Anti-obesity effects of α-cyclodextrin-stabilized 4-methylthio-3-butenyl isothiocyanate from daikon (Raphanus sativus var. longipinnatus) in mice

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4-Methylthio-3-butenyl isothiocyanate (MTBI) is a pungent bioactive constituent found in daikon. However, MTBI is immediately hydrolyzed to 3-hydroxy-methylene-2-thioxopyrrolidine in grated daikon. In this study, we evaluated whether MTBI in grated daikon complexed with α-cyclodextrin (αCD) has anti-obesity effects in mice. C57BL/6J mice were fed a normal diet (normal group), high-fat diet (HFD, control group), HFD with αCD (αCD group), or HFD with MTBI-αCD (MTBI-αCD group) for 16 weeks. The results showed that the final body weight, epididymal white adipose tissue weight, and plasma triglyceride and total cholesterol levels were significantly lower in the MTBI-αCD group than in the control group. The cell size in epididymal adipose tissue was significantly smaller and the accumulation of lipids in the liver was significantly smaller in the MTBI-αCD group than in the control group. Furthermore, real-time polymerase chain reaction showed that the mRNA expression level of tumor necrosis factor-alpha was suppressed in the MTBI-αCD group. We also observed low superoxide dismutase activity in the MTBI-αCD group, possibly because MTBI-αCD has the potential to resist HFD-induced oxidative injury. In conclusion, MTBI-αCD exerted anti-inflammation and antioxidant effects to suppress lipid accumulation in epididymal adipose tissue and the liver. These effects then prevented HFD-induced obesity in mice.

Key Words: 4-methylthio-3-butenyl isothiocyanate, αCD, anti-obesity effect, antioxidant effect, high-fat diet

Daiikon (Japanese white radish, Raphanus sativus L.) has been widely used in Japanese cuisine for more than 1,000 years.1) Japanese people often eat grated daikon with soy sauce, grilled fish, or soba. Daikon is rich in nutrients and vitamins and provides multiple benefits. It is known to help digestion, fight different types of cancer, and prevent irritation of the throat. (1-3) 4-Methylthio-3-butenyl isothiocyanate (MTBI), a kind of isothiocyanate, is a typical pungent constituent of daikon, enzymatically formed from the corresponding glucosinolates (Fig. 1). (4,5) The isothiocyanate most widely known in Japan is allyl isothiocyanate (AITC), derived from wasabi (Wasabia japonica Matsum.), and various effects of isothiocyanates have been reported by many researchers. (6,7) Previous studies have reported antimicrobial, (8,9) antioxidant, (10,11) anticancer and anti-obesity activities of isothiocyanates. (12-14) However, many isothiocyanates, including MTBI and AITC, are very reactive and unstable in aqueous solutions. Uda et al. (8) have reported that MTBI is degraded to a watersoluble product and that is releases methane thiol in an aqueous medium. In grated daikon, MTBI is hydrolyzed to 3-hydroxy-methylene-2-thioxopyrrolidine (Fig. 1).

Cyclodextrins (CDs) are a family of cyclic oligosaccharides, typically containing six (αCD), seven (βCD), or eight (γCD) 1,4-linked D-glucose units. In aqueous solutions, CDs can accommodate a variety of organic compounds in their central cavities and form inclusion complexes. CDs can improve the solubility, stability, and bioavailability of hydrophobic or lipophilic molecules through complex formation. Therefore, CDs are widely used in pharmaceuticals and nutraceuticals. αCD is an ideal dietary fiber because of its relatively small cavities and high resistance to enzymatic hydrolysis. (12) Oral supplementation of αCD provides health benefits, such as improving the blood lipid profile and/or lowering the body weight. (15-18) Additionally, βCD is widely used to mask odor or pungent taste. It has been reported that the stability of AITC was improved by βCD. (16-18)

In recent decades, the prevalence of metabolic syndrome, especially obesity, has become an urgent worldwide public health problem. (19) This condition is a cluster of risk factors, including central obesity, atherogenic dyslipidemia, hypertension, and glucose intolerance, and is a strong predictor of cardiovascular diseases, diabetes, or stroke. (20-22) Metabolic syndrome is associated with oxidative stress, resulting from an imbalance between the production and scavenging of reactive oxygen species (ROS). (23) As Western-type diet is becoming popular, the number of metabolic syndrome patients has been increasing in Japan and other Asian countries. Obesity, the most prominent manifestation of metabolic syndrome, mainly involves an increase in the size of adipocytes. Animal experiments on obesity reveal that weight gain is accompanied by adipose tissue accumulation via the conversion of excess energy to lipids. (11)

In our previous study, we found that MTBI was stabilized in grated daikon by CDs, (24,25) among which αCD was the most effective and sufficiently stabilized MTBI. In this study, we prepared a freeze-dried powder of MTBI in grated daikon with αCD in an attempt to improve the stability of MTBI. Furthermore, we

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evaluated the anti-obesity effects of the MTBI-αCD in high-fat diet (HFD)-induced C57BL/6J mice and elucidated the potential mechanism.

**Materials and Methods**

**Materials.** Oyada Karami daikon, which maintains the highest level of MTBI, was purchased from Kanematsu Co., Ltd. (Nagano, Japan). CAVAMAX W6 FOOD (αCD) was supplied by CycloChem Co., Ltd. (Kobe, Japan). All other chemicals were of analytical grade.

**Complexation of MTBI with αCD.** To make MTBI-αCD, we grated Oyada Karami daikon in a food processing machine with the addition of 5% (w/w) αCD, freeze-dried the mixture, and then ground it into powder. We measured the amount of MTBI in MTBI-αCD by gas chromatography on a GC-17A instrument (Shimadzu Corp., Kyoto, Japan) with a flame ionization detector at room temperature and a DB-5 column (30 m × 0.53 mm i.d.; Agilent Technologies, Inc., Santa Clara, CA), with the column temperature held at 250 °C. The front inlet temperature was 250 °C; helium gas was used as a carrier gas, and the injection volume was 1 μl.

**Animal experiments.** Six-week-old male C57BL/6J mice were purchased from SLC Japan, Inc. (Shizuoka, Japan) and were housed in a room with controlled temperature (23 ± 1 °C), humidity (55–60%), and lightning (12-h light/dark cycle), with free access to food and water. After one week of acclimation, the mice were divided into four groups and were fed experimental diets for 16 weeks. The normal group was fed a normal diet [MF diet, 5% (w/w) fat; Oriental Yeast Co., Ltd., Tokyo, Japan]. The control group was fed HFD [20% (w/w) fat]. The other two groups were fed HFD with either αCD or the MTBI-αCD powder. MTBI-αCD was added to a final MTBI concentration of 0.05% (w/w) in the diet. The dose of αCD was equivalent between the αCD and MTBI-αCD groups. The composition of the experimental diets is shown in Table 1. The body weight and food intake were monitored every 3–4 days. At the end of the experiment, all mice were fasted for 14 h. Subsequently, blood samples were collected through the abdominal vena cava with a heparinized syringe under isoflurane anesthesia and separated by centrifugation at 1,200 × g for 10 min. The plasma, epididymal adipose tissue, and the liver were stored at –80 °C until analysis. All experiments were approved by the Experimental Animal Research Committee of Kobe Women’s University and were performed in accordance with the Guidelines for Animal Experimentation of Kobe Women’s University.

**Biochemical analyses.** Plasma triglycerides (TG) and total cholesterol (TCHO) were assayed using a Fuji Dry-Chem system (Fuji Medical Co., Tokyo, Japan). Plasma insulin level was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi Co., Ltd., Gunma, Japan). Glycated hemoglobin (HbA1c) was measured using a DCA 2000 analyzer (Bayer Corp., Vienna, Austria). Superoxide dismutase (SOD) in the liver was measured using an assay kit with water-soluble tetrazolium salt (Dojindo Laboratories, Kumamoto, Japan). 100 mg liver samples were homogenized in 600 μl of phosphate-buffered saline, then centrifuged at 1,500 × g for 10 min at 4 °C. An aliquot of each supernatant was diluted at 1:4 in assay buffer before measuring SOD activity.

**Intraperitoneal glucose tolerance test.** An intraperitoneal glucose tolerance test (IGTT) was conducted after feeding the mice for 14 weeks with the experimental diets. Mice were intraperitoneally administered 2 g of glucose per kg of body weight following a 13-h fast. Blood glucose level was measured using blood collected from the tail vein at 0, 15, 30, 60, 90, and 120 min after glucose administration using a Glutestace (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan).
Histological examination. For histological analysis, epididymal adipose tissue and liver tissue were fixed in 10% formalin, embedded in paraffin, sectioned, then stained with hematoxylin and eosin. The stained areas were observed under a light microscope (BA210E-T3M; Shimadzu Corp.) equipped with a camera. The mean adipocyte size and the mean lipid droplet size in the liver were quantified using Photoshop software (Adobe Inc., San Jose, CA) or WinROOF software (Mitani Corp., Fukuji, Japan).

Real-time quantitative reverse transcription polymerase chain reaction. The total RNA was extracted from the liver using a High Pure RNA tissue kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer’s protocol. The RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Schwerte, Germany). Primers were purchased from Nihon Gene Research Laboratories, Inc. (Sendai, Japan). Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using a LightCycler Nano (Roche Diagnostics, Mannheim, Germany) and the One Step SYBR PrimeScript PLUS RT-PCR kit (Takara Bio, Inc., Kusatsu, Japan). Amplification reactions were performed in a total volume of 20 μl containing 50 ng of template RNA, under the following conditions: reverse transcription at 42°C for 5 min; initial denaturation at 95°C for 10 s, followed by 40 cycles at 95°C for 5 s, 55°C for 30 s, and 72°C for 30 s. The primer sets are shown in Table 2.

Analysis of water-soluble metabolites in the liver. Metabolites were extracted and derivatized as described previously, with the following modifications. To extract water-soluble metabolites from the liver, 10 mg liver samples were mixed with 1.0 ml of a MeOH:H₂O:CHCl₃ mixture (2.5:1:1), and then 10 μl of 0.5 mg/ml 2-isopropylmalic acid (internal standard) was added. The mixture was sonicated for 20 s with a handheld sonicator (UR-21P; Tomy Seiko Co., Ltd., Tokyo, Japan), incubated at 37°C for 30 min, then centrifuged at 15,000 × g for 3 min at 4°C. A 900 μl aliquot of the supernatant was transferred to a new 2-ml tube, and chloroform (500 μl) was added to each tube, followed by vortexing and centrifugation at 15,000 × g for 3 min at 4°C. A 500 μl aliquot of each supernatant was transferred to a new 1.5-ml tube, mixed with 200 μl of Milli-Q water by vortexing, and centrifuged at 15,000 × g for 3 min at 4°C. Then, a 500 μl aliquot of each supernatant was transferred to a new 1.5-ml tube and evaporated in a vacuum centrifuge dryer for 1 h, followed by freeze-drying overnight.

For oximation, 50 μl of methoxyamine hydrochloride in pyridine (20 mg/ml) was added to freeze-dried supernatant, followed by vortexing and sonication in a water bath for 20 min. After incubation for 90 min at 30°C, 25 μl of N-methyl-N-(trimethylsilyl)-trifluoroacetamide was added for trimethylsilylation, followed by incubation at 37°C for 30 min and centrifugation at 15,000 × g for 5 min. The supernatant was analyzed on a GCMS-QP2010 Ultra gas chromatograph–mass spectrometer (Shimadzu, Kyoto, Japan) using a fused silica capillary column (30 m × 0.25 mm i.d.) coated with 0.25-μm CP-SIL 8 CB low bleed/MS (Agilent Technologies Inc.). The flow rate of helium was 1.12 ml/min. The column temperature was held at 80°C for 2 min isothermally, and then increased by 15°C/min to 330°C and held at 330°C for 6 min isothermally. The front inlet temperature was 230°C. The interface and ion source temperatures were 250°C and 200°C, respectively. A mass spectrometer (MS) scan started at 3.55 min and ended at 24.00 min, with spectra recorded over a mass range of 85–500 m/z at a scan speed of 10,000 amu/s. The injection volume was 1 μl and the split mode was 25:1 (v/v).

Data were preprocessed using the free MetAlign software (RIKILT, Wageningen University and Research Centre, Netherlands). Aloutput was used for peak identification, prediction, and integration of the data exported from MetAlign, with user-defined retention times and a library of spectra. Aloutput is an analysis tool for GC/MS-based metabolomics, written in Visual Basic and available in Microsoft Excel for Windows. Peak intensity data were calculated using Aloutput based on the weight of the liver.

Statistical analysis. Data are expressed as mean ± SD. Comparison between the normal and control groups was performed using a Student’s t test, and multiple comparisons among groups (excluding the normal group) were performed using Dunnett’s test. P<0.05 was considered to be statistically significant.

Results

Complexation of MTBI with αCD. The amount of MTBI in the MTBI-αCD complex was 6.3 mg/g as measured by GC. MTBI was stable in the test diet throughout the animal experiment.

IGTT. The highest blood glucose level was observed 30 min after glucose injection in each group, and glucose was gradually metabolized. However, glucose metabolism was significantly slower in the control group than in the normal group. Thus, the blood glucose level in the control group was higher than glucose in the other groups even at 120 min after glucose injection (Fig. 2A). The blood glucose area under the curve of the control group was also significantly higher than the normal group. The MTBI-αCD and αCD groups exhibited significant suppression of HFD-induced elevation in blood glucose level (Fig. 2B).

Body weight, food intake, and organ weights. HFD administration led to a significantly increased body weight (Fig. 3). The food intake was not significantly different among the groups (Supplemental Fig. 1). The body weight was significantly lower in the MTBI-αCD group than in the control group after 20 days of feeding the experimental diet. Although the MTBI-αCD group was fed HFD, the body weight gain in the MTBI-αCD group was similar to that in the normal group that were fed the normal diet. There was no significant difference in the body weight between the αCD and control groups. The water intake in the normal group was slightly higher than that in the other groups at the start of the experiment, but there were no differences in water intake among the four groups after 40 days of feeding the experimental diets (data not shown).

HFD administration led to a significant increase in the epididymal fat weight in the control group compared with that in the normal group, whereas MTBI-αCD significantly suppressed the HFD-induced elevation in the epididymal fat weight. However, there was no difference between the control and αCD groups (Fig. 4A). Liver weight did not significantly differ among the four groups (Fig. 4B).

Serum biochemical parameters. HFD administration resulted in increased plasma TG and TCHO levels (Fig. 5A and B), while MTBI-αCD significantly suppressed the HFD-induced increase in plasma TG and TCHO levels. The HbA1c level was significantly lower in the MTBI-αCD group than in the control group (Fig. 5C). However, there was no difference between the normal and control groups. The plasma insulin level was below the limit of detection of ELISA (<39 pg/ml) in more than half of the normal mice (Fig. 5D), while insulin levels did not differ among the groups that were fed HFD.

Histological evaluation. Histological evaluation revealed that HFD administration led to an increase in the size of cells in

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sequences (5′-3′)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Gapdh</td>
<td>F: AAATGGTTGAAGTGTGGTG</td>
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<td></td>
<td>R: TGAAGGGTCGGTATGG</td>
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<tr>
<td>Tnf-α</td>
<td>F: TGGGACAGTGACCTGGACTG</td>
<td>28</td>
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<tr>
<td></td>
<td>R: TTCGGGAAAGCCCATTTGAG</td>
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</tr>
</tbody>
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Gapdh, glyceraldehyde 3-phosphate dehydrogenase; Tnf-α, tumor necrosis factor-α; F, forward; R, reverse.

*See online. https://doi.org/10.3164/jcbn.19-11

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epididymal adipose tissue compared with that in the normal diet-fed mice (Fig. 6). However, MTBI-αCD effectively reduced the size of adipocytes compared with the control and αCD groups. Microscopic examination showed that there was negligible accumulation of lipid droplets in the liver of the mice that were fed the normal diet (Fig. 7), while HFD induced accumulation of lipid droplets in the liver. In the αCD group, the lipid accumulation tended to be lower than the control group, while the MTBI-αCD group had even lower accumulation than the αCD group.

SOD activity in the liver. The SOD activity in the liver tended to be higher in the control group than in the normal group (Fig. 8). These results indicated that in the control group, the SOD activity increased to alleviate HFD-induced oxidative stress. However, the SOD activity in the MTBI-αCD group was significantly lower than that in the control group. The αCD group had slightly lower SOD activity that was not significantly different from the control group.

Gene expression in the liver. To determine the mechanism underlying MTBI-αCD anti-obesity effects, we examined the mRNA expression level of the gene encoding tumor necrosis factor-α (Tnf-α), a proinflammatory cytokine, by real time qRT-PCR (Fig. 9). HFD administration led to an increase in the mRNA expression of Tnf-α in the liver, whereas MTBI-αCD suppressed this increase. However, the mRNA expression level of Tnf-α was not significantly different between the αCD and control groups.

Analysis of water-soluble metabolites in the liver. To clarify the anti-obesity effect of MTBI-αCD, we analyzed the metabolomic data of the three groups except the αCD group. In this study, 114 metabolites, including amino acids, sugars, and organic acids, were identified (Supplemental Table 1*). Among

*See online. https://doi.org/10.3164/jcbn.19-11
them, amino acids related to obesity and inflammation are shown in Fig. 10.\(^{(30)}\) Principal component analysis (PCA) of the metabolomic data performed using MetaboAnalystR software (ver. 3.4.3).\(^{(31)}\) The performance parameter \(R^2(X)\) was 37.6% and the parameter \(R^2(Y)\) was 33.9%, indicating that the model was reliable and highly predictive power. It showed a clear separation of liver metabolites in the MTBI-αCD group from the other two groups (Fig. 11).

Fig. 4. Comparison of epididymal fat weight (A) and liver weight (B) in mice that were fed the normal and experimental diets. Data are expressed as mean ± SD. Normal: \(n=5\); control: \(n=8\); αCD: \(n=8\); MTBI-αCD: \(n=9\). *p<0.01 compared with the control group.

Fig. 5. Plasma TG (A), TCHO (B), HbA1c (C), and insulin (D) levels in mice that were fed the normal and experimental diets. Data are expressed as mean ± SD. Normal: \(n=5\); control: \(n=8\); αCD: \(n=8\); MTBI-αCD: \(n=9\). *p<0.01 compared with the control group.
Discussion

In our previous study, we demonstrated that MTBI was stabilized in grated daikon by forming complexes with CDs.\(^{24,25}\) Our previous results suggested that the improved stability of MTBI was due to CDs, which could prevent the hydrolysis of MTBI in grated daikon. \(\alpha\)CD provided better stabilization of MTBI than did \(\beta\)CD and \(\gamma\)CD. Ohta \textit{et al.}\(^{32}\) have reported the isothiocyanate group of AITC is located close to the secondary hydrogen group, and the hydrophobic allyl group is located within the hydrophobic cavity of \(\alpha\)CD. We speculate that MTBI forms a complex with \(\alpha\)CD through interactions (Fig. 12), similar to what has been reported for AITC.\(^{32}\) The reactive site of MTBI can be protected from nucleophilic attack of water by the steric hindrance of the \(\alpha\)CD torus. In addition, by forming the inclusion complex with \(\alpha\)CD, MTBI in MTBI-\(\alpha\)CD could be stably stored for a long time.

To the best of our knowledge, our \textit{in vivo} study demonstrated, for the first time, that the supplementation of MTBI, stabilized in daikon with \(\alpha\)CD, was effective in the prevention of diet-induced obesity, amelioration of insulin resistance, and attenuation of lipid accumulation in epididymal adipose tissue and the liver.

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**Fig. 6.** Histological staining of epididymal adipose tissue sections and the mean areas of adipocytes in mice that were fed the normal and experimental diets. Data are expressed as mean \(\pm\) SD. Normal: \(n=5\); control: \(n=8\); \(\alpha\)CD: \(n=8\); MTBI-\(\alpha\)CD: \(n=9\). \(p<0.05\) and \(*p<0.01\) compared with the control group.

**Fig. 7.** Histological staining of liver sections and lipid droplet areas in the liver of mice that were fed the normal and experimental diets. Data are expressed as mean \(\pm\) SD. Normal: \(n=5\); control: \(n=8\); \(\alpha\)CD: \(n=8\); MTBI-\(\alpha\)CD: \(n=9\).
Vivarelli et al.\(^{33}\) have reported that *Raphanus sativus* Sango freeze-dried sprout juice, rich in anthocyanins and isothiocyanates, showed hypolipidemic, antioxidant, and anti-obesity effects in Sprague–Dawley rats with HFD-induced obesity. Unlike our findings, it decreased food intake compared with that in the mice that were fed HFD. In another study, You et al.\(^{34}\) have reported that MTBI decreased the hepatic lipid content, the level of low-density lipoprotein cholesterol, and the severity of insulin resistance, caused by HFD in Sprague–Dawley rats; however, there were no changes in the body weight and serum lipid profile, in contrast with our findings. We also observed that the mean areas of adipocyte cells and lipid droplets in the liver were also significantly smaller in the MTBI-αCD group than in the control group. These results suggest that MTBI-αCD ameliorated the HFD-induced obesity by reducing lipid accumulation in epididymal adipose tissue and the liver of mice. Accumulation of visceral fat...
respectively; the percent of explained variances is shown in brackets.

The X- and Y-axes represent the first and second principal components, respectively; the percent of explained variances is shown in brackets.

In another study, MTBI was shown to exert a chemoprotective effect, attributed to its antioxidant activity, which is higher than that of vitamins C and E. AITC allyl isothiocyanate

Oxidative stress is regulated by the balance between the ROS production and the activity of antioxidant enzymes. Therefore, to evaluate the effect on oxidative injury by supplementing MTBI-CD, we measured the SOD activity in the liver and found that it tended to be higher in the control group to reduce HFD-induced oxidative stress. Our results potentially suggest that the MTBI-CD group had low SOD activity because MTBI inhibited oxidative injury. In addition, MTBI-CD suppressed HFD-induced increase in Tnf-α mRNA expression, which is associated with inflammation and oxidative stress in the liver.

We also prepared mice fed HFD with daikon powder not including MTBI and αCD. In this group, the epididymal adipocyte size and SOD activity in the liver were significantly lower than that in the control group but were not robust. They were not significantly lower than that in the MTBI-αCD group. Furthermore, other results of mice fed HFD with daikon powder not including MTBI and αCD did not differ compared with the control group (data not shown). These results indicated that MTBI-αCD treatment was important to suppress HFD-induced obesity.

In this study, analysis of hepatic metabolites showed that HFD feeding caused metabolic disorder and decreased hepatic amino acids levels especially glycine. Glycine, a major amino acid in mammalians, is synthesized from serine and threonine in hepatocytes. Serine and threonine are both ketogenic amino acids, and a close relationship exists in metabolic disorders between metabolism of amino acids and the development of a fatty liver and other metabolic diseases. Leucine and Isoleucine, which are branched-chain amino acids (BCAAs), have been reported to mediate anti-obesity effects, especially in rodent models. Moreover, glycine and phenylalanine have been reported to prevent various types of liver injury in rodent models. (44)

In our study, these amino acid levels in the control group were decreased compared with those in the normal group indicating that HFD-induced liver injury might occur, whereas those in MTBI-αCD-fed mice liver were elevated compared with that in the control group, indicating that MTBI-αCD feeding could restore it.

It has been reported that MTBI activates transient receptor potential ankyrin 1 (TRPA1), a nonselective cation channel. In addition, a recent study investigated the effect of TRPA1 on ghrelin secretion. Ghrelin is a kind of orexigenic hormones that stimulates food intake and body weight gain. Cinnamaldehyde, is well-known as the principal agonist of TRPA1, decreased ghrelin secretion and gastric emptying, partially through TRPA1 activation in ghrelin secreting cells (mouse ghrelinoma 3-1). MTBI-αCD may decrease the secretion of ghrelin through TRPA1 activation and accordingly suppressed body weight gain.

In summary, HFD induced obesity through increasing oxidative injury and causing metabolic disorder in the mouse liver. However, MTBI-αCD treatment was able to inhibit the accumulation of fat in epididymal adipose tissue and the liver and suppressed HFD-induced obesity by reducing inflammation and oxidative injury in mice. Further study of antimiometabolic syndrome activity of MTBI is necessary to elucidate its mechanism of action.

**Author Contributions**

Conceptualization: HO, NI, KT, YY and NS.
Investigation: HO, SI, NN, NI, AI and CU.
Formal analysis: HO, NI, YY and NS.
Project administration: KT, YY and NS.
Writing – original draft: HO and NI.
Writing – review & editing: HO, NI, YY and NS.

**Abbreviations**

AITC: allyl isothiocyanate
CD: cyclodextrin
ELISA: enzyme-linked immunosorbent assay
Gapdh  glyceraldehyde 3-phosphate dehydrogenase
HbA1c  glycated hemoglobin A1c
HFD  high-fat diet
IGTT  intraperitoneal glucose tolerance test
MS  mass spectrometry
MTBI  4-methylthio-3-butenyl isothiocyanate
PCA  principal component analysis
qRT-PCR  real-time quantitative reverse transcription polymerase chain reaction
ROS  reactive oxygen species
SOD  superoxide dismutase
TCHO  total cholesterol
TG  triglycerides
TMS  trimethylsilyl
TRPA1  transient receptor potential ankyrin 1
Tnf-α  tumor necrosis factor-α

Clinical Trial Register and Registration Number
All experiments were approved by the Experimental Animal Research Committee of Kobe Women’s University and were performed in accordance with the Guidelines for Animal Experimentation of Kobe Women’s University (permit number A92).

Conflict of Interest

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