Original paper

Anti-obesity Effects of Wasabi Leaf Extract on Rats Fed a High-fat Diet are Related to Upregulation of mRNA Expression of β3-adrenergic Receptors in Interscapular Brown Adipose Tissue

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Wasabi is a plant of Japanese origin belonging to the Brassicaceae family. Although the wasabi rhizome is a popular condiment in Japan, the leaf is typically discarded. For utilization of the wasabi leaf, we investigated its anti-obesity effect on Wistar rats fed a high-fat diet containing wasabi leaf extract (WLE) prepared with 50% ethanol. At the experimental endpoint, WLE had significantly decreased the body weight of rats and upregulated the mRNA expression of the β3-adrenergic receptor (Adrb3) in the interscapular brown adipose tissue (BAT). WLE may have promoted lipid absorption from the dietary fat, as the fecal lipid content was considerably lower in the WLE group. These results suggest that WLE suppressed obesity in rats fed a high-fat diet, which was related to the upregulation of Adrb3 mRNA expression in the interscapular BAT without increased fecal lipid excretion. Thus, wasabi leaves may be used as a functional food material for the suppression of obesity.

Keywords: anti-obesity, wasabi leaf, high-fat diet, β3-adrenergic receptor, fecal lipid

Introduction

Obesity, a state of excess fat accumulation in the adipocytes, occurs because energy intake exceeds energy consumption. It is a risk factor for lifestyle-related diseases such as hypertension, hyperlipidemia, and diabetes. Thus, the weight loss properties of certain foods, including the inhibition of fat absorption from the intestine and improvement of lipid metabolism, have been studied in obesity prevention (Kamiya et al., 2012; Maeda et al., 2009; Nishi et al., 2007).

Dietary fat is digested by pancreatic lipase in the intestine, and is then absorbed. When energy intake exceeds its expenditure, fat accumulates in adipocytes. The β3-adrenergic receptor (Adrb3), which is expressed predominately in adipocytes, activates protein kinase A (PKA) by sympathetic nerve stimulation. Successively, PKA upregulates the mRNA expression of uncoupling protein 1 (UCP1), which induces thermogenesis from stored energy in
adipocytes. On the other hand, PKA also activates hormone-sensitive lipase (HSL), which promotes lipolysis from stored energy in adipocytes. Then, free fatty acids (FFA) generated by lipolysis are consumed through ATP synthesis by oxidative phosphorylation and/or UCP1.

Adrb3 plays an important anti-obesity role because its agonists show anti-obesity effects in animals (Cawthorne et al., 1992). Anti-obesity compounds upregulate Adrb3 mRNA expression in animal brown adipose tissue (BAT) and white adipose tissue (WAT) (Cawthorne et al., 1992; Emilsson et al., 1998; Ito et al., 2010; Maeda et al., 2009). Therefore, Adrb3 mRNA expression is related to the anti-obesity effect.

Wasabi [Wasabia japonica (Miq.) Matsumura] is a plant of Japanese origin belonging to the Brassicaceae family. Grated wasabi rhizome, which contains various isothiocyanates, is a popular condiment in Japan. These isothiocyanates have many physiological functions, including anti-microbial (Isshiki et al., 1992), anti-platelet (Morimitsu et al., 2000), anti-cancer, anti-oxidative (Morimitsu et al., 2000, 2002), anti-inflammatory (Uto et al., 2005), anti-diabetic (Fukuchi et al., 2004; Yoshida et al., 2011), and anti-allergic (Nagai and Okunishi, 2009; Yamada-Kato et al., 2012) activities. Furthermore, isothiocyanates are known ligands of transient receptor potential ankyrin 1 (TRPA1) (Jordt et al., 2004; Terada et al., 2015; Uchida et al., 2012).

Wasabi leaves, which contain lower amounts of isothiocyanates than the rhizomes (Etoh et al., 1990; Kumagai et al., 1994), are not consumed and are usually discarded. There are some reports regarding the physiological functions of these leaves, including anti-fungal (Pedras et al., 1999), anti-oxidative (Hosoya et al., 2008; Lee et al., 2010; Sekiguchi et al., 2010), anti-cancer (Okamoto et al., 2008), anti-inflammatory (Yoshida et al., 2015), anti-influenza virus (Mochida and Ogawa, 2008), and collagen synthesis (Nagai et al., 2010) activities. In a study identifying compounds besides isothiocyanates in wasabi leaves, polyphenols such as flavonoids (Hosoya et al., 2005), phenylpropanoids (Hosoya et al., 2008), terpenoids (Yoshida et al., 2015), and carotenoids (Yoshida et al., 2015) were isolated.

The anti-obesity effects of wasabi leaves have been studied. Ogawa et al. (2010) reported that a hot-water extract of wasabi leaves suppressed the differentiation of 3T3-L1 preadipocytes. Furthermore, Misawa et al. (2015) reported that polyphenols contained in wasabi leaves suppressed the differentiation of 3T3-L1 preadipocytes. Specifically, phenylpropanoids, such as 5-hydroxy ferulic acid methyl ester, sinapic acid methyl ester and ferulic acid methyl ester, and carotenoids, such as all-trans-lutein, suppressed triglyceride accumulation in differentiating 3T3-L1 preadipocytes. Yamasaki et al. (2013) reported that C57/BL mice fed a high-fat diet containing a hot-water extract of wasabi leaves experienced anti-obesity effects. Furthermore, Lee et al. (2010) reported that Sprague–Dawley rats fed a diet containing cholesterol and wasabi leaf powder showed anti-hypercholesterolemic effects.

### Materials and Methods

#### Preparation of wasabi leaf 50% ethanol extract

The extract from harvested wasabi leaves was obtained according to the method described by Okamoto et al. (2008). The powdered extract prepared using 50% ethanol was used as the wasabi leaf extract (WLE).

#### Animals

Male 4-week-old Wistar rats (n = 30; Nihon Clea, Tokyo, Japan) were used. Rats were housed individually in stainless-steel cages under controlled temperature (24°C ± 1°C), humidity (50%), and lights on from 08:30 am to 08:00 pm. After one week of acclimatization, rats were divided into three groups according to their body weights (n = 10 each). The standard group was fed an AIN-76 diet (Oriental Yeast, Tokyo, Japan), the high-fat group was fed a high-fat diet, and the WLE group was fed a high-fat diet containing 5% (w/w) WLE throughout the experimental period of 4 weeks (Table 1). The rats were allowed free access to water, and food intake and body weight were recorded three times per week. At the end of the experimental period, rats were fasted overnight, and then anesthetized using diethyl ether. Rats were then sacrificed by decapitation, and blood was collected from the jugular vein and left to stand for 30 min at room temperature. Serum was separated from blood by centrifugation at 3,000 rpm for 15 min at 4°C and stored at −80°C until analysis. The mesenteric, perinephric, epididymal, and inguinal WAT; interscapular BAT; liver, kidney, and pancreas; and gastrocnemius muscle were quickly removed and stored at −80°C.

All rats were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Table 1. Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient (g/100g diet)</th>
<th>standard</th>
<th>high fat</th>
<th>WLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>48.3</td>
<td>43.3</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>20.0</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>AIN-76</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Mineral mix (AIN-76)</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Vitamin mix (AIN-76)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>WLE</td>
<td>0.0</td>
<td>0.0</td>
<td>5.0</td>
</tr>
<tr>
<td>(Dietary fiber contained in WLE 5g)</td>
<td>-</td>
<td>-</td>
<td>(0.135)</td>
</tr>
</tbody>
</table>

Total 100.0 100.0 100.0

Abreviations: WLE, wasabi leaf extract

These reports suggest that wasabi leaves suppress obesity.

High-fat diets are widely used to induce obesity in animal experiments (Kamiya et al., 2012; Maeda et al., 2009; Nishi et al., 2007; Yamasaki et al., 2013). The high-fat diet experimental period in the present study induced a marked weight increase. For utilization of wasabi leaf, we studied the anti-obesity effects of a wasabi leaf ethanol extract in rats fed a high-fat diet.
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Serum biochemical analyses The concentrations of total cholesterol (TC), triglyceride (TG), phospholipid (PL), FFA, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the serum were measured using commercial kits (cholesterol E-test Wako, triglycerides E-test Wako, phospholipid C-test Wako, NEFA C-test Wako, creatinine test Wako, transaminase test Wako, and transaminase test Wako, respectively, Wako Pure Chemicals, Osaka, Japan).

Histological analysis After mesenteric WAT was obtained, the tissue was dipped into a 10% formalin neutral buffer solution. After embedding in paraffin the tissue was cut into 2–3 μm sections and stained using hematoxylin and eosin. The number of adipocytes in the unit visual field was counted.

Fecal lipid analysis During the final 3 days of the experimental period, feces were collected and freeze-dried. After the freeze-dried feces were weighed, they were incubated in 5 mL of a chloroform–methanol mixture (2:1 v/v) overnight at 4°C to extract lipids. The extracted lipids were dried by heating at 75°C and redissolved in 5 mL of methanol containing 5% KOH. The mixture was incubated for 45 min at 75°C to saponify the lipids, followed by the addition of 5 mL of 1N HCl containing 2% TritonX-100. The FFA concentration was measured using a commercial kit (NEFA C-test Wako, Wako Pure Chemicals).

Quantitative real-time polymerase chain reaction (PCR) Total RNA was extracted from the interscapular BAT using the RNeasy Mini Kit (Qiagen, Tokyo, Japan) and from the mesenteric WAT using Qiazol (Qiagen). Total RNA was reverse transcribed into cDNA using reverse transcriptase (SuperScript™III, Invitrogen Japan K.K., Tokyo, Japan). Real-time PCR reactions were conducted using the ABI Prism 7000 Sequence Detection System (Applied Biosystems Japan Ltd., Tokyo, Japan) with TaqMan® Universal PCR Master Mix (Applied Biosystems Japan). The primers and probes of β-actin (used as an internal control) were synthesized according to the method described by Oishi et al. (2003). The primers and probes of UCP1 and Adrb3 were TaqMan® Gene Expression Assays (Applied Biosystems Japan).

Statistical analyses The results are expressed as mean ± standard deviation. The results were tested by an ANOVA, followed by Fisher’s test to identify significant differences. All statistical analyses were performed using Stat View version 5.0 (Abacus Concepts, CA, USA). A level of p < 0.05 was considered statistically significant.

Results and Discussion In the present study, we investigated the anti-obesity effects of a wasabi leaf ethanol extract in rats fed a high-fat diet. The final body weight was significantly lower in the WLE group than in the high-fat group (Table 2). No significant differences were observed in total food intake among the three groups, and the decreased body weight of rats indicated that WLE suppressed diet-induced obesity (Table 2). Although no significant differences were observed in the weights of WATs and BAT between the WLE and high-fat groups (Table 2), the number of adipocytes in the mesenteric WAT in the unit visual field was significantly increased in the WLE group.
compared to the high-fat group (Fig. 1 and Table 3). Therefore, WLE may reduce the size of adipocytes. On the other hand, although the weights of mesenteric, perinephric, epididymal, and inguinal WATs were determined, other WATs (e.g., omental, retroperitoneal, and subcutaneous WATs) were not assessed. Therefore, the weights of other WATs might be lower. In addition, Yamasaki et al. (2013) reported that C57/BL mice fed high-fat diet containing a hot-water extract of wasabi leaves for 163 days showed anti-obesity effects, such as low epididymal WAT weight. The experimental period in this study was 4 weeks (28 days); thus, a longer experimental period may produce lower epididymal WAT weights.

No significant differences were observed in the weights of liver, kidney, pancreas, and gastrocnemius muscle between the WLE and high-fat groups (Table 2). There were also no differences in creatinine, AST, and ALT between the WLE and high-fat groups (Table 4). In this study, 5-week-old rats were fed a high-fat diet. Previous studies have reported on 5-week-old rats fed a high-fat diet (Bonilla et al., 2000; Kobayashi et al., 2014; Nomura et al., 2008) Therefore, the rats’ age is not anticipated as a source of error in our experiment. While the final body weight of the WLE group was significantly lower than that of the high-fat diet group, WLE is not thought be associated with side effects or growth inhibition.

The concentration of TC was significantly lower in the high-fat and WLE groups than the standard group (Table 4), although there were no differences in the concentrations of TG, PL, and FFA among the three groups. Margareto et al. (2001) reported that the concentrations of FFA and other lipid metabolism markers may be low during the accumulation of excess fatty acids (energy) when animals are fed a high-fat and -energy diet. Therefore, the lower TC concentration in the high-fat and WLE groups may be a result of the high-fat diet.

### Table 3. The number of adipocytes in mesenteric white adipose tissues

<table>
<thead>
<tr>
<th></th>
<th>standard</th>
<th>high fat</th>
<th>WLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of adipocytes (cells/visual field)</td>
<td>68.1 ± 8.8 a</td>
<td>57.5 ± 7.0 b</td>
<td>64.5 ± 9.2 c</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n = 10).
Different symbols express p < 0.05 as compared with the other groups.
Abbreviations: WLE, wasabi leaf extract

### Table 4. Serum variables measured

<table>
<thead>
<tr>
<th></th>
<th>standard</th>
<th>high-fat</th>
<th>WLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (TC) (mg/dL)</td>
<td>88.2 ± 36.4 a</td>
<td>60.8 ± 22.3 b</td>
<td>59.7 ± 14.7 b</td>
</tr>
<tr>
<td>Triglycerides (TG) (mg/dL)</td>
<td>112.0 ± 61.1</td>
<td>78.6 ± 25.5</td>
<td>69.7 ± 27.6</td>
</tr>
<tr>
<td>Phospholipid (PL) (mg/dL)</td>
<td>139.7 ± 62.0</td>
<td>95.1 ± 38.4</td>
<td>103.2 ± 25.9</td>
</tr>
<tr>
<td>Free fatty acid (FFA) (mg/dL)</td>
<td>1.37 ± 0.48</td>
<td>1.27 ± 0.51</td>
<td>1.09 ± 0.56</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.99 ± 0.42</td>
<td>1.22 ± 0.55</td>
<td>1.07 ± 0.69</td>
</tr>
<tr>
<td>Asparate aminotransferase (AST) (IU/L)</td>
<td>203.5 ± 47.7</td>
<td>159.0 ± 80.3</td>
<td>229.9 ± 55.0</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT) (IU/L)</td>
<td>27.2 ± 2.88</td>
<td>16.3 ± 9.63</td>
<td>21.2 ± 6.26</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n = 10).
Different symbols express p < 0.05 as compared with the other groups.
Abbreviations: WLE, wasabi leaf extract

![Fig. 1. Histological analysis of the mesenteric white adipose tissue (WAT) stained with hematoxylin and eosin](image-url)
Wasabi Leaf Extract Suppresses Obesity

No significant difference was observed in Adrb3 mRNA levels in the epididymal WAT among the three groups (Table 5). There were no differences in UCP-1 mRNA levels in the interscapular BAT between the high-fat and WLE groups (Table 5). Adrb3 mRNA levels in the interscapular BAT were significantly increased in the WLE group compared to the high-fat group (Table 5). Dietary WLE upregulated Adrb3 mRNA expression in BAT. Therefore, Adrb3 protein expression might be upregulated, activating PKA. Although the activated PKA upregulates the mRNA expression of UCP1, UCP1 mRNA expression was not upregulated in the WLE group. On the other hand, PKA also activates HSL, which promotes lipolysis from stored energy in adipocytes, and then FFA generated by lipolysis are consumed through ATP synthesis by oxidative phosphorylation and/or UCP1. Therefore, the lipid consumption absorbed from the diet may be affected by the increased HSL activation in the WLE group. These data suggest that the upregulation of Adrb3 mRNA expression is related to the WLE-associated decrease in rat body weight.

Fecal lipid content was significantly lower in the WLE group than in the high-fat group (Table 6). WLE may promote lipid absorption from dietary fat. Orlistat, which is a powerful lipase inhibitory drug, exerts its effects by reducing lipid absorption from dietary fat. Although orlistat reduces body weight, side effects such as flatulence with discharge, increased defecation, and fecal incontinence with increasing fecal lipid excretion are known (Hauptman et al., 2000; Hvizdos and Markham, 1999). WLE significantly decreased the body weight of rats despite the promotion of lipid absorption. Therefore, the absorbed lipids may be consumed as energy. The promotory effect of lipid absorption may be induced by an increase in energy expenditure following WLE treatment as a biological adaptation. WLE may function as an anti-obesity food without increasing fecal lipid excretion.

The fecal dry weight was significantly greater in the WLE group than in the high-fat group (Table 6). Because the dietary fiber contained in WLE is low, it is not thought to considerably increase the fecal dry weight (Table 1). Future research on the promotion of fecal excretion is anticipated.

Isothiocyanates are known ligands of TRPA1 (Jordt et al., 2004; Terada et al., 2015; Uchida et al., 2012). TRPA1 activation results in sympathetic nerve stimulation. Sympathetic nerve stimulation induced by exercise or cold activates Adrb3 in adipocytes. Although Etoh et al. (1990) and Kumagai et al. (1994) reported that the amount of isothiocyanates contained in wasabi leaves is lower than that found in wasabi rhizomes, a high-fat diet containing a powdered wasabi rhizome 50% ethanol extract did not have anti-obesity effects in rats (data not shown). Therefore, we propose that WLE does not contain a sufficient level of isothiocyanates to activate TRPA1.

Yamasaki et al. (2013) reported that C57/BL mice fed high-fat diet with a wasabi leaf hot-water extract (without specific pungent components such as allyl isothiocyanate) showed anti-obesity effects. Furthermore, polyphenols are known for their anti-obesity effects (Kamiya et al., 2012; Nishi et al., 2007). Misawa et al. (2015) reported that polyphenols contained in wasabi leaves

<table>
<thead>
<tr>
<th>Table 5. The mRNA expression in the epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>standard</strong></td>
</tr>
<tr>
<td>Epididymal WAT</td>
</tr>
<tr>
<td>Adrb3</td>
</tr>
<tr>
<td>Interscapular BAT</td>
</tr>
<tr>
<td>UCP1</td>
</tr>
<tr>
<td>Adrb3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n = 5). Different symbols express p < 0.05 as compared with the other groups. Abreviations: WLE, wasabi leaf extract; Adrb3, β3-adrenergic receptor; UCP1, uncoupling protein 1

<table>
<thead>
<tr>
<th>Table 6. Fecal excretion in 3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>standard</strong></td>
</tr>
<tr>
<td>Dried fecal weight (g/24h)</td>
</tr>
<tr>
<td>Fecal lipids (mg/24h)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (n = 10). Different symbols express p < 0.05 as compared with the other groups. Abreviations: WLE, wasabi leaf extract
suppress the differentiation of 3T3-L1 preadipocytes. In particular, phenylpropanoids, such as 5-hydroxy ferulic acid methyl ester, sinapic acid methyl ester and ferulic acid methyl ester, and carotenoids, such as all-trans-lutein, suppressed triglyceride accumulation during the differentiation of 3T3-L1 preadipocytes. Therefore, these polyphenols may be candidates as key compounds with anti-obesity effects.

We report that WLE suppressed obesity in rats fed a high-fat diet, and that this effect was related to the upregulation of Adrb3 mRNA expression in the interscapular BAT without increased fecal lipid excretion. These results indicate that wasabi leaves are a potential functional food material for obesity suppression. Future research on wasabi leaves is required.

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References


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Vitaminol, 55, 195-200.


