Young Persimmon Ingestion Suppresses Lipid Oxidation in Rats

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(Received July 17, 2014)

Summary Persimmon is widely eaten in Asia and the nutritional components of young and mature persimmons differ. Although raw young persimmon has a strong bitter taste and is difficult to eat, the beneficial health effects of young persimmon powder have attracted attention in recent years. Young persimmon has been suggested to have hypolipidemic activity as well as other biological effects. However, there has been little investigation of the beneficial effects of young persimmon. In the present study, we investigated the antioxidative effects of persimmon in an animal study and compared the effects of young persimmon and mature persimmon. Six-week-old male F344 rats were divided into three groups and fed a standard diet, young persimmon diet, or mature persimmon diet for 4 wk. The young persimmon and mature persimmon groups were fed a diet containing 5% (w/w) freeze-dried young or mature persimmon. We analyzed phosphatidylcholine hydroperoxide (PCOOH) levels in the rats as a biomarker of membrane lipid peroxidation. Our study showed that plasma PCOOH levels were significantly lower in the young persimmon group (36.1 ± 28.5 pmol/mL plasma) than in the control group (120 ± 66 pmol/mL plasma). No significant difference was observed between the mature persimmon group (57.3 ± 15.6 pmol/mL plasma) and the control group. It is possible that ascorbic acid and soluble tannin contribute to the difference in the antioxidant effects of young and mature persimmons. These results indicated that intake of young persimmon contributes to the reduction of plasma phospholipid hydroperoxide levels in rats.

Key Words persimmon, ascorbic acid, tannin, antioxidant, phospholipid hydroperoxide

The increased prevalence of diabetes and arteriosclerosis is a worldwide problem. The use of food products to prevent these lifestyle-related diseases has recently attracted significant attention. It is well known that oxidative stress contributes to these diseases (1, 2). In 1992, Miyazawa et al. developed a method to analyze phosphatidylcholine hydroperoxide (PCOOH) levels in the body using chemiluminescence detection coupled with high performance liquid chromatography (the CL-HPLC method) (3, 4). PCOOH is the primary product of membrane lipid oxidation in the body and is considered a biomarker of oxidative stress. Miyazawa et al. also found that plasma PCOOH levels were elevated in patients with diabetes, arteriosclerosis, hyperlipidemia and intake of high-fat diet (5–7).

Meanwhile, several epidemiologic and in vivo studies have reported that the risk of developing lifestyle-related diseases is reduced by increased intake of fruits and vegetables (8, 9). This may be due to the fact that fruits and vegetables are rich in antioxidant agents that reduce oxidative stress (10, 11).

Persimmon (Diospyros kaki) is widely cultivated in Japan and contains a variety of antioxidants such as ascorbic acid, carotenoids, flavonoids, polyphenols, tannins, and vitamin E (12). In addition, the antioxidative components of persimmon differ depending on the fruit’s stage of development. Greater accumulation of ascorbic acid and soluble tannin are observed in young persimmons (Table 1) (13, 14). Given these differences, young persimmons may have especially beneficial health effects. The antioxidant activity of mature persimmons was shown to be much lower than that of young persimmons in an in vitro study (15). Ethanol extraction of young persimmons showed significant 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and inhibited production of copper-oxidized low-density lipoproteins. Matsumoto et al. found that intake of young persimmon reduced plasma cholesterol levels in vivo (16, 17). However, there have been no previous in vivo studies of the antioxidant effects of young persimmon. Thus, we investigated the antioxidant effects of persimmon in an animal study and compared the antioxidant activity of young and mature persimmon.

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**MATERIALS AND METHODS**

**Materials.** A high-fat diet (Quick fat®) was obtained from CLEA Japan, Inc. (Tokyo, Japan). All other reagents used were of analytical grade and obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

**Preparation of diet.** Diospyros kaki were used in this study. Young persimmons (which are green) were harvested in October 2011, in Akita Prefecture, Japan, and mature persimmons (which are orange) were harvested in the same place in November 2011. Whole fruits containing peel were sliced 1–2 cm thick, frozen at approximately −80°C, dried with a freeze-dryer, and then powdered with a mill. The high-fat diet was used as the basal diet. This was because, from our previous study, 4-wk intake of a high-fat diet showed a significant increase in plasma and liver phospholipid hydroperoxide (PLOOH) levels in rats (7). In the present study, the micronutrient composition of the diet was 6.8% moisture, 24.8% crude protein, 14.4% crude fat, 2.45% crude fiber, 5% crude ash and 46.7% nitrogen-free extract (calculated from mean values) and energy was 415.1 kcal/100 g diet. Dried and powdered persimmon fruits were added and mixed well with the basal diet at a concentration of 5% (w/w, excluding water). The groups were designated as follows: control group, basal diet (QF); young persimmon group, basal diet (QF) 95%+young persimmon 5%; mature persimmon group, basal diet (QF) 95%+mature persimmon 5%.

**Animal study.** F344 rats (6-wk-old males) were purchased from CLEA Japan, Inc. After 1 wk of acclimatization, the rats were assigned to 3 groups of 6 such that the average body weight value and standard deviation were the same in each group, and were fed the diets for 4 wk. Animals were housed two per cage with a member of the same group. Animals were maintained under controlled environmental conditions (relative temperature, humidity, and 12 h-dark-light cycle) with free access to distilled water and the experimental diet. Daily food intake amount and body weight were measured every day. At the end of the study, after fasting for 12 h, animals were sacrificed by decapitation and their organs were dissected and blood samples were removed. Blood was treated with EDTA (ethylene-diamine-tetraacetic acid), and plasma was isolated by centrifugation at 1,000 × g for 15 min at 4°C. Liver, heart, lung, spleen, kidney, and adipose tissue (epididymal fat, mesenteric fat and perinephric fat) were removed and weighed. All samples were stored at approximately −80°C until analysis. All experiments were performed according to the Regulations for Animal Experiments and Related Activities at Tohoku University. The permit number for this animal experiment protocol is 23-Noudou-23.

**Hepatic and plasma parameters.** Livers were homogenized in normal saline containing 1 mM EDTA. Glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), blood glucose, triglyceride (TG), phospholipid (PL), and total cholesterol were measured in plasma samples by Mitsubishi Chemical Medience (Tokyo, Japan).

**Plasma and liver PCOOH levels.** PCOOH was extracted from plasma and liver by using chloroform and methanol (Folch method) (18). PCOOH levels were measured by CL-HPLC (3, 4). The column was a Finepak SIL NH2-5 (4.6×250 mm; Japan Spectroscopic (Tokyo, Japan)), the mobile phase was 2-propanol–methanol–water (135 : 45 : 20, v/v/v), and the flow rate was 1 mL/min. Post-column CL detection was carried out using a CLD-100 detector (Tohoku Electronic Industries (Sendai, Japan)). A mixture of luminol and cytochrome c in 50 mM borate buffer (pH 10.0) was used as the hydroperoxide-specific post-column CL reagent. Calibration was carried out using standard PCOOH (19).

**Statistical analysis.** Data were expressed as means ± standard deviations (SD). The Kruskal-Wallis/Steel-Dwass test and post-hoc tests were used for multiple comparisons. Differences were considered significant at p<0.05.

**RESULTS**

**Food intake and body weight**

The mean amounts of total food intake throughout the experimental period of the control group, young persimmon group and mature persimmon group were 793.8±21 g/rat, 841.6±38.8 g/rat, and 866.2±18.9 g/rat, respectively. The calculated calories of each group were 3,295 kcal, 3,494 kcal and 3,596 kcal. No differences were observed in total food intake among the rat groups. Figure 1 shows the final body weight of rats after the 4-wk study period. No significant difference was observed among the groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Harvesting date in 1998</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October 6</td>
</tr>
<tr>
<td>Maturity stage</td>
<td>Breaker</td>
</tr>
<tr>
<td>Fruit firmness (kg/cm)</td>
<td>15.8</td>
</tr>
<tr>
<td>Soluble solid concentrations (%)</td>
<td>12.8</td>
</tr>
<tr>
<td>Organic acid (mg/100 mL extracted juice)</td>
<td>750.4</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100 mL extracted juice)</td>
<td>24.3</td>
</tr>
<tr>
<td>Soluble tannins (mg/100 g wet weight)</td>
<td>17.1</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of persimmon at different harvesting stages (modified from Ramin and Tabatabaie (13)).
**Organs and adipose tissue weight**

There was no significant difference in the weight of organs except for epididymal fat. Epididymal fat weights were higher in the mature persimmon group (Table 2, \( p < 0.05 \)).

**Plasma parameters**

There was no significant difference among groups in GOT, GPT, blood glucose, TG, PL or total cholesterol after the 4-wk study period (Table 3).

**PCOOH levels in liver and plasma**

The young persimmon-supplemented group showed a significant decrease of PCOOH levels in plasma compared to that of the high-fat diet group (Fig. 2, \( p < 0.05 \)). The mature persimmon-supplemented group also showed a trend towards lower plasma PCOOH. Similar results were observed for liver parameters (Fig. 3). These results suggest that PCOOH accumulation in plasma is significantly attenuated by young persimmon intake.

**DISCUSSION**

Many natural products contain antioxidant molecules such as carotenoids, flavonoids, polyphenols, ascorbic acid, and vitamin E, and supplementation

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**Table 2. Final tissue weights.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Heart</th>
<th>Epididymal fat</th>
<th>Mesenteric fat</th>
<th>Perinephric fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (g)</td>
<td>8.39</td>
<td>0.69</td>
<td>0.55</td>
<td>0.04</td>
<td>1.69 0.10</td>
<td>0.74 0.04</td>
<td>4.89 0.68</td>
</tr>
<tr>
<td>Young persimmon (g)</td>
<td>8.28</td>
<td>0.52</td>
<td>0.56</td>
<td>0.01</td>
<td>1.69 0.09</td>
<td>0.74 0.05</td>
<td>4.99 0.51</td>
</tr>
<tr>
<td>Mature persimmon (g)</td>
<td>8.29</td>
<td>0.78</td>
<td>0.57</td>
<td>0.03</td>
<td>1.71 0.08</td>
<td>0.77 0.08</td>
<td>5.97 0.74*</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD (n=6). Mean values were significantly different in the Kruskal-Wallis/Steel-Dwass test for multiple comparisons. * \( p < 0.05 \).

**Table 3. Plasma parameters of the study.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Young persimmon</th>
<th>Mature persimmon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>GOT (U/L)</td>
<td>180.7</td>
<td>16.7</td>
<td>193.5</td>
</tr>
<tr>
<td>GPT (U/L)</td>
<td>59.8</td>
<td>5.1</td>
<td>59.2</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>128.7</td>
<td>15.0</td>
<td>123.0</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>155.2</td>
<td>34.0</td>
<td>150.3</td>
</tr>
<tr>
<td>PL (mg/dL)</td>
<td>111.5</td>
<td>11.1</td>
<td>103.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>50.7</td>
<td>4.6</td>
<td>44.2</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD (n=6). No significant difference among groups as determined using the Kruskal Wallis test. \( p < 0.05 \).
with these can help to prevent peroxidative degeneration of plasma and tissue lipids (20–22). Persimmons are widely eaten in Japan and contain many beneficial antioxidants. Young persimmon is particularly rich in ascorbic acid and soluble tannin (Table 1) (13, 14). However, to the best of our knowledge, there is little information about the effects of young persimmon on human health. Recent animal studies have shown that intake of young persimmon reduced plasma cholesterol levels in mice (16, 17). Although the health benefits of young persimmon are gradually becoming clear, the antioxidant effects of young persimmon intake have not previously been investigated. In this study, dietary supplementation with young or mature persimmon was used to determine persimmon antioxidant activity in rats. PCOOH is a primary product of lipid oxidation and a reliable biomarker of oxidative stress. CL-HPLC can detect PCOOH levels sensitively. In the present study, we analyzed PCOOH levels as an oxidative stress marker in rats.

In this study, only epididymal fat showed a weight increase in the mature persimmon group (Table 2). The mature persimmon group tended to ingest a higher total calorie intake (3,596 kcal) compared to the other groups (3,295 kcal for the control group and 3,494 kcal for the young persimmon group). It is known that high-calorie intake increases epididymal fat weight in rats (7). Therefore, the observed increase in epididymal fat (Table 2) would be due to the difference in total calorie intake among the rats. Nevertheless, the difference (epididymal fat weight and calorie intake) seems not to be closely related to plasma or liver PCOOH levels, because of the absence of differences in total food intake, other organ weights and plasma parameters among the rat groups (Fig. 1, Tables 2 and 3).

In our previous study (7), a 4-wk intake of a high-fat diet led to a significant increase in plasma total cholesterol, liver PLOOH and plasma PLOOH levels for rats. However, in this study, we couldn’t find higher plasma total cholesterol, and thus we couldn’t demonstrate any hypolipidemic effects of young persimmon (Table 3) because our previous study used two times higher crude fat contents in the diet (HFD32®, CLEA Japan, Inc.; micronutrient composition of the diet was 6.2% moisture, 25.5% crude protein, 32% crude fat, 2.9% crude fiber, 4% crude ash and 29.4% nitrogen-free extract (calculated from mean values) and energy was 507.6 kcal/100 g diet) compared to the present study. Hence, we think that the fat content of the present diet may be too low to increase the blood cholesterol levels. The feeding period may be another important issue. Several studies showed that hypolipidemic effects of young persimmon were seen in a 10-wk feeding period (16, 17). Therefore, if the feeding period of the present study was longer, hypolipidemic action might be observed.

As shown in Fig. 2, plasma PCOOH levels in the young persimmon group (36.1 ± 28.5 pmol/mL plasma) were significantly lower than in the control group (120 ± 66 pmol/mL plasma). Meanwhile, there was no significant difference in plasma PCOOH levels between the mature persimmon group (57.3 ± 15.6 pmol/mL plasma) and control group. These results indicate that accumulation of plasma PCOOH is attenuated by the intake of young persimmon. The findings raise the question of why young persimmon shows greater antioxidant activity than mature persimmon. The most likely contributors to the antioxidant effects of young persimmon are carotenoids, ascorbic acid, vitamin E, and soluble tannin.

The nutrient values of mature persimmon (but not young persimmon) have been reported by the USDA as: carotenoids 488 µg and vitamin E 0.73 mg/100 g wet weight persimmon (23). Zhao et al. reported that total carotenoid levels in mature persimmon are higher than in young persimmon (24). The maximum content of total carotenoid they analyzed was around 100 µg/100 g wet weight of mature persimmon. However, considering the results of our previous study (22, 23), these carotenoids and vitamin E contents would be too low to prevent lipid peroxidation in vivo.

Meanwhile, Ramin and Tabatabaie investigated the characteristics of persimmon at different harvesting stages (Table 1) (13). They mentioned that the ascorbic acid and soluble tannin levels of green-colored persimmon harvested in October were much higher than those of orange-colored persimmon harvested in November. In the present study, we harvested green-colored young persimmon in October, and orange-colored mature persimmon in November. We thought that the young persimmon used in the present study was rich in ascorbic acid and soluble tannin compared to mature persimmon. Furthermore, Del Bubba et al. measured the soluble tannin levels in persimmons and found them to range from 0.2–1.2 g gallic acid equivalent/100 g wet weight persimmon (14). They investigated the soluble tannin and ascorbic acid content of persimmon at different harvesting stages and found that young persimmon (harvested in August) had two-fold greater soluble tannin and four-fold greater ascorbic acid content compared to mature persimmon (harvested in October). Soluble tannin is a phenolic compound and is mainly composed of epicatechin, epigallocatechin, epicatechin-3-O-gallate, and epigallocatechin-3-O-gallate (12, 26). Soluble tan-
nin has antioxidant effects and was shown to reduce PCOOH levels in vivo (27, 28). Meanwhile, ascorbic acid is a well-known dietary antioxidant that reduces lipid peroxidation levels in the body (29). Thus, soluble tannin and ascorbic acid may be responsible for the difference in antioxidant capacity of young and mature persimmon. Compared to the control group, the plasma PCOOH level of the mature persimmon group tended to decrease (Figs. 2 and 3). This would be because mature persimmon contains a considerable amount of ascorbic acid and soluble tannin.

Liver PCOOH levels did not differ among groups in spite of the decreased in plasma PCOOH levels (Figs. 2 and 3). In contrast, our previous study showed that plasma and liver PLOOH levels of high-fat diet-fed rats were significantly decreased by daily intake of vitamin E (tocotrienol) (7). Hence, soluble tannin/ascorbic acid and tocotrienol may show different antioxidant ability. Further studies are needed to evaluate the mechanisms and relationship between persimmon antioxidants and PLOOH generation in the body. Furthermore, a clinical study is needed to clarify the possibility of mitigating lifestyle-related diseases in the future.

Acknowledgments

The present study was supported in part by a Grant-in-Aid for Scientific Research (Kakenhi Kiban(s) 20228002) from the Japanese Society for the Promotion of Science (JSPS, Tokyo, Japan). Taiki Miyazawa was supported by a JSPS Postdoctoral Fellowship for Research Abroad (http://www.jsps.go.jp/).

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