Effects of Yuzu (Citrus junos Siebold ex Tanaka) peel on the diet-induced obesity in a zebrafish model

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ABSTRACT

Effects of yuzu peel (Citrus junos Siebold ex Tanaka), yuzu pomace after hexane extraction, and auraptene on metabolic disorders in zebrafish with diet-induced obesity (DIO) were evaluated. All materials tested exhibited anti-obesity effects. Yuzu peel significantly suppressed the rise in plasma triacylglycerol (TG) and liver lipid accumulation. The hepatic mRNA expression of ppars (peroxisome proliferator-activated receptor, alpha b) and its target genes were significantly upregulated by yuzu peel, which suggests enhanced fatty acid β-oxidation in liver. In visceral adipose tissue, yuzu peel significantly increased the mRNA expression of pparγ (peroxisome proliferator-activated receptor, gamma) and adipocytokine β2 (adiponectin, C1Q and collagen domain containing, b), which play roles in adipose differentiation and maintenance. Our findings suggest that yuzu peel exerts anti-obesity effects by activating hepatic PPARα and adipocyte PPARγ pathways. Additionally, the anti-obesity effects of yuzu pomace suggest a novel application to achieve complete use of yuzu instead of disposal as industrial waste.

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PPARs
Yuzu peel
Yuzu pomace
Zebrafish

1. Introduction

Obesity is one of the most challenging public health problems in developed countries and it is of growing concern in developing countries. The prevalence of obesity has increased so rapidly (worldwide obesity has nearly doubled since 1980) that it is now considered a global epidemic (WHO, 2013). Obesity increases the likelihood of various adverse health consequences, particularly cardiovascular diseases, type 2 diabetes...
mellitus, dyslipidaemia, nonalcoholic fatty liver, and certain types of cancer (Haslam & James, 2005). Dieting and physical exercise are the mainstays of treatment for obesity. If diet and exercise are not effective, anti-obesity drugs may be taken, in the context of a suitable diet, to reduce appetite or inhibit fat absorption (Aronne, Powell, & Apovian, 2011; Christensen, Kristensen, Bartels, Blikdãl, & Astrup, 2007). However, most of these drugs are associated with side effects such as high blood pressure, restlessness, insomnia, and drug addiction (Bessesen, 2008). For this reason, a variety of natural products have been studied for their potential to treat obesity with minimal side effects (Hirai et al., 2010; Yun, 2010).

Yuzu originated in China, but also grows wild in Japan and Korea. The fruit looks like a very small grapefruit with an uneven skin, but is rarely eaten as a fruit because of its tart flavour. Yuzu produces what has been described as a pleasant citrus fragrance with a floral overtone, and is widely used in Japanese and Korean cuisines. Examples include Yuzu-ponzu (a dressing made from soy sauce and yuzu juice), Yuzu-koshó (a seasoning made from yuzu peel, chili peppers and salt) and Yüja-cha (a traditional Korean herbal tea made from sliced yuzu peel and combined with honey or sugar prepared as fruit pre-dressing made from soy sauce and yuzu juice), Yuzu pomace after hexane extraction, and auraptene (one of the bioactive compounds present in yuzu peel) on body weight, plasma TG, fat storage in the liver and adipose tissue, and expression of lipid metabolism-related genes in DIO zebrafish.

2. Materials and methods

2.1. Animals and maintenance

Zebrafish species (AB strain; the Zebrafish International Research Centre, Eugene, OR, USA) were maintained under standard laboratory conditions at 28 °C with a light:dark cycle of 14:10 h (Westerfield, 2007). Fish were fed twice daily with commercial dry food (Hikari Tropical Fancy Guppy; Kyorin, Hyogo, Japan) and once daily with live Artemia nauplii (Kitamura, Kyoto, Japan). The zebrafish used in this study were 3-month-old females bred in our facility.

2.2. Preparation of yuzu peel, yuzu pomace, and auraptene-containing gluten granules

Yuzu peel and pomace were provided by Tsuji Oil Mill Co., Ltd (Matusaka, Mie, Japan). The fruit pulp of yuzu was removed and the peel was sliced and stored at −20 °C for ≤ 2 months. Hexane extraction was performed on the yuzu peel to extract the essential yuzu oil, and the remaining pomace was heated to 100 °C for 20 min to remove the residual hexane (1–2,000 ppm of hexane remained at this stage. Details for this analytical method are provided in Supplementary File S1). Samples were then stored at −20 °C until use. Before treatment of zebrafish, yuzu peel (containing essential oil) and pomace (not containing essential oil) were lyophilized and ground into granules using a mortar and a 700 μm mesh sieve (AS ONE Corporation, Osaka, Japan). After lyophilization, a trace amount of hexane (9.1 ppm) remained in pomace.

For oral administration of auraptene to adult zebrafish, we used gluten as a carrier material. Auraptene (LKT Laboratories, St. Paul, MN, USA) was suspended in 50% ethanol and mixed with gluten powder (Wako Pure Chemicals, Osaka, Japan). The wet dough was kneaded, freeze-dried, and ground to granules using a mortar and a 700 μm mesh sieve (AS ONE Corporation, Osaka, Japan). The major component of the essential oil extracted by

2.3. Quantification of bioactive compounds in yuzu peel and yuzu pomace

Levels of the primary bioactive compounds in yuzu peel and pomace were quantified by Tsuji Oil Mill Co., Ltd (measurement of auraptene) and Japan Food Research Laboratories (Nagoya, Aichi, Japan) (Table 1). Auraptene, hesperidin and naringin were measured using high performance liquid chromatography (HPLC) (Ogawa et al., 2000). Limonene was measured using gas chromatography-mass spectrometry (GC-MS) (Lesjak et al., 2014). Eriocitrin was measured using liquid chromatography-mass spectrometry (LC-MS/MS). Dietary fibre was measured using an enzymatic-gravimetric method (AOAC Official Methods of Analysis, 2000; Ajila & Rao, 2013). Details for these analysis methods are provided in Supplementary File S2. The major component of the essential oil extracted by
Table 1 – Contents of the primary bioactive compounds in yuzu peel and pomace.

<table>
<thead>
<tr>
<th>Compound (units)</th>
<th>Yuzu peel</th>
<th>Yuzu pomace</th>
<th>Referencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auranetin (mg/g)b</td>
<td>0.3 ± 0.02</td>
<td>0.1 ± 0.02</td>
<td>0.375</td>
</tr>
<tr>
<td>Limonene (mg/100 g)c</td>
<td>150.77 ± 5.21 (83%)d</td>
<td>9.99 ± 0.46</td>
<td>73.16%e</td>
</tr>
<tr>
<td>Hesperidin (mg/100 g)d</td>
<td>92.57 ± 0.15</td>
<td>127 ± 3.61</td>
<td>74.47–96.24</td>
</tr>
<tr>
<td>Naringin (mg/100 g)b</td>
<td>52.23 ± 0.21</td>
<td>68.23 ± 0.6</td>
<td>81.51–98.1</td>
</tr>
<tr>
<td>Eriocitrin (mg/100 g)b</td>
<td>ND</td>
<td>1.01 ± 0.07</td>
<td>ND</td>
</tr>
<tr>
<td>Dietary fibre (g/100 g)b</td>
<td>6.4 ± 0.02</td>
<td>8.35 ± 0.08</td>
<td>4.4 ± 0.3</td>
</tr>
</tbody>
</table>

ND, <0.5 mg/100 g.

a Data are the mean ± SD (n = 3) on a dry weight basis.

b Data are the mean ± SD (n = 3) on a fresh weight basis.

c Contents in yuzu peel.

Data are the mean ± SD (n = 3) on a dry weight basis.

d Proportion of limonene in hexane-extracted yuzu peel oil.

e Proportion of limonene in yuzu peel oil extracted using a cold-pressing method.

hexane was limonene (83%, as measured by Tsuji Oil Mill Co., Ltd), with the remainder being aromatic compounds (data not shown).

2.4. Feeding zebrafish

The experimental protocol for induction of obesity and administration of yuzu peel, pomace, and auraptene is shown in Supplementary Fig. S1. During the first 4 weeks, 3-month-old female zebrafish were randomly divided with 15 fish per 2 L tank for dietary restriction, which was performed by feeding once daily (approximately 4 mg/fish/day) with Hikari Tropical Fancy Guppy diet. After dietary restriction, zebrafish were assigned to one of six treatment groups (non-DIO, DIO, DIO + yuzu peel, DIO + yuzu pomace, DIO + LA, DIO + HA) with five fish per 2 L tank. The details for feeding the zebrafish are shown in Supplementary Table S1. Yuzu peel, pomace granules, auraptene-containing granules were fed to zebrafish 20 min before Artemia feeding. During feeding, the tank water flow was stopped for 2 h. Leftover food was removed once daily by vacuuming to avoid water pollution.

2.5. Measurement of body weight and plasma TG

The body weight of the zebrafish was measured weekly during the overfeeding treatment. Fish were fasted overnight and anesthetized by placing them in a tank containing 500 ppm of 2-phenoxyethanol (2-PE; Wako Pure Chemicals). Body weight (g) was measured after the body surface was dried with soft tissue paper (Kimwipe; Nippon Paper Crecia, Tokyo, Japan).

At the end of the experiment, blood samples were collected from individual zebrafish as described previously (Zang, Shimada, Nishimura, Tanaka, & Nishimura, 2013). In brief, glass microcapillary needles were prepared by pulling a glass capillary (GD-1; outer diameter, 1.0 mm; Narishige, Tokyo, Japan) with a needle puller (PC-10; Narishige). The tips of needles were cut obliquely and heparinized using an aspirator tube assembly (Drummond, New Bethlehem, PA, USA). Before blood collection, the body surface of anesthetized zebrafish was dried with soft tissue paper. The heparinized needle was inserted at 30–45° into the blood-collection site (along the body axis and posterior to the anus in the region of the dorsal aorta). Once the needle was felt to touch the spine, suction was applied to the mouthpiece-end of the aspiration tube to collect the blood.

Once the appropriate amount of blood was collected, suction was stopped. The needle was removed and the blood sample expelled from the needle onto a clean area of a piece of parafilm. Two microlitres of blood was obtained using a pipette set and then diluted with 6 μL of saline for determination of plasma TG concentration. The blood samples were centrifuged for 3 min at 680 g at room temperature and the plasma was harvested. TG was measured using a Wako L-type TG kit (Wako Pure Chemicals) according to the manufacturer’s protocol.

2.6. Feeding volume assay

Feeding volume of Artemia was measured weekly during overfeeding treatment as previously described (Hasumura et al., 2012; Tainaka et al., 2011). Briefly, freshly hatched Artemia were uniformly suspended in the water, and then fed to zebrafish in a 2 L fish tank. For a blank control, Artemia were placed in a 2 L tank without zebrafish (i.e., containing only system water). After 2 h, the number of Artemia not eaten by the zebrafish was counted three times and subtracted from the number in the blank tank to determine the feeding volume in each tank.

2.7. CT measurement of visceral adipose tissue volume

After blood collection, zebrafish were euthanized by immersion in an ice–water bath (5 parts ice/1 part water at ≤ 4 °C) for ≥ 20 min (Matthews & Varga, 2012) and a 3D micro-CT scan was performed using an in vivo System R_mCT 3D micro-CT scanner (Rigaku, Tokyo, Japan) as described previously (Hasumura et al., 2012). The 3D images were reconstructed and viewed using i-View type R software (J. Morita Mfg, Kyoto, Japan), and visualized and analysed using CTAtlas Metabolic Analysis Ver. 2.03 software (Rigaku). Measurement of visceral adipose tissue volume was limited to the abdominal cavity, and the initial point of the abdominal cavity was set from the cleithrum to the anus.

2.8. Oil red O staining

Scissors and forceps were used to strip off one side of the abdominal wall, which was then fixed in 4% formaldehyde solution in PBS (Histo-Fresh; FALMA, Tokyo, Japan) at 4 °C for 24 h. The fixed liver tissues were isolated and placed in 30%
Table 2 – Primer pair sequences, accession numbers and product sizes of the studied genes.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Accession number</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fasn</td>
<td>XM_005169478</td>
<td>AATGTTCCGATGTAGGCC</td>
<td>AGCATATCTCGGCTG ACGTT</td>
<td>250</td>
</tr>
<tr>
<td>acacb</td>
<td>XM_678989</td>
<td>GTGGCTGAGAAAACACCTGT</td>
<td>CCAATGGAATGAGCTGC TCC</td>
<td>152</td>
</tr>
<tr>
<td>acadm</td>
<td>NM_213010</td>
<td>TGGAGAACTGCTGTTTATAA</td>
<td>AGAGAGCTGCTGCTGCTT</td>
<td>170</td>
</tr>
<tr>
<td>acox1</td>
<td>NM_001005933</td>
<td>ACACGACAGCAAGG T TACG</td>
<td>TGAAGGCGCATAAACGAGACG</td>
<td>177</td>
</tr>
<tr>
<td>pparab</td>
<td>NM_001102567</td>
<td>CGTCGATCGTTTTAAG</td>
<td>AGGCCCTCTCGG AATCGACA</td>
<td>250</td>
</tr>
<tr>
<td>sox3b</td>
<td>NM_213010</td>
<td>CTGCCAGATGCACCTTCTT</td>
<td>GCCTGAGGCGCATGAAATG</td>
<td>175</td>
</tr>
<tr>
<td>pparγ</td>
<td>NM_131467</td>
<td>TGCGCCGATACACAAAGA GA</td>
<td>TCAAGTACTCGG G AATCGT</td>
<td>152</td>
</tr>
<tr>
<td>adipopoq</td>
<td>BC165538</td>
<td>ACAAAGACAGCAAAGG C ATC</td>
<td>AAAACCGGAAAGGTTG GAT</td>
<td>166</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>18S rRNA</td>
<td>TGCATGGCCTGTT TTTAGTG</td>
<td>AGTCTCGGTCGTTATCGGAAATG</td>
<td>62</td>
</tr>
</tbody>
</table>

* Sequences are given in the 5’–3’ order.

sucrose solution for 1 h at room temperature. Liver tissues were embedded in Tissue-Tek OCT compound (Sakura Finetek Japan, Tokyo, Japan) and rapidly frozen in liquid nitrogen-cooled isopentane (Wako Pure Chemicals). Frozen livers were serially sectioned at 8 μm thickness with a HM-550 cryostat (Microm, Walldorf, Germany), placed on slides, and dried at room temperature. Sections were then stained with 0.3% oil red-O (Wako Pure Chemicals) in 60% isopropanol solution for 15 min at 37 °C as described previously (Tainaka et al., 2011). Sections were visualized under a BX41 microscope (Olympus, Tokyo, Japan).

2.9. RNA extraction, cDNA synthesis, and quantitative real-time PCR

Zebrafish from which total RNA was to be extracted were given a laparotomy, immediately transferred into tubes containing 3 mL of RNAlater (Qiagen, Hilden, Germany), and stored at 4 °C for gene expression analysis. Liver and visceral adipose tissues were dissected using forceps and subjected to RNA extraction. Total RNA was extracted from liver using Isogen (Nippongene, Tokyo, Japan) and rapidly frozen in liquid nitrogen-cooled isopentane (Wako Pure Chemicals). Frozen livers were serially sectioned at 8 μm thickness with a HM-550 cryostat (Microm, Walldorf, Germany), placed on slides, and dried at room temperature. Sections were then stained with 0.3% oil red-O (Wako Pure Chemicals) in 60% isopropanol solution for 15 min at 37 °C as described previously (Tainaka et al., 2011). Sections were visualized under a BX41 microscope (Olympus, Tokyo, Japan).

2.10. Statistical analysis

All results are presented as means and standard errors (SE). Differences between two groups were examined for statistically significance using Student’s t-test. For multiple comparisons, one-way ANOVA followed by Bonferroni–Dunn multiple-comparison procedure was used. A *P-value < 0.05 was considered statistically significant.

3. Results

3.1. Yuzu peel and pomace exhibits high anti-obesity effects in DIO zebrafish

Zebrafish in the DIO group exhibited a significantly higher body weight (P < 0.001) and plasma TG (P < 0.05) compared with non-DIO zebrafish, indicating successful establishment of a DIO model (Fig. 1A and 1B). The DIO group fed yuzu peel (6 mg/g/
A

Body weight (g)

Week

non-DIO

DIO

DIO + yuzu peel

DIO + yuzu pomace

B

Plasma TG (mg/dl)

non-DIO

DIO

DIO + yuzu peel

DIO + yuzu pomace

C

Artemia numbers / fish

Week

non-DIO

DIO

DIO + yuzu peel

DIO + yuzu pomace

D

Visceral adipose tissue volume (mm3)

non-DIO

DIO

DIO + yuzu peel

DIO + yuzu pomace
day) showed a trend towards reduced diet-induced body weight gain ($P = 0.13$), and had significantly lower plasma TG ($P < 0.05$) compared with DIO zebrafish. In addition, zebrafish that received yuzu pomace (6 mg/g/day) gained significantly less body weight ($P < 0.05$) and showed a trend towards reduced plasma TG ($P = 0.09$) compared with those in the DIO group. No significant differences in feeding volume were observed between the DIO, DIO + yuzu peel, and DIO + yuzu pomace groups, indicating no appetite suppression in response to either yuzu peel or pomace during the feeding experiment (Fig. 1C).

3D micro-CT analysis (Fig. 1D) showed that visceral adipose tissue volume in the DIO group was significantly greater ($P < 0.01$) than that in the non-DIO group. The yuzu pomace group had significantly reduced visceral adipose tissue volume ($P < 0.05$) while the yuzu peel group had a trend towards a reduction of adipose tissue volume ($P = 0.16$) compared with DIO zebrafish. Additionally, Oil red O staining confirmed that DIO zebrafish fed yuzu peel and yuzu pomace had much lower lipid accumulation (numbers and size of red spots) in their liver compared with the DIO group (Fig. 1E).

3.2. Auraptene exhibits anti-obesity effects in DIO zebrafish

Auraptene is an abundant prenyloxycoumarin found in yuzu peel. We hypothesized that auraptene might play a role in preventing lipid metabolism abnormalities induced by overfeeding. Therefore, we performed the same DIO experiment and fed DIO zebrafish a low-auraptene diet (10 μg/g/day, a 5.5-fold greater level than that found in yuzu peel) or a high-auraptene diet (20 μg/g/day, 11-fold greater than in yuzu peel), prepared as described in section 2.2. After a 4-week administration of auraptene, zebrafish in the DIO + LA group showed a slight trend towards lower body weight compared with those in the DIO group (Fig. 2A). No changes in plasma TG or appetite suppression were observed after auraptene administration during the feeding experiment (Fig. 2B and 2C). In addition, auraptene administration showed a dose-dependent trend towards lower visceral adipose tissue volume (Fig. 2D). Moreover, DIO zebrafish fed auraptene had less lipid accumulation in liver than those in the DIO group (Fig. 2E).

3.3. Effects of yuzu peel, pomace, and auraptene on the expression of lipid metabolism genes in liver and visceral adipose tissue

In the liver tissue at week 6 of the experiment, there were no significant differences between the five DIO groups in the mRNA expression levels of two lipogenic enzymes, *fasn* or *acox1*, which are key enzymes for de novo fatty acid synthesis (Fig. 3). The mRNA expression levels of *acadm* (a mitochondrial β-oxidation enzyme, which catalyses the initial reaction in the β-oxidation of C4 to C12 straight-chain acyl-CoAs), *acox1* (a peroxisomal β-oxidation enzyme, which is required for β-oxidation of long-chain fatty acids), and *pparab* (a nuclear receptor protein that regulates β-oxidation), were significantly higher in the DIO + yuzu peel group than in the DIO group ($P < 0.05$).

In the visceral adipose tissue, no significant differences were observed in the expression of *fasn*, *acacb*, or *socs3b* (a protein that suppresses leptin signalling) between the groups (Fig. 4). The expression level of *pparab* had a trend towards up-regulation by yuzu peel but this was not statistically significant (data not shown). However, the gene expression of *acox1*, *pparg* (a regulator of adipocyte differentiation), and *adipoqb* (adiponectin, an antilipogenic protein) were significantly higher in the DIO + yuzu peel group compared with the DIO group ($P < 0.05$).

4. Discussion

Yuzu has traditionally been used in cuisines, and is also used industrially in sweet production, beverages, cosmetics and perfumery. In commercial food processing, large amounts of residual yuzu are commonly discarded as industrial waste after squeezing yuzu for juice (in which case it is mainly the peel that is discarded) or after extracting essential oil from yuzu peel (in which case pomace is discarded). This is a major problem that needs to be resolved; understanding how to more completely use yuzu fruit will contribute to the reduction of this industrial waste. The primary aim of this study was to evaluate the anti-obesity effects of yuzu peel and pomace in DIO zebrafish. We demonstrated here that yuzu peel and pomace treatment exhibit powerful efficacy against body weight gain, dyslipidaemia and hepatic steatosis (Fig. 1). We thus identified a potential use for current waste products from the processing of yuzu, which may eventually enable a more complete use of yuzu and a reduction of the waste from its processing.

Yuzu peel contains a wide variety of bioactive components, such as flavonoids (e.g. hesperidin, naringin and eriocitrin), anthocyanins, phenolic acids, carotenoids, tannins, vitamins, dietary fibres, and aromatics (e.g. limonene) (Nile & Park, 2014). In this study, we measured the contents of several compounds with bioactivity in yuzu peel and pomace (Table 1). Among these components, we focused on auraptene, the most abundant prenyloxycoumarin found in plants of the genus *Citrus* (Epifano, Genovese, & Curini, 2008). Auraptene has various notable biological functions, including anti-cancer, anti-inflammatory, anti-bacterial, and angiogenic activity in vitro (Genovese & Epifano, 2011; Wang et al., 2012). Yuzu peel used in this study contained a relatively high level of auraptene (0.3 ± 0.02 mg/g dry weight), similar to that reported in a previous study (0.376 mg/g dry weight) (Ogawa et al., 2000). It has been reported that auraptene administration ameliorates...
Fig. 3 – Effects of yuzu peel, pomace, and auraptene on the expression of lipid metabolism genes in liver. *fasn and acacb play important roles in lipogenesis. acadm, acox1 and pparab are closely related to fatty acid oxidation (*n = 5). Values are means ± SE. *P < 0.05 vs. the DIO group.
Fig. 4 – Effects of yuzu peel, pomace, and auraptene on the expression of lipid metabolism genes in visceral adipose tissue. socs3b is a negative regulator of leptin signalling. pparg and adipoqb play roles in adipose differentiation (n = 5). Values are means ± SE. *P < 0.05 vs. the DIO group, **P < 0.01 vs. the DIO group.
hepatic TG accumulation in the livers of obese rats (Nagao et al., 2010), which is consistent with our results in zebrafish livers (Fig. 2E). However, there were no significant differences in plasma TG levels or adipose tissue volume between the auraptene group and the DIO group (Fig. 2B and 2D). This suggests that the therapeutic property of auraptene is limited to improvement of hepatoosteatosis, but not of systemic lipid metabolism including visceral adiposity.

In addition to auraptene, some bioactive molecules in yuzu peel have been reported to induce PPARα mRNA expression with consequent anti-obesity properties. For example, limonene protected against the development of dyslipidaemia by activating PPARα transactivation in obese mice (Jing et al., 2013). Hesperidin and naringin are the most abundant flavonoids in yuzu fruit (Nile & Park, 2014). The amounts of these flavonoids can be affected by various factors, such as production area, measurement site and stage of maturation. We found that hesperidin and naringin levels were high in our Japanese yuzu peel (92.57 ± 0.15 and 52.23 ± 0.21 mg/100 g fresh weight, respectively); these levels are similar to those observed in Korean yuzu peel (74.47–96.24 and 81.51–98.1 mg/100 g fresh weight, respectively) (Yoo et al., 2004). Hesperidin and naringin attenuated hyperlipidaemia and hepatic steatosis, partly by regulating fatty acid and cholesterol metabolism through enhancing hepatic and adipocyte PPARγ expression in type-2 diabetic animals (Jung, Lee, Park, Kang, & Choi, 2006; Sharma et al., 2011). In this study, administration of yuzu peel increased the mRNA expression of markers of lipid oxidation (pparab and acadm in liver, ppara in adipose tissue, and acox1 in both) and mature adipocytes (adipoq in adipose tissue) in DIO zebrafish without affecting markers of lipogenesis (fasn and acacb in adipose tissue and liver) (Figs 3 and 4). It is well known that activation of PPARα (the human homolog of zebrafish pparα) causes lipid clearance via enhancement of fatty acid β-oxidation in liver (Berger, Akiyama, & Meinke, 2005; Kersten, 2002). The expression levels of PPARα target genes (ACOX1 and ACADM) were also enhanced which consequently promote mitochondrial and peroxisomal fatty acid β-oxidation pathway to increase lipolysis (Rakhshandehroo, Knoch, Muller, & Kersten, 2010). PPARγ (the human homolog of zebrafish pparγ) is predominantly expressed in adipose tissue and is critical for adipocyte differentiation, maintenance, and regulation of adipose tissue lipid metabolism (Spiegelman, 1998). In addition, PPARγ activates the transcription of ADIPOQ (the human homolog of zebrafish adipoq) (Lee, Olson, & Evans, 2003), which plays a role in energy homeostasis through the regulation of fatty acid metabolism in muscle and liver (Berg, Combs, & Scherer, 2002). These observations suggest that the systemic anti-dyslipidaemia effect of yuzu peel is dependent on the PPARγ-ADIPOQ axis, which may be conserved in vertebrates. Our results suggest that various bioactive compounds in yuzu peel can improve dyslipidaemia and hepatic steatosis via activation of hepatic PPARα and adipocyte PPARγ pathways.

It is notable that yuzu pomace also improved visceral adiposity and hepatic steatosis (Fig. 1D and 1E), while the gene expression profiles are different from those of the peel group. Yuzu contains an almost threefold greater amount of total dietary fibre than other citrus fruits (Yoo, Hwang, Park, & Moon, 2009). The content of dietary fibre in yuzu pomace was higher than in peel (8.35 ± 0.08 and 6.4 ± 0.02 g/100 g fresh weight, respectively) (Table 1). It is well known that dietary fibre can suppress body weight gain in obese people, because of its low digestibility and low absorption (Fukada, Furutani, Shimizu, & Masumoto, 2013; Lattimer & Haub, 2010). In addition, while most of the auraptene and limonene were lost after hexane extraction, both hesperidin and naringin remained and were further concentrated (Table 1). The greater amount of fibre in combination with hesperidin and naringin in pomace may have helped to suppress the body weight increase in the DIO group, which was not seen in response to yuzu peel (Fig. 1A). Furthermore, we applied yuzu pomace to cultured red seabream (Pagrus major), and found that yuzu pomace-supplemented bait prevented dark muscle discoloration and decreased white adipose tissue weight (data not shown). Overall, our results suggest a possible application of yuzu peel or pomace for reducing obesity and related diseases, making use of material which is currently disposed of as industrial waste.

5. Conclusions

Using DIO zebrafish, we clarified the anti-obesity effects of yuzu peel, pomace and the bioactive component auraptene. We established that the therapeutic mechanism of yuzu peel is through activation of hepatic PPARα and adipocyte PPARγ pathways. Our findings demonstrate that yuzu peel may be an ideal natural product with actions against obesity and related diseases. In addition, we showed that yuzu pomace is effective in obesity phenotypes, which suggests a novel use for citrus fruit pomace. This study is the first to identify new approaches to achieve the complete use of yuzu, and thus provides a valuable insight into improving the processing of citrus fruit to reduce waste.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2014.08.002.

REFERENCES


