Specified Kiwifruit Extract Blocks Increase of Body Weight and Visceral Fat in High-fat-diet-fed Mice by Inhibiting Intestinal Lipase

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Oligomeric polyphenol-enriched specified kiwifruit extract (SKE) is reported to inhibit pancreatic lipase activity in vitro. Therefore, in this work, we investigated the effects of SKE on body weight, visceral fat, and lipid metabolism in mice fed a high-fat diet for 6 weeks. Body weight gain was significantly and dose-dependently suppressed in the groups fed a high-fat diet containing SKE (HF + SKE groups) compared to the high-fat diet control group (HF group). Moreover, Dose-dependent increases in triglyceride (TG) content in feces were observed in the HF + SKE groups. Since we confirmed that SKE inhibits lipase in vitro, these findings indicate that SKE suppresses the hydrolysis of TG by inhibiting lipase activity in the intestinal tract, thereby restricting the absorption of lipids, increasing the excretion of TG in feces, and suppressing the increase in body weight and visceral fat caused by the high-fat diet.

Keywords: oligomeric polyphenol, lipase inhibitory activity, lipid, absorption, triglyceride

Introduction

Fat is a primary macronutrient that provides the energy needed to maintain physical functions and carry out daily activities. Nevertheless, the World Health Organization (WHO) suggests reducing the amount of total fat intake to less than 30 % of total energy intake in the adult population, since excessive intake of fats can cause a variety of health problems (WHO, 2015). Ingested dietary fat, which consists mainly of triglyceride (TG), is emulsified by bile acid in the upper part of the small intestine, and hydrolyzed into free fatty acids and 2-acylmonoglyceride by pancreatic lipase. The resulting micelles are absorbed in the intestinal tract and resynthesized to TG, which is incorporated into lipoproteins, which are subsequently distributed throughout the whole body via the lymphatics, and stored in liver and fat tissues. Therefore, it is considered that the absorption of excess dietary fats can be suppressed by regulating pancreatic lipase activity (Ioannides-Demos et al., 2005, Okuda and Han, 2001).

Polyphenols are promising candidates to suppress fat absorption by regulating pancreatic lipase activity (Buchholz and Melzig, 2015; Lunagariya et al., 2014). Polyphenols are widely found in fruits and vegetables, and more than 500 individual types have been identified (Pérez-Jiménez et al., 2010). They exhibit various biological activities, including antioxidant, anticancer, fat absorption-inhibitory and anti-diabetes activities (Scalbert et al., 2005; Manach et al., 2004; Manach et al., 2005; Williamson and Manach, 2005). For this study, we are interested in kiwifruit, which contains many kinds of nutrient components, including folic acid, carotenoids (β-carotene, lutein and zeaxanthin), potassium, dietary fiber, vitamins and several kind of polyphenols (Ward and Courtney, 2013; Ferguson, 2013; Drummond, 2013; Nishiyama, 2007).

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Previous studies have reported that kiwifruit shows antioxidant action, alters the serum lipid profile, and protects against infection and anemia (Stonehouse et al., 2013; Brevik et al., 2011). Kiwifruit contains only 0.1% polyphenols, but an oligomeric polyphenol-enriched specified kiwifruit extract (SKE) was reported to exhibit pancreatic lipase inhibitory activity in vitro (Eidenberger et al., 2014). However, it has not been established whether SKE also inhibits fat absorption in vivo. In this study, therefore, we investigated the effects of SKE on body weight, visceral fat, and lipid metabolism in C57BL/6J mice fed a high-fat diet for 6 weeks.

Materials and Methods

Materials
Specified kiwifruit extract (SKE) (Developed product code: Act, Omnica, Lot. 20160701) was used. 380 kg of kiwifruits (Actinidia chinensis) was extracted with aqueous ethanol solution (v/v=30/70), followed by purification with ion-exchange resin to give approximately 1 kg of SKE, which contains mainly proanthocyanidins. SKE is oligomeric polyphenol-enriched and specified by the contained amount of total polyphenol within 40 ± 5% as a catechin by Folin-Ciocalteu method.

Lipase-inhibitory activity
SKE (2 g) was suspended in 50 mL of a 60% aceton aqueous solution. The suspension was stirred at room temperature for 12 h, and then centrifuged at 2800 g for 10 min. The supernatant was concentrated under reduced pressure at 45°C. The residue was diluted with 50% aqueous methanol solution (50 mL). The suspension was ultrasonicated for 5 min and centrifuged at 2800 g for 5 min. The supernatant was filtered through a 0.22 μm membrane filter (Millipore Corporation, Bedford, USA). The prepared solution was stored in a refrigerator, and was used at a concentration of 2 g/50 mL as SKE.

Lipase-inhibitory activity was measured using a Lipase Kit (DS Pharma Biomedical Co., Ltd., Osaka, Japan), with dimercaptopropanol tributyrate (BALB) as the substrate. The lipase solution contained 25 mU porcine pancreatic lipase type II (Sigma-Aldrich, St Louis, MO, USA) in citrate phosphate buffer (pH 7.4). The reaction mixture, which consisted of 25 μL lipase, 370 μL of chromogenic reagent, and 50 μL sample solution, was preincubated at 30°C for 5 min. SKE was diluted to give sample solutions containing 5, 10, 15, 30 and 40 mg/mL; 50% aqueous methanol was used as a control and the lipase inhibitor orlistat (0.02 μg/mL) was used as a positive control. After preincubation, 50 μL substrate was added to the reaction mixtures, which were then incubated at 30°C for 30 min in the dark. The enzyme reaction was terminated by the addition of 1 mL stop reagent, and the absorbance at 412 nm was measured with a spectrophotometer, Labsystems Multiskan JX (Thermo Fisher Scientific K.K., Kanagawa, Japan). A blank solution was prepared similarly, except that the stop reagent was added before the substrate.

The lipase-inhibitory activity (%) was calculated using the following formula: 1 - (sample absorbance) / (control absorbance) × 100

Animals and diets
All animal experiments were approved by the Takasaki University of Health and Welfare Animal Experiment Center Ethics Committee and were conducted in accordance with guidelines for the management and use of laboratory animals. Male 7-week-old C57BL/6J mice (Japan SLC, Inc., Shizuoka, Japan) were housed individually on a 12 h cycle (light period 08:00 to 20:00 hours; dark period 20:00 to 08:00 hours) at a room temperature of 23°C and humidity of 60%. Food and water were provided ad libitum. After preliminary feeding AIN-93G (Clea Japan, Inc., Tokyo, Japan) for 1 week, mice were divided into 4 groups in such a manner that the mean body weights of the groups were not significantly

Table 1. Composition of the experimental diets

<table>
<thead>
<tr>
<th>Component (g)</th>
<th>STD</th>
<th>HF</th>
<th>HF+1%SKE</th>
<th>HF+3%SKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>β-cornstarch</td>
<td>39.75</td>
<td>22.75</td>
<td>21.75</td>
<td>19.75</td>
</tr>
<tr>
<td>α-cornstarch</td>
<td>13.2</td>
<td>13.2</td>
<td>13.2</td>
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<tr>
<td>Sucrose</td>
<td>10.25</td>
<td>10.25</td>
<td>10.25</td>
<td>10.25</td>
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<tr>
<td>Soy bean oil</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Lard</td>
<td>0</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>SKE</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

| Calories (kcal) | 395.8 | 480.8 | 480.5 | 479.9 |
| Protein (% kcal) | 20 | 17 | 17 | 17 |
| Carbohydrate (% kcal) | 64 | 38 | 38 | 38 |
| Fat (% kcal)    | 16 | 45 | 45 | 45 |
| Total (% kcal)  | 100 | 100|100 |100 |
Kiwifruit Extract Blocks Increase of Body Weight

Table 1 shows the feed composition and feed caloric ratio of each group. The AIN-93G feed was crushed and used for the standard diet group (STD; n = 8). The high-fat diet group (HF; n = 8) received AIN-93G supplemented with lard so that the lipid calories amounted to 45% of the total calories. For the SKE feeding groups, the same high-fat diet was supplemented with 1% or 3% SKE (HF + 1% SKE; n = 8, HF + 3% SKE; n = 8). The amounts of SKE were established by preliminarily study. The amounts referred to the concentration corresponding to approximately 20 times the IC\textsubscript{50} of pancreatic lipase inhibition obtained in vitro. Mice in each group received the appropriate diet for 6 weeks. Body weight was measured once a week and food intake was measured every 2 to 3 days. Feces were collected during the last 7 days and weighed.

Organs and blood biochemistry At 6 weeks after the start of feeding, blood was collected in a heparinized syringe from the postcaval vein under inhalation anesthesia with isoflurane. The blood was centrifuged (1200 g, r.t, 10 min) and biochemical parameters of the obtained plasma were measured at an external laboratory (Oriental Yeast Co., Ltd., Tokyo, Japan). Liver, kidney and adipose tissue from three sites (retroperitoneum including perirenal, mesentery and epididymal regions) were collected after lethal exsanguination, and weighed.

Quantitation of hepatic and fecal lipid The experiments were performed in accordance with existing reports (Kazmi et al., 2013; Kobayashi et al., 2014) as follows; Briefly, the liver (wet weight 100 mg) was homogenized in 2 mL of chloroform/methanol mixed solution (2/1 (v/v)), and 100 μL of the supernatant was evaporated to dryness. Isopropanol containing 10% TritonX-100 (100 μL) was added to the residue, and TG and total cholesterol (TC) were measured using triglyceride E-test kit and cholesterol E-test kit, respectively (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Chloroform/methanol mixed solution (2/1 (v/v), 5 mL) was added to the lyophilized and pulverized feces (300 mg), and lipid extraction was performed in the same manner as described for the liver.

Statistical analysis All data are presented as the mean ± standard deviation (SD). Student's t test was used to compare the STD and the HF groups. Food intake was analyzed using an analysis of covariance (ANCOVA) with body weight as a covariate. Dose-response relationships between the HF and the HF + SKE groups in lipase activity, body weight, visceral fat weight, TG in plasma, TG in the liver, TC in the liver, TG in feces and fecal weight were analyzed using Williams' multiple comparison test. Liver and kidney weight, and plasma parameters except TG, were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was defined as \( P < 0.05 \).

Results Lipase inhibition First, we confirmed the in vitro lipase-inhibitory activity of SKE (Fig. 1). SKE inhibited the lipase activity in a concentration-dependent manner, and the IC\textsubscript{50} value was 22.2 mg/mL. SKE at concentrations above 30 mg/mL showed more potent lipase-inhibitory activity than orlistat at a concentration of 0.02 μg/mL.

Body weight and food intake The HF group showed a significant weight gain compared with the STD group after 3 weeks. In the HF + SKE groups, significant suppression of the body weight gain was observed after 3 weeks, and the body weights during 3 to 6 weeks were almost the same as those in the STD group (Fig. 2). Feeding amount with body weight as a covariate during the last 7 days was decreased in the HF group. In the HF + SKE groups, no significant difference was observed compared with the HF group (Fig. 3).

Organ weight The fat weights of all the measured sites normalized by body weight on the final day were significantly increased in the HF group compared with the STD group. In the HF + SKE groups, this increase was significantly
suppressed in a dose-dependent manner (Fig. 4). Liver weight normalized by body weight was significantly decreased in the HF group compared with the STD group, but no significant change was observed in the HF + SKE groups compared to the HF group. A similar tendency was observed for kidney weight normalized by body weight, although the difference between the HF group and the STD group was not significant (Fig. 5).

**Blood biochemistry** Blood biochemical parameters are shown in Table 2. Plasma TG concentration was significantly increased in the HF group compared to the STD group, whereas it was significantly decreased in the HF + SKE groups compared to the HF group. There was no significant change in plasma aspartate transaminase (AST), alanine aminotransferase (ALT), TC, non-esterified fatty acids (NEFA), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glucose or insulin concentration in the HF + SKE groups compared to the HF group.

**Lipid content in the liver** In the HF + 1% SKE and HF + 3% SKE groups, the TG concentration in the liver decreased concentration-dependently compared with the HF group (Fig. 6A). There was no difference in TC content in the liver (Fig. 6B).

**Fecal weight and lipid content in feces** Fecal dry weight during the last 7 days shown in Figure 7A. The fecal weight in the HF + 3% SKE group was 1.35 times greater than that in the HF group. A dose-dependent increase TG content in feces

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**Fig. 2. Effect of SKE on body weight**
Time courses of body weight during the adaptation and treatment periods. The data are mean ± SD (n = 8 in each group). *p < 0.05, **p < 0.01, compared with HF. #p < 0.05, ##p < 0.01, compared with STD.

**Fig. 3. Effect of SKE on food intake**
Food intake during the 6-week treatment period is shown. The data are mean ± SD (n = 8 in each group). ##p < 0.01, compared with STD.
Fig. 4. Effect of SKE on visceral fat weight
(A) Retroperitoneal fat weight. (B) Mesenteric fat weight. (C) Epididymal fat weight. (D) Visceral fat weight. Visceral fat was excised from each site after 6 weeks treatment and weighed. The data are mean ± SD (n = 8 in each group). *p < 0.05, **p < 0.01, compared with HF. #p < 0.05, ##p < 0.01, compared with STD.

Fig. 5. Effect of SKE on liver and kidney weights
(A) Liver weight. (B) Kidney weight. Organs were excised from each site after 6 weeks treatment and weighed. The data are mean ± SD (n = 8 in each group). ##p < 0.01, compared with STD.
Table 2. Effects of SKE on biochemical parameters in plasma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>STD</th>
<th>HF</th>
<th>HF+1%SKE</th>
<th>HF+3%SKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dL)</td>
<td>40.6 ± 13.2</td>
<td>68.0 ± 18.7</td>
<td>36.9 ± 16.4</td>
<td>50.6 ± 19.6</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>59.8 ± 26.5</td>
<td>46.4 ± 18.2</td>
<td>41.1 ± 5.9</td>
<td>48.8 ± 31.4</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>22.1 ± 11.0</td>
<td>12.3 ± 4.7</td>
<td>16.1 ± 3.2</td>
<td>15.8 ± 4.5</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>119.8 ± 17.3</td>
<td>141.5 ± 13.4</td>
<td>151.0 ± 7.9</td>
<td>131.5 ± 16.2</td>
</tr>
<tr>
<td>NEFA (μEq/L)</td>
<td>830.6 ± 151.2</td>
<td>774.6 ± 80.5</td>
<td>787.4 ± 138.8</td>
<td>801.8 ± 112.1</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>4.4 ± 1.8</td>
<td>4.6 ± 0.9</td>
<td>4.8 ± 1.0</td>
<td>5.5 ± 0.9</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>70.3 ± 7.6</td>
<td>79.6 ± 3.7</td>
<td>86.4 ± 3.8</td>
<td>73.4 ± 9.7</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>307.4 ± 16.1</td>
<td>319.8 ± 25.4</td>
<td>312.5 ± 33.1</td>
<td>301.5 ± 31.3</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.4 ± 0.1</td>
<td>0.9 ± 0.5</td>
<td>0.5 ± 0.2</td>
<td>0.9 ± 0.4</td>
</tr>
</tbody>
</table>

Fig. 6. Effects of SKE on lipids in liver
(A) Amount of TG in liver. (B) Amount of TC in liver. The data are mean ± SD (n = 7 or 8 in each group). *p < 0.05, compared with HF.

Fig. 7. Effect of SKE on fecal amount during the last 7 days of treatment
(A) Amount of feces during the last 7 days. (B) Amount of TG in feces during the last 7 days. The data are mean ± SD (n = 7 or 8 in each group). **p < 0.01, compared with HF.
was observed in the HF + SKE groups (Fig. 7B). TG content in feces was increased significantly in the HF + 3% SKE group compared to the HF group.

Discussion

In this study, we found that SKE dose-dependently suppressed the increases in body weight and visceral fat induced by a high-fat diet in mice, without affecting feed intake. In addition, SKE suppressed the HF-diet-induced increase of TG concentration in both plasma and liver. On the other hand, the groups that received SKE showed an increase of TG content in feces. Since we confirmed that SKE inhibits lipase in vitro, these findings might indicate that SKE slows the absorption of lipids, thereby excreting a part of TG in feces, and suppressing the increase in body weight caused by the high-fat diet.

We also considered whether SKE might affect cholesterol or carbohydrate metabolism. However, there was no significant difference of TC in plasma or liver in the HF + SKE groups compared to the HF group. HDL-C levels were within the physiological range in all groups, and SKE had no effect on LDL-C. Furthermore, blood glucose and insulin concentrations were unchanged in all groups. These results indicate that SKE had little or no effect on cholesterol or carbohydrate metabolism. Similarly, apple polyphenol extracts have shown inhibition of pancreatic lipase and TG absorption in mice (Sugiyama et al., 2007). Moreover, the apple polyphenols have been reported to inhibit adipocyte differentiation (Shoji et al., 2000). However, there was also a report that kiwifruit extracts did not inhibit adipocyte differentiation (Abe et al., 2010).

Since there are conflicting reports on the adipocyte differentiation inhibitory action in polyphenols, a consensus has not been determined. Further studies are needed to determine the effect of these polyphenol extracts.

The liver weight was decreased in the HF group, compared to the STD group, due to the increased body weight, but the liver weight and kidney weight (per body weight) in the SKE-treated groups showed no significant difference from the values in the standard diet control group. Moreover, there was no significant change in blood AST and ALT concentrations in the HF + SKE groups compared to the HF group. These findings suggest that SKE did not impair liver or kidney function.

In conclusion, our findings indicate that SKE suppresses the absorption of TG by inhibiting lipase activity in the intestinal tract, causing inhibition of body weight increase. This action is likely due to the oligomeric polyphenols contained in SKE, but further studies will be needed to identify the active components, and also to confirm the efficacy and safety of SKE during longer-term administration. Even when the concentration of the functional components may be equivalent, the properties may differ depending on the raw materials, extraction method and volume. Therefore, it is necessary for foods with such functions to be supplied under certain standards. Nevertheless, our findings suggest that SKE is a promising candidate for controlling body weight in humans.

Conflict of interest

Satomi Iwata and Junko Hirota are employees of Omnica Co., Ltd. The other authors have no potential conflict of interest.

References


