycerol Increases UCP1 Content in Brown Adipose Tissue and Suppresses Accumulation of Visceral Fat in Mice Fed a High-Fat and High-Sucrose Diet

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The administration of such a transient receptor potential vanilloid 1 (TRPV1) agonist as capsaicin, which is a pungent ingredient of red pepper, promotes energy metabolism and suppresses visceral fat accumulation. We have recently identified monoacylglycerols (MGs) having an unsaturated long-chain fatty acid as the novel TRPV1 agonist in foods. We investigated in this present study the effects of dietary MGs on uncoupling protein 1 (UCP1) expression in interscapular brown adipose tissue (IBAT) and on fat accumulation in mice fed with a high-fat, high-sucrose diet. The MG30 diet that substituted 30% of all lipids for MGs (a mixture of 1-oleoylglycerol, 1-linoleoylglycerol and 1-linolenoylglycerol) significantly increased the UCP1 content of IBAT and decreased the weight of epididymal white adipose tissue, and the serum glucose, total cholesterol and free fatty acid levels. The diet containing only 1-oleoylglycerol as MG also increased UCP1 expression in IBAT. MGs that activated TRPV1 also therefore induced the expression of UCP 1 and prevented visceral fat accumulation as well as capsaicin.

Key words: monoacylglycerol; transient receptor potential vaniloid 1 (TRPV1); uncoupling protein 1; obesity; capsaicin

Obesity is a risk factor for hyperglycemia, hyperlipidemia and hypertension. It is important for preventing obesity to decrease the energy intake and increase the energy expenditure. It has been reported that capsaicin (CAP), a pungent ingredient of red pepper, is the functional ingredient for increasing energy expenditure. CAP promotes energy metabolism (oxygen consumption) by increasing adrenaline secreted from the adrenal medulla and upregulating uncoupling protein 1 (UCP1) in interscapular brown adipose tissue (IBAT) by activating the sympathetic nerve.[1–4] Since CAP has the effect of increasing energy expenditure, the ingestion of CAP could suppress the accumulation of visceral fat in rats fed with a high-fat diet.[5]

The CAP receptor was identified as vanilloid receptor subtype 1 in 1997,[6] and has recently been called transient receptor potential vanilloid 1 (TRPV1). TRPV1 is mainly expressed in the primary afferent nerve.[6] Recent studies have indicated that stimulating the sensory nerve by activating TRPV1 was essential to CAP-induced energy expenditure. CAP-induced adrenaline secretion was completely dissipated in rats in which the CAP-sensitive sensory nerve had been denervated by an excess CAP treatment,[7] suggesting that the TRPV1-expressing sensory nerve was indispensable for CAP-induced adrenaline secretion. The effects of suppressing an accumulation of visceral fat, increasing oxygen consumption and promoting thermogenesis by CAP were canceled in TRPV1 knockout mice.[8,9]

We searched in our previous study for the food compound which would activate TRPV1 as the functional compound increasing energy expenditure, and identified 1- or 2-monoacylglycerols (MGs) having oleic, linoleic, and α-linolenic acids from wheat flour, onion (*Allium cepa*) and myoga (*Zingiber mioga*) as TRPV1 agonists.[10] The potency and efficacy of these MGs for activating TRPV1 were respectively about 50 times weaker than and half that of CAP.[10] These MGs had very low pungency, unlike CAP. We identified capsiate from non-pungent red pepper and [10]-shogaol from ginger as TRPV1 agonists having low pungency which increased the adrenaline secretion and energy expenditure.[11–13] MGs as activators of TRPV1 may therefore be useful food ingredients to increase energy expenditure.

We examined in this present study whether MGs could prevent visceral fat accumulation and increase

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Abbreviations: BAT, brown adipose tissue; CAP, capsaicin; HFS, high-fat and high-sucrose; IBAT, interscapular brown adipose tissue; MG, monoacylglycerol; 1-MG(18:1), 1-oleoylglycerol; 1-MG(18:2), 1-linoleoylglycerol; 1-MG(18:3), 1-linolenoylglycerol; NEFA, non-esterified fatty acid; T-Cho, total cholesterol; TG, triacylglycerol; TG(18:1), trioleoylglycerol; TRPV1, transient receptor potential vanilloid 1; UCP, uncoupling protein; WAT, white adipose tissue

UCP1 expression in IBAT in mice fed with a high-fat and high-sucrose diet. Two sources of 1-MG were used in two experiments: synthetic 1-MG comprising 1-MG(18:1), 1-MG(18:2) and 1-MG(18:3) was used in experiment 1, and high-purity 1-MG(18:1) was used in experiment 2.

Materials and Methods

Monoacylglycerols. We used two kinds of 1-MG, one being enzymatically synthesized as described next, and the other being commercially available 1-oleolyglycerol (SunSoft 8070V, purity > 90%) from Taiyo Kagaku Co. (Yokkaichi, Mie, Japan).

1-MG was synthesized by an enzymatic method.[14] Briefly, a mixture of 9.23 mol glycerol (purity > 99%; Wako Pure Chem. Ind., Osaka, Japan), 0.71 mol oleic acid (purity > 60%, Wako) and 65,000 unit lipase G (Amano Enzymes, Aichi, Japan) in 6 mL of water was shaken at 40 °C for 4 d. The reactants were extracted with *n*-hexane. Diacylglycerol as a byproduct was removed from the extract by 7-times partitioning with hexane/methanol/H_2O (400/87/13, by vol.). The remaining fatty acid in the methanol layer was removed by passing through activated alumina to afford 1-MG (0.55 mol, 77.5% yield). The chemical structure of synthetic 1-MG was confirmed by ^{1}H-NMR spectral data (α-400 instrument, Jeol, Tokyo, Japan). The purity of 1-MG was >97% by TLC with flame ionization detection (Iatroscan MK-5, Mitsubishi Kagaku Iatron, Tokyo, Japan). The composition of 1-MG was detected by a reversed-phase HPLC analysis (UV at 220 nm) to be 1-oleoylglycerol (1-MG(18:1), 47.6%), 1-linoleoylglycerol (1-MG(18:2), 46.7%) and 1-linolenoylglycerol (1-MG(18:3), 1.00%).

Experimental diets. The compositions of the experimental diets are shown in Table 1. The high-fat and high-sucrose (HFS) diet was based on AIN-93G[15] and used to induce obesity in the short term.[16] The HFS diet contained, on an energy basis, 32% carbohydrate including sucrose, 20% protein, and 48% fat. The increased lipid (lard) and sucrose was subtracted from β-corn starch in the basal diet. The control HFS diet (TG diet) had 30% (w/w) of the total lipid substituted by trioleoylglycerol (TG(18:1); Wako). The MG15 diet contained 15% 1-MG and 15% TG(18:1) of the total lipid, while the MG30 diet contained 30% 1-MG of the total lipid. Synthetic 1-MG containing 1-MG(18:1), 1-MG(18:2) and 1-MG(18:3) was used in experiment 1, and high-purity 1-MG(18:1) was used in experiment 2. The respective amounts of energy in TG(18:1) and 1-MG were 38.9 and 35.1 kJ/g which were calculated according to the method of Livesay.[17]

Table 1. Composition of the Experimental Diets

Experimental diet	TG diet	MG15 diet	MG30 diet
Sucrose (g)	31.2	31.2	31.2
β-Corn starch (g)	7.9948	7.9948	7.9948
Casein (g)	25.0	25.0	25.0
Soybean oil (g)	7.0	7.0	7.0
Lard (g)	11.2	11.2	11.2
Trioleoylglycerol (g)	7.8	3.9	
Monoaclyglycerol (g)		3.9	7.8
Cellulose (g)	5.0	5.0	5.0
Mineral mix (g)	3.5	3.5	3.5
Vitamin mix (g)	1.0	1.0	1.0
L-Cystine (g)	0.3	0.3	0.3
BHT (g)	0.0052	0.0052	0.0052
Energy (kJ/100 g)	2062.2	2047.5	2032.9

All ingredients are listed in grams per 100 g of diet. An AIN-93G mineral mixture and AIN-93G vitamin mixture with choline bitartrate (Oriental Yeast Co., Tokyo, Japan) were used as the mineral and vitamin mix. BHT is described as an abbreviated form of *t*-butylated hydroxytoluene. Synthetic 1-monoaclyglycerols were used in experiment 1, and high-purity 1-oleolyglycerol was used in experiment 2. The energy of trioleoylglycerol and monoaclyglycerol were calculated as 38.9 and 35.1 kJ/g, respectively.

Animals and experimental procedures (experiments 1 and 2). Five-week-old C57BL/6Cr male mice (Japan SLC, Shizuoka, Japan) were used. The animals were individually housed in a room with a controlled temperature (23 ± 1 °C), humidity (55 ± 5%), and light cycle (lights on from 07.00 to 19.00). The animal experiments were approved by the Animal Care and Use Committee of the University of Shizuoka.

Each diet was supplied in a specialized feeding basket for mice (1-8179-01, As One Co., Osaka, Japan). After a 1-week adaptation period with a standard MF laboratory diet (Oriental Yeast Co., Tokyo, Japan) in individual cages, the mice were randomly assigned to three experimental groups: the TG, MG15 and MG30 groups (n = 6 in each group,). The mice were fed with the relevant experimental diets for 30 d by the pair-feeding method and received tap water *ad libitum*. The food intake and body weight of the mice were measured every morning. Experiments 1 and 2 were performed with the same protocol, except for the experimental diet that was fed the different sources of 1-MG.

On day 28 of feeding with the experimental diet, the mice were transferred into individual metabolic chambers, and feces were collected for 24 h. The feces were freeze-dried, and fats were extracted with $CHCl_3$:MeOH = 2:1. The extract (organic layer) was washed with a 0.9% NaCl aqueous solution. After drying with Na_2SO_4, the organic solvent was evaporated under an N_2 stream. The resulting fat was weighed to calculate the apparent fat digestibility as (weight of dietary fat per day − weight of fat in the faces per day)/(weight of dietary fat per day) × 100.

After 30 d of the feeding experiment, the mice were fasted for 8 h from 06.00 and dissected under urethane/α-choloralose anesthesia (1 and 0.1 g/kg, i.p, respectively). Blood was collected from the inferior vena cava, and the tissues (heart, spleen, liver, kidney, pancreas, IBAT, mesenteric white adipose tissue (WAT), perirenal WAT, and epididymal WAT) were extirpated. IBAT and liver were frozen with liquid nitrogen and kept at −80 °C until the UCP1 or hepatic lipid content assay. The collected blood was stood at room temperature for 30 min and then stood on ice. Serum was separated by centrifugation. Serum glucose, triglyceride (TG), total cholesterol (T-Cho), and free fatty acid (non-esterified fatty acid, NEFA) were measured by using commercial kits (Wako) with a 96-well microplate at a one-twentieth scale of that in the supplier's manual. Absorbance was measured by a microplate reader (Spectramax 190, Molecular Devices, Missisauga, Ontario, Canada). The hepatic lipid content was measured by extracting lipids from the liver with $CHCl_3$:MeOH = 2:1 (v/v) after washing the lipids with 0.75% KCl.

Measurement of the UCP1 content in IBAT. The mitochondrial fraction was prepared according to the method of Cannon and Lindberg.[18] Individual IBAT was minced and homogenized with 300 µL of a sucrose buffer (0.3 M sucrose, 10 mM Tris, and 2.2 mM ethylenediaminetetraacetic acid at pH 7.2) in a test tube. After centrifuging at 8,500 × *g* for 10 min at 4 °C, the supernatant containing the microsomal fraction was discarded. The remaining pellet was rehomogenized with 50 µL of the sucrose buffer and transferred to a microtest tube. The sucrose buffer (450 µL) was added, and the tube was shaken well. The resulting suspension was centrifuged at 700 × *g* for 10 min at 4 °C. The supernatant containing the mitochondrial fraction was transferred to a microtest tube and centrifuged at 8,500 × *g* for 10 min at 4 °C. The supernatant was discarded, and 50 µL of 0.25 M sucrose was added to the residue and homogenized. After a 40-fold dilution with 0.25 M sucrose, the mitochondrial protein content in the resulting solution was measured by using a micro-BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA).

The UCP1 content of the mitochondrial fraction in IBAT was measured by Western blotting. An equivalent amount of the mitochondrial protein of each sample was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12%), and the protein was transferred to a PVDF membrane. This membrane was soaked in a 5% skim milk aqueous solution for 1 h at room temperature and then washed with a Tween (1%)–PBS solution. The membrane was then soaked with a primary antibody (the anti-UCP1 rabbit polyclonal antibody, Calbiochem, Darmstadt, Germany) in Tween–PBS (1:1,000 or 1:2,000) for 1 h at room temperature. After washing 3 times with Tween–PBS for 10 min each, the membrane was treated with a

Table 2. Effect of 1-Monoacylglycerol Supplementation on the Body Weight, Energy Intake, Fat Digestibility, and Organ Weights of Mice Fed with the High-Fat and High-Sucrose Diets for 30 d (experiment 1)

	TG group	MG15 group	MG30 group
Final body weight (g)	27.8 ± 0.561	28.3 ± 0.39	26.9 ± 0.665
Total energy intake (kJ)	1446 ± 23.1	1467 ± 15.4	1456 ± 15.4
Apparent fat digestibility (%)	99.0 ± 0.112	99.3 ± 0.027	99.1 ± 0.0956
Heart (mg)	97.4 ± 2.59	95.5 ± 1.47	106.4 ± 7.15
Spleen (mg)	55.9 ± 1.59	59.7 ± 1.93	63.3 ± 5.7
Kidney (mg)	292 ± 5.85	305 ± 13.8	319 ± 16.0
Liver (mg)	1056 ± 20.2	1051 ± 33.2	892 ± 53.3*
Pancreas (mg)	239 ± 5.79	257 ± 5.59	268 ± 20.2
Mesenteric WAT (mg)	460 ± 42.8	448 ± 34.5	342 ± 52.7
Perirenal WAT (mg)	410 ± 23.3	491 ± 40.1	334 ± 66.1
Epididymal WAT (mg)	973 ± 90.9	941 ± 44.4	667 ± 112*
Interscapular BAT (mg)	95.8 ± 13.4	96.3 ± 4.72	83.2 ± 16.9

Each value is expressed as the mean ± SEM (n = 6).
*$p < 0.05$ (*vs.* the TG group, one-way ANOVA with Dunnett's multiple-comparison test)

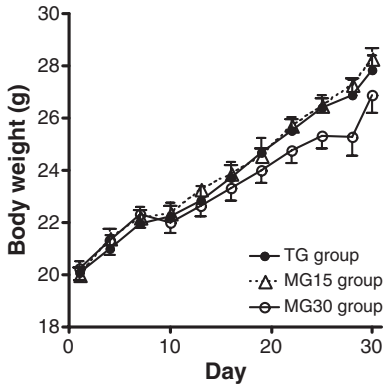

Fig. 1. Body Weight Change in Mice Fed with the Experimental Diets (experiment 1).
Each value is presented as the mean ± SEM (n = 6).

Table 3. Serum Parameters for Mice Fed with the High-Fat and High-Sucrose Diets for 30 d (experiment 1)

	TG group	MG15 group	MG30 group
Glucose (mg/dL)	364 ± 14.5	326 ± 6.79	288 ± 11.8*
TG (mg/dL)	59.9 ± 7.88	54.8 ± 3.30	45.0 ± 1.77
T-Cho (mg/dL)	118 ± 7.14	108 ± 2.49	85.0 ± 10.7*
NEFA (mEq/L)	1.38 ± 0.0851	1.39 ± 0.0600	1.12 ± 0.0839*

Each value is expressed as the mean ± SEM (n = 6).
*$p < 0.05$ (*vs.* the TG group, one-way ANOVA with Dunnett's multiple-comparison test)

secondary antibody (the ECL anti-IgG antibody, GE Healthcare UK, Buckinghamshire, UK) in Tween–PBS (1:10,000 or 1:20,000) for 1 h. After washing 3 times with Tween–PBS, an ECL substrate (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) was added, and the mixture was reacted for 1 min at room temperature. The stained protein was detected by using an LAS 3000 chemical luminescence detector (Fujifilm Corp., Tokyo, Japan) and analyzed with MultiGauge ver. 3.0 software (Fujifilm Corp.). The protein content of UCP1 was normalized to that of the TG-group loaded on the same gel slab. After the Western blotting experiment, the membrane was stained by Coomassie Brilliant Blue, and the equivalent amount of protein loaded into each lane was confirmed.

Statistical analysis. All data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were carried out by using Prism 4 software (GraphPad Software, San Diego, CA, USA). Differences among more than 3 groups were subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple-comparison test. Differences are considered significant at $p < 0.05$.

Results

Dietary 1-monoacylglycerols prevented the accumulation of visceral fat, hyperglycemia and hypercholesterolemia by feeding the HFS diet

The results of experiment 1, in which synthetic 1-MG (a mixture of 1-MG having oleic, linoleic and α-linolenic acids) was added to the experiment diet, are summarized in Table 2 and Fig. 1. There were no differences in the total energy intake and apparent fat digestibility during the experimental period. The body weight gain in one month for both the TG group and MG15 group showed similar values. On the other hand, the body weight for the MG30 group tended to be lower than that for either the TG or MG15 group after the 20th day. However, these values do not show a statistically significant difference for any period. After one month of the feeding experiment, the weight of epididymal WAT in the MG30 group had significantly decreased compared with the TG group. Moreover, the weight of mesenteric and perirenal WAT in the MG30 group tended to be lower than that in the TG group, although the values do not show any significant difference. The weight of the liver in the MG30 group was significantly lower than that in the TG group. The total lipid content in the liver of the MG30 group also tended to be lower than that in the TG group, although there was no significant difference (5.52 ± 0.295% for the TG group, 4.66 ± 0.508% for the MG30 group, n = 6). There were no differences among the three groups for the other organs (heart, spleen, kidney, pancreas and IBAT). Table 3 shows the results for the serum parameters. The value for serum glucose in the TG group was 364 mg/dL, although it was a fasting serum sample, suggesting that feeding the HFS diet for 30 d induced hyperglycemia. Substituting TG with 1-MG in the HFS diet decreased serum glucose in a dose-dependent manner, and the serum glucose value in the MG30 group was significantly lower than that in the TG group. Moreover, serum total cholesterol and NEFA in the MG30 group were also significantly lower than the values in the TG group. Serum TG tended to decrease in a 1-MG dose-dependent manner without any significant difference. These results suggest that 1-MG probably prevented HFS-induced hyperglycemia and hypercholesterolemia, and possibly hyperlipidemia.

Experiment 2 was performed with the same protocol as that in experiment 1, except for dietary high-purity 1-MG(18:1) being used instead of synthetic 1-MG. 1-MG(18:1) had the same activation potency toward TRPV1 as 1-MG(18:2) and 1-MG(18:3),[10] and high-purity 1-MG(18:1) was commercially available. There were no differences in the total energy intake and apparent fat digestibility during the experimental period (Table 4). There were also no significant differences in the body weight, or in the weight of each visceral fat and other organs among the three experimental groups (Table 4). Furthermore, substituting 1-MG(18:1)

Table 4. Effect of 1-Monoacylglycerol Supplementation on Body Weight, Energy Intake, Fat Digestibility, and Organ Weights of Mice Fed with the High-Fat and High-Sucrose Diets for 29 d (experiment 2)

	TG group	MG15 group	MG30 group
Final body weight (g)	27.3 ± 0.823	27.2 ± 0.356	27.3 ± 0.683
Total energy intake (kJ)	1508 ± 40.8	1509 ± 8.65	1511 ± 12.7
Apparent fat digestibility (%)	99.4 ± 0.0322	99.4 ± 0.0838	99.4 ± 0.0717
Heart (mg)	100 ± 2.94	99.0 ± 1.99	97.8 ± 3.05
Spleen (mg)	70.1 ± 2.62	65.0 ± 3.41	65.8 ± 3.78
Kidney (mg)	316 ± 6.22	308 ± 8.37	322 ± 9.98
Liver (mg)	1216 ± 59.2	1169 ± 42.8	1072 ± 30.4
Pancreas (mg)	163 ± 9.85	150 ± 5.58	165 ± 13.3
Mesenteric WAT (mg)	428 ± 48.3	484 ± 27.9	461 ± 43.7
Perirenal WAT (mg)	346 ± 51.1	378 ± 29.8	379 ± 59.6
Epididymal WAT (mg)	763 ± 96.9	830 ± 58.7	747 ± 91.0
Interscapular BAT (mg)	118 ± 13.4	122 ± 5.21	119 ± 7.13

Each of these values is expressed as the mean ± SEM (n = 6).

in the HFS diet did not alter the serum glucose, TG total cholesterol or NEFA values (data not shown).

Dietary 1-monoacylglycerol upregulated UCP1 protein expression in IBAT

There was no significant difference of mitochondrial protein in IBAT among the three experimental groups in experiment 1 (data not shown). The amount of UCP1 protein in the mitochondrial protein was measured by Western blotting, showing no difference in the UCP1 protein content in IBAT between the TG and MG15 groups. On the other hand, the UCP1 protein content in the MG30 group was about 2 times higher than that in the TG group (Fig. 2A). The MG30 diet only containing 1-MG(18:1) as MG also induced a significant increase in the UCP1 protein content of IBAT in experiment 2 (Fig. 2B).

Discussion

1-MGs having an unsaturated long-chain fatty acid have been identified as novel TRPV1 agonists in foods.[10] Oral administration of the TRPV1 agonist has increased energy metabolism *via* the sensory-central-sympathetic nerve axis[12] and the amount of UCP1 protein in IBAT,[4] and prevented the accumulation of visceral fat.[5] We examined in this study whether an HFS diet containing 1-MGs would alter several obesity-related markers in mice to clarify the effect of 1-MGs on obesity and its related incidence. When 30% of the lipid in the HFS diet was substituted by 1-MGs having oleic, linoleic, and α-linolenic acids, the levels of epididymal WAT, serum glucose, total cholesterol and free fatty acids were decreased, and UCP1 protein in IBAT was increased, although there was no difference in the total energy intake and apparent fat digestibility during experimental period.

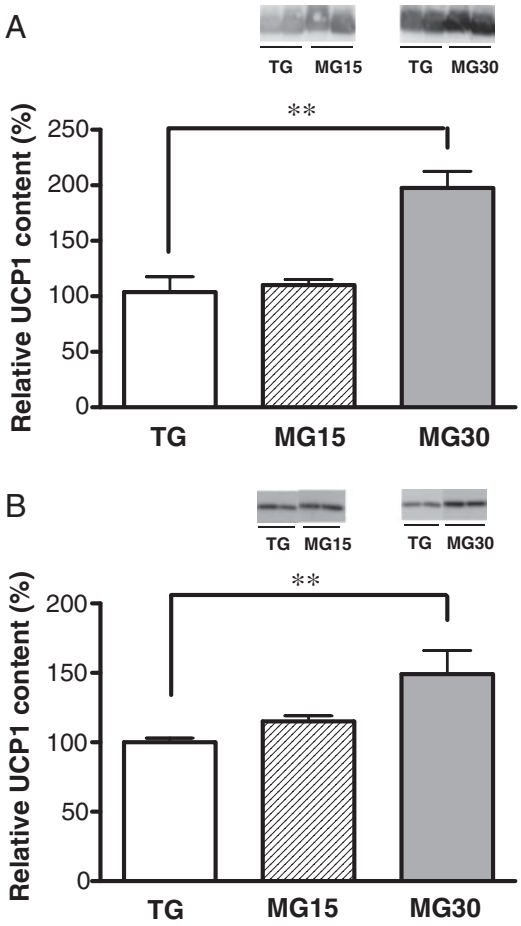

Fig. 2. Contents of UCP1 Protein in IBAT of the Mice Fed with the Experimental Diets.

Western blots are shown of UCP1 in IBAT mitochondrial protein (15 μg) from experiment 1 (A) and in IBAT mitochondrial protein (2.5 μg) from experiment 2 (B). The UCP1 antibody specific bands were 32 kDa. The protein content of UCP1 is expressed as a percentage of that of the TG group. Each value is the mean ± SEM (n = 6). $^{**}p < 0.01$ (*vs.* the TG group, one-way ANOVA with Dunnett's multiple-comparison test)

BAT is the major tissue producing non-shivering thermogenesis in cold exposure and diet-induced thermogenesis,[19] including CAP intake.[20,21] IBAT is heavily innervated by the sympathetic nerve, and noradrenaline release from the end of the activated sympathetic nerve promotes thermogenesis by activating the β-adrenergic receptor.[19] Moreover, chronic β-adrenergic receptor stimulation of BAT increases the amount of UCP-1 protein. The amount of UCP1 protein is therefore accepted to be an indirect index of the sympathetic activity. The expression of UCP1 protein in the MG30 group was significantly more than that in the TG group in both experiments 1 and 2. These results suggest the possibility of activation of sympathetic nerve-innervating IBAT by the 1-MG intake. Our study has demonstrated for the first time that dietary 1-MGs having unsaturated long-chain fatty acid, which are TRPV1 agonists, induced the expression of UCP1 protein in IBAT as well as CAP. The adiposity suppressing effect observed with the 1-MG intake might therefore be partially involved in the activation of the sympathetic nerve and thermogenic protein UCP1. On the other hand, the HFS diet in which 30% of the lipid was substituted by 1-MG(18:1) increased the UCP1

protein level in IBAT, but did not alter the weight of visceral WAT or the serum parameters (experiment 2). However, the increase of UCP1 protein in experiment 2 was smaller than that in experiment 1. It is accordingly assumed that the increase of UCP1 and/or the sympathetic nerve activity of organs other than IBAT might have been too small to prevent visceral fat deposition.

Dietary CAP has activated TRPV1 and prevented obesity-induced hyperglycemia and hyperlipidemia (TG or T-Cho) in previous studies,[8,22,23] although details of the mechanism are still unclear. However, it is thought that CAP activates the sympathetic nerve by activating the TRPV1-expressing sensory nerve, and that this secretes adrenaline from the adrenal gland. This adrenaline increases the glucose and lipid metabolism in such organs as the liver and adipose tissue. The result is that the accumulation of fat and glycogen is suppressed and fat accumulation in WAT and the liver is also suppressed.[12] The MG30 diet including 1-MG(18:1), 1-MG(18:2) and 1-MG(18:3), which activated TRPV1, also significantly decreased the levels of serum glucose, T-Cho and NEFA, and tended to decrease serum TG in this study. An accumulation of abdominal visceral fat is linked to hyperglycemia and hyperlipidemia;[24] therefore, preventing an accumulation of visceral fat by the MG diet might improve obesity-induced hyperglycemia and hyperlipidemia.

The ingestion of a TRPV1 agonist, *e.g.*, CAP, increases energy expenditure by activating the sensory nerve expressing TRPV1. However, there was no direct evidence for the sensory nerve being stimulated by the agonist. Kawabata *et al.* have reported that capsaicin enhanced energy expenditure by the TRPV1-expressing sensory nerve innervating the gastrointestinal tract.[9] Belza *et al.* have reported that the ingestion of CAP-containing tablets which dissolve in the stomach increased the energy expenditure, whereas the same tablets dissolving in the small intestine had no such effect.[25] These reports suggest that the local action of CAP in gastric mucosa is a prerequisite for exerting the thermogenetic effect. Orally administered 1-MGs might therefore increase the expression of UCP1 protein and prevent WAT accumulation by activation of the TRPV1-expressing sensory nerve distributed in the upper gastrointestinal tract.

The MG30 diet including 1-MG(18:1), 1-MG(18:2) and 1-MG(18:3) showed a stronger effect than the MG30 diet composed only of 1-MG(18:1) for upregulating UCP1 protein and preventing WAT accumulation, hyperglycemia and hypercholesterolemia in the diet-induced obese mice. 1-MG(18:2) or 1-MG(18:3) might therefore have a strong anti-obese effect such as that for decreasing fat accumulation. These different results among the 1-MGs cannot be explained by their TRPV1 activation potency, because the EC_{50} and maximum activity values for TRPV1 were the same levels.[10] One possibility is that the lipophilicity of 1-MGs might be involved in accessibility to the TRPV1-expressing nerve in the gastrointestinal tract. The terminal of sensory nerve is covered with epithelial tissue, making it necessary for the compounds to penetrate the tissue to reach the nerve ending. The lipophilicity of a compound is an important factor for penetrating tissue and mucosa: too high lipophilicity is inferior for crossing into tissue due to too high affinity to the lipid bilayer of cells. In fact, agonists like capsiate and olvanil with high lipophilicity have difficulty reaching the epithelium-covered TRPV1-expressing trigeminal nerve endings in the mouth and eye.[11] 1-MG(18:1) has the highest lipophilicity among these 1-MGs.[10] Irritant responses caused by injecting 1-MGs into the rat hind paw to activate TRPV1 also tended to be attenuated in the order of 1-MG(18:3), 1-MG(18:2) and 1-MG(18:1),[10] suggesting that 1-MG(18:1) would be inferior to 1-MG(18:2) or 1-MG(18:3) for activating TRPV1 *in vivo*. It is necessary to investigate the differences and mechanism for the anti-obese effect by 1-MG(18:1), 1-MG(18:2) and 1-MG(18:3).

This study has revealed that diet-induced obesity could be prevented by replacing 30% of the lipid with 1-MGs having an unsaturated long-chain fatty acid in a high-fat diet. TRPV1 agonists have been noted as functional compounds for increasing energy expenditure and preventing obesity. However, it is difficult to ingest an effective amount of them as food because many TRPV1 agonists have strong pungency. 1-MGs having an unsaturated long-chain fatty acid have been identified as non-pungent TRPV1 agonists.[10] In addition, MG has been used as an emulsifying additive for such processed foods as ice cream and margarine. The results of the present study might suggest new applications for 1-MGs as functional food additives for preventing obesity and controlling the metabolic syndrome.

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