Original paper

Influence of Sweet Potato (Ipomoea batatas L.) Leaf Consumption on Rat Lipid Metabolism

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The nutrient content of the leaves of sweet potato cultivar Koganesengan was determined to be 35.0 kcal energy, 89.0 g water, 3.9 g protein, 0.8 g fat, 1.2 g carbohydrates, 1.2 g minerals, and 4.1 g dietary fiber/100 g fresh weight. The polyphenol content of the leaves was 11.5 g chlorogenic acid equivalents per 100 g dry weight. Some reports have described that the consumption of dietary fiber and polyphenols is effective in treating dyslipidemia. Therefore, we investigated the influence of sweet potato leaves on lipid metabolism in rats fed a high-fat diet. After 35 days of rearing, the weight gain and adipose tissue weight were lower in the rats fed a high-fat diet supplemented with sweet potato leaves than in those not fed sweet potato leaves. Plasma triglyceride and total cholesterol levels, and liver total cholesterol level were significantly lower in rats fed sweet potato leaves compared to rats fed the high-fat diet alone. These results suggested that the simultaneous intake of sweet potato leaf and the high-fat diet inhibited the excessive accumulation of adipose tissue in rats.

Keywords: sweet potato leaf, polyphenol, caffeoylquinic acid, lipid metabolism

Introduction

In Japan, the tuberous root of sweet potatoes is widely used for fresh and processed foods, distilled spirits, starch, purple pigments and so on. In contrast, the uses for the stems and leaves of sweet potatoes remain limited. Yet, in Africa, Southeast Asia, Taiwan, and Korea, the stems and leaves of sweet potatoes are regularly eaten as a leafy vegetable. In fact, the stems and leaves are rich in protein, vitamins, and minerals, and have greater nutritional value than other beta-carotene-rich vegetables (Ishida et al., 2000, Antia et al., 2006). Furthermore, sweet potato stems and leaves are distinguished by their extremely high functional components content, such as lutein and polyphenols, which far exceeds that of other vegetables (Ishiguro et al., 2006). The primary polyphenols in the stems and leaves are caffeic acid (CA) and caffeoylquinic acids (CQAs), including 3-O-chlorogenic acid (ChA), 3,4-di-O-caffeoylquinic acid (3,4-DCQA), 3,5-di-O-caffeoylquinic acid (3,5-DCQA), 4,5-di-O-caffeoylquinic acid (4,5-DCQA), and 3,4,5-tri-O-caffeoylquinic acid (3,4,5-TCQA). These polyphenols have many functions, including antioxidant, antimutagenicity (Yoshimoto et al., 2002) and cancer cell growth-suppressing activities (Kurata et al., 2007). While CA and its respective CQAs are present in all sweet potato varieties, their contents differ (Islam et al., 2002). The sweet potato cultivar Koganesengan has many applications, including in distilled spirits and food processing. This is also the most popular cultivar in Japan, and produces large amounts of stems and leaves that are easily procured. Therefore, we expect that this cultivar will be used as a vegetable.

The number of patients with lifestyle-related diseases is
currently growing in Japan, due to the effects of overeating and aging. Dyslipidemia, one of the lifestyle-related diseases, is suspected in 14 million people, and might affect 42 million people if those at risk are included. Dyslipidemia is the persistent state of high total-cholesterol (T-CHO) levels, high LDL-cholesterol (LDL-C) levels, low HDL-cholesterol (HDL-C) levels, or high triglyceride (TG) levels, and is a major factor in arteriosclerosis. Since reducing body fat percentage is effective in improving this condition, dietary and exercise therapies are prescribed. Recommended dietary therapies include adjusting the total energy intake and nutrient partitioning, together with the intake of dietary fiber. Several recent reports have demonstrated that body fat may be decreased through the consumption of polyphenols (Aoki et al., 2007; Bose et al., 2008; Murase et al., 2011). Therefore, we investigated the influence of sweet potato leaves, which represents an abundant untapped biomass resource rich in both dietary fiber and polyphenols, on lipid metabolism in rats fed a high-fat diet (HFD).

Materials and Methods

Sweet potato sample preparation The sweet potato leaves used in the experiment were harvested in June, 2013 from plants of the cultivar Koganesengan, cultivated in a research field of the Miyakonojo Research Station, Kyushu Okinawa Agricultural Research Center NARO. Samples for nutritional composition analysis were washed after harvest, and only the leaves were removed. The leaves were immediately refrigerated and transported to the Japan Food Research Laboratories (Fukuoka, Japan) for analysis. Samples for other analyses were washed after harvest and only the leaves were removed, which were immediately frozen at -30°C and freeze-dried. The dried samples were crushed by a mixer, sealed in air-tight bags, and preserved at -30°C until use.

Nutritional composition analysis The nutritional composition (water content, protein, fat, minerals, carbohydrates, dietary fiber, energy, sodium) of the fresh sweet potato leaf was analyzed by the Japan Food Research Laboratories. Water content was determined by the vacuum oven method (AOAC 925.45). Protein content was calculated from the nitrogen content (%N × 6.25), analyzed by the Kjeldahl method (AOAC 981.10). Fat content was determined by the acid hydrolysis method (AOAC 922.06). Ash content was measured by the ashing method (AOAC 923.03). Carbohydrate content was calculated. Fiber was measured by the enzymatic-gravimetric method (AOAC 985.29). Sodium was determined using an atomic absorption spectrophotometer (AA240FS; Varian Technologies Japan Ltd., Tokyo, Japan).

Materials CA was purchased from Wako Pure Chemical Industries (Osaka, Japan). ChA was obtained from Sigma Chemical (St. Louis, MO). 3,4-DCQA, 3,5-DCQA, 4,5-DCQA, and 3,4,5-TCQA were purified (> 97%) from sweet potato leaves (Kurata et al., 2011).

Measurement of polyphenols and caffeoylquinic acids The total polyphenol content of the extract solution was measured by a partially modified Folin-Ciocalteu method (Coseteng and Lee, 1987), and calculated as ChA equivalents using ChA as the standard substance. Measurement of CQAs was performed by high performance liquid chromatography (HPLC) using the method of Okuno et al. (2010). The HPLC system consisted of a DGU-12A degasser, SIL-10A auto-injector, CTO-10A column oven, SPD-10A UV-VIS detector, SCL-10A VP system controller, and two LC-10AD pumps (Shimadzu, Kyoto, Japan). The system was controlled by an LC solution (version 1.24 SP1) workstation (Shimadzu). The column was a YMC-Pack ODS-AM AM12S03-L546WT (4.6 i.d. × 75 mm, 3-µm particle; YMC, Kyoto, Japan). The mobile phase consisted of water containing 0.2% (v/v) formic acid (A) and acetonitrile (B).

Animal dosing experiment Four-week-old male Sprague-Dawley (SD) rats were reared in separate cages at room temperature (23°C ± 2°C) with a controlled light-dark cycle (lights on 07:00 – 19:00). The animals were given standard commercially available rodent feed (CE-2; CLEA Japan, Tokyo, Japan). Then, after preliminary rearing for 7 days, they were divided into 4 groups of seven rats each, ensuring equal average body weight among groups. Subsequently, the feed of each group was changed to High Fat Diet 32 (CLEA Japan; containing 24.5% casein, 20% safflower oil, 15.88% beef tallow, 8.25% maltodextrin, 6.928% lactose, 6.75% sucrose, 5.5% cellulose powder, 5.0% egg white powder, 5.0% mineral mixture, 1.4% vitamin mixture, 0.43% L-cystine, 0.36% choline bitartrate, 0.002% tert-butyl hydroquinone) supplemented with 0%, 1%, 3%, and 5% of freeze-dried powder of sweet potato leaves (SPLP), respectively. The rats were reared for 35 days, with each group being provided feed under the same intake amount conditions, and water ad libitum. On the 35th day of rearing, the animals were fasted overnight, and then a small amount (~1.0 mL) of blood was collected from the caudal vein, and plasma was obtained by a conventional method. The plasma was preserved at -30°C until lipid components and glucose level analyses. The rats were then anesthetized with diethyl ether, and euthanized by exsanguination from the abdominal artery. The blood, liver, kidneys, and epididymal adipose tissue were resected, and each component was weighed. It has been reported that epididymal adipose tissue was mainly influenced as the visceral adipose tissue in high-fat diet intake examinations (Cho et al., 2010, Murase et al., 2011, Qin and Anderson, 2012). The liver was preserved at -30°C until the measurement of lipid components. The animal experiments in this study were performed in accordance with the animal experiment guidelines of the Kyushu Okinawa Agricultural Research Center NARO, Japan.

Analysis of lipid components and blood glucose level Plasma TG, T-CHO, HDL-C, and glucose levels were measured using commercially available kits (Triglyceride E-Test, Cholesterol E-Test, HDL-Cholesterol E-Test, Glucose CII-Test; Wako Pure
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Chemical Industries, Tokyo, Japan). Liver lipids were measured by TG and T-CHO kits after thawing the cryopreserved liver samples and extracting the lipids by the method of Folch et al. (1957).

Measurement of plasma antioxidant capacity Plasma antioxidant capacity was measured using a commercially available kit (“PAO” Colorimetric assay for Total Antioxidant Capacity; Japan Institute for Control of Aging, NIKKEN SEIL Co. Ltd., Shizuoka, Japan).

Statistical analysis The experimental results were subjected to an analysis of variance (ANOVA) and significant differences were obtained. The Tukey-Kramer method was used to identify the significance of mean differences between groups. The level of significance between groups was taken as 5%.

Results and Discussion

Basic components and polyphenols in sweet potato leaves The nutritional contents per 100 g of sweet potato leaves (cultivar Koganesengan) were as follows: 89.0 g water, 3.9 g protein, 0.8 g fat, 1.0 g carbohydrates, 1.2 g minerals, and 4.1 g dietary fiber. The leaves also contain 35.0 kcal energy and 3.0 mg of sodium per 100 g (Table 1). These values are similar to those reported by Ishida et al. (2000) and show that sweet potato leaves are high in protein and dietary fiber, and low in carbohydrates.

Islam et al. (2002) analyzed over 1,500 varieties of sweet potato and reported that sweet potato leaves are rich in polyphenols. They classified the varieties with a total polyphenol content of 5.0 – 9.0 g of ChA equivalents per 100 g dry weight as the intermediate group, and those with contents higher than this as the high-content group. Leaves of the cultivar Koganesengan used in this study contained 11.50 g of ChA equivalents per 100 g dry weight (Table 2); thus, this cultivar belongs to the high-content group. The main components of the polyphenols were CQAs, with the following contents per 100 g dry weight: 1.42 g ChA, 1.81 g 3,4-DCQA, 3.12 g 3,5-DCQA, 0.48 g 4,5-DCQA, and 0.10 g 3,4,5-TCQA. Of these, the content of 3,5-DCQA was the highest, accounting for up to 45% of the total CQAs. Furthermore, when the three types of dicaffeoylquinic acid (DCQA) isomer (3,4-DCQA, 3,5-DCQA, and 4,5-DCQA) were combined, they amounted to 78% of the total CQAs, which corresponds to 5.41 g of total DCQAs per 100 g dry weight. To our knowledge, this DCQA content is higher than that reported from other plants. Moreover, according to a report by Kurata et al. (2011), the content of 3,4,5-TCQA in plants is very small; therefore, the value of 0.10 g per 100 g dry weight obtained in this study is exceptionally large. Overall, our data confirm that sweet potato leaves are rich in dietary fiber and polyphenols, and are thus useful as a healthful leafy vegetable.

Consumption trial of sweet potato leaves and a high-fat diet in SD rats As SPLP contains a large amount of dietary fiber and polyphenols, both of which have been shown to have anti-obesity effects (Isken et al., 2010, Shimoda et al., 2006, Rodriguez de Sotillo and Hadley 2002, Thom, 2007), we next investigated the anti-obesity effects of sweet potato leaves using rats fed a HFD.

We established four SD rat groups and fed them a HFD supplemented with 0 (control), 1, 3, and 5% (v/v) SPLP, respectively (Table 3). After rearing for 35 days, the expected obesity was observed in the control group, whereas weight gain was suppressed in a dose-dependent manner in all of the groups fed the SPLP-supplemented HFD. Statistically significant suppression was observed for the 5% SPLP group compared to the control.
Furthermore, a clear dose-dependent decrease in adipose tissue weight was observed for the 1%, 3%, and 5% SPLP groups. Despite the small reduction in feed and energy intake, which might have affected the weight gain and adipose tissue weight observed in the 3% SPLP group, the 5% SPLP group showed significant suppression in both weight gain and adipose tissue weight without such a reduction in feed intake. Furthermore, the reduced energy content in the feed caused by the addition of SPLP could be one of the causes of suppressed weight gain and adipose tissue weight. However, because the addition of only 1% SPLP caused an 18% reduction in adipose tissue weight, energy reduction does not seem to be a major cause of the suppression. Collectively, our results support the hypothesis that sweet potato leaves have an anti-obesity effect when consumed with a HFD.

The mechanism of suppression of weight gain and adipose tissue weight is not known. However, because SPLP contains insoluble dietary fiber (Ishida et al., 2000), it is possible that excretion was promoted in the SPLP-supplemented groups (Isken et al., 2010). Alternatively, as reported by Isken et al. (2010), it is possible that lipid absorption in the small intestine was blocked by the insoluble dietary fiber. Because ChA in coffee has also been reported to reduce body fat (Rodriguez de Sotillo and Hadley, 2002, Shimoda et al., 2006, Thom, 2007, Murase et al., 2011), ChA might also have contributed to the anti-obesity effect of SPLP.

To test the possibility that the reductions in weight gain and adipose tissue weight were caused through altered blood lipid composition, we next investigated the changes in blood components. As shown in Table 5, plasma TG levels decreased as the amount of added SPLP increased. The plasma TG level in the 5% SPLP group was significantly lower and approximately 38% of that in the control group. The T-CHO level in the 1% and 3% SPLP groups was significantly lower and approximately 80% of that in the control group. Although not significant, the 5% SPLP group also showed a lower T-CHO level (approx. 85% of the control group). In contrast, no significant differences were found among groups for HDL-C levels. The effect of SPLP addition on T-CHO level was not dose-dependent. It is possible that the T-CHO level is more sensitive to the addition of SPLP compared to TG-level, and the effect was already saturated at 1% addition.

**Table 4.** Effects of sweet potato leaf consumption on body weight, feed intake and tissue weight in SD rats

<table>
<thead>
<tr>
<th></th>
<th>HFD</th>
<th>HFD +SPLP</th>
<th>1%</th>
<th>3%</th>
<th>5%</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>147.3 ± 5.16</td>
<td>147.3 ± 4.80</td>
<td>147.3 ± 5.09</td>
<td>147.3 ± 4.84</td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td>425.0 ± 24.97</td>
<td>409.2 ± 10.53</td>
<td>403.2 ± 8.11</td>
<td>390.6 ± 15.69</td>
<td></td>
</tr>
<tr>
<td>Feed intake (g/rat/35d)</td>
<td>588.4 ± 25.91</td>
<td>567.8 ± 23.23</td>
<td>548.2 ± 9.80</td>
<td>581.5 ± 20.94</td>
<td></td>
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<tr>
<td>Energy intake (kcal/rat/35d)</td>
<td>3020.93 ± 133.01</td>
<td>2903.64 ± 118.81</td>
<td>2782.01 ± 49.74</td>
<td>2928.27 ± 105.45</td>
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Tissue weight (g /100 g of body weight)

<table>
<thead>
<tr>
<th></th>
<th>HFD</th>
<th>HFD +SPLP</th>
<th>1%</th>
<th>3%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>21.75 ± 1.70</td>
<td>20.58 ± 2.91</td>
<td>20.70 ± 2.38</td>
<td>20.88 ± 2.05</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>3.19 ± 0.33</td>
<td>3.08 ± 0.20</td>
<td>3.11 ± 0.17</td>
<td>2.85 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>8.85 ± 1.32</td>
<td>7.23 ± 1.01</td>
<td>7.01 ± 0.51</td>
<td>6.25 ± 0.95</td>
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</tbody>
</table>

Values are means ± SD (n=7).
Means in the same row and with different letters are significantly different (p < 0.05).

**Table 5.** Effects of sweet potato leaf consumption on plasma and liver lipids and plasma glucose

<table>
<thead>
<tr>
<th></th>
<th>HFD</th>
<th>HFD +SPLP</th>
<th>1%</th>
<th>3%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma lipids</td>
<td></td>
<td></td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>178.63 ± 44.76</td>
<td>134.08 ± 44.41</td>
<td>110.67 ± 61.23</td>
<td>66.96 ± 35.29</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>93.64 ± 17.29</td>
<td>74.31 ± 6.31</td>
<td>76.43 ± 6.66</td>
<td>79.64 ± 17.29</td>
<td></td>
</tr>
<tr>
<td>HDL- cholesterol (mg/dl)</td>
<td>63.46 ± 10.37</td>
<td>57.48 ± 6.90</td>
<td>60.92 ± 8.90</td>
<td>61.85 ± 8.70</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>139.53 ± 19.30</td>
<td>120.99 ± 8.41</td>
<td>127.55 ± 7.31</td>
<td>124.74 ± 8.19</td>
<td></td>
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<tr>
<td>Liver</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/g)</td>
<td>95.27 ± 10.51</td>
<td>118.94 ± 14.12</td>
<td>110.68 ± 15.08</td>
<td>111.14 ± 28.32</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/g)</td>
<td>8.57 ± 0.39</td>
<td>7.99 ± 0.94</td>
<td>7.49 ± 0.37</td>
<td>7.07 ± 1.03</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD (n=7).
Means in the same row and with different letters are significantly different (p < 0.05).
supplemented groups suggest that the dietary fiber in SPLP blocked lipid absorption in the small intestine, as reported by Isken et al. (2010). However, other reports have suggested that ethanol extracts of sweet potato leaves suppress LDL oxidation (Nagai et al., 2011, Taira et al., 2012), with the main components of the extract being polyphenols. Thus, polyphenols could also affect the blood lipid composition, along with the dietary fiber. To further elucidate the mechanism, the detailed composition and characteristics of the dietary fiber in sweet potato leaves should be clarified to determine whether they block lipid absorption in experimental animals. Furthermore, the effect of polyphenol extracts from sweet potato leaves on lipid metabolism should be examined.

It has been reported that the consumption of polyphenols, such as sulforaphane, curcumin, and coffee polyphenols, may suppress hepatic lipid accumulation (Asai and Miyazawa, 2001, Murase et al., 2011, Kikuchi et al., 2015). Although there was no significant difference in the weight of livers among the groups in this study (Table 4), a dose-dependent decrease in liver T-CHO levels was detected in the SPLP-supplemented groups (Table 5), suggesting that the accumulation of liver cholesterol was suppressed by SPLP addition. In contrast, liver TG was slightly increased in all SPLP-supplemented groups, although this was not a statistically significant increase. The reason for this increase is not known. Further studies are necessary to elucidate the detailed effect of SPLP on hepatic lipid accumulation.

Interestingly, the blood glucose level was also reduced in the SPLP-supplemented groups compared to the control group. This observation is consistent with the previous reports by Gao et al. (2008) and Kurata et al. (2011), which suggests that the CQAs in sweet potato leaves block α-glucosidase and aldose reductase, and thus contribute to diabetes prevention. A significant reduction in the blood glucose level was found in the 1% SPLP group, suggesting that the CQAs included in SPLP are effective even at low concentrations. Although the relationship between blood glucose level and lipid metabolism is not known, these results suggest that SPLP has pleiotropic functions when consumed with a HFD.

We also measured blood antioxidant activity in terms of plasma antioxidation power after consumption of the SPLP-supplemented HFD for 35 days. The antioxidant activity of the blood increased dose-dependently with the amount of SPLP, and a statistically significant increase was observed in the 5% SPLP group compared to the control group (Fig. 1). Because polyphenols have antioxidant activity, this result implies that the polyphenols in SPLP are absorbed by the digestive tract, enter the blood, and affect blood antioxidation activity. However, direct detection of DCQAs in blood is necessary to verify its absorption from the digestive tract. Collectively, our data suggest that sweet potato leaves are a novel leafy vegetable with high dietary fiber content, and might have anti-obesity effects through improvement of lipid metabolism when consumed with high fat foods. Further studies are necessary to confirm the anti-obesity effect of SPLP and identify the SPLP component that directly contributes to improvement of lipid metabolism.

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References


