Consumption of Japanese Yam Improves Lipid Metabolism in High-Cholesterol Diet-Fed Rats

Yuri KUSANO1, Nobuko TSUJIHARA2, Hironori MASUI1, Hana KOZAI1 and Wakako TAKEUCHI2

1 College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 478–8501, Japan
2 Faculty of Human Life and Environmental Sciences, Nagoya Women’s University, Nagoya 467–8610, Japan
3 Department of Human Life and Environmental Sciences, Mukogawa Women’s University, 6–46 Ikebiraki-cho, Nishinomiya, Hyogo 663–8558, Japan

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Summary We investigated the effects of dietary Japanese yam (Dioscorea japonica Thunb.) on lipid metabolism. Male Wistar rats (6 wk old) were fed a high-cholesterol diet for 6 wk and then supplemented with 26% of Japanese yam or 0.5% of its constituent diosgenin for a further 4 wk of high-cholesterol feeding (C6-J4 and C6-D4 groups, respectively). In the C6-J4 group, body weight gains significantly decreased, but skeletal muscle fiber sizes in quadriceps significantly increased compared with the other groups. Furthermore, Japanese yam supplementation resulted in the reduction of triglyceride contents in their liver, quadriceps, and intra-abdominal visceral fat. Diosgenin supplementation resulted in an increase in the numbers of skeletal muscle fibers and decrease in the fat accumulations in liver and of the lipid contents in quadriceps. Although quadriceps cholesterol contents decreased concomitantly with increased serum HDL-cholesterol in both the groups, fecal bile acid, fecal cholesterol contents, and fecal weight were higher in the C6-J4 group than in the C6-D4 group. Meanwhile, we demonstrated that Japanese yam inhibited micellar cholesterol solubility in vitro in a concentration-dependent manner. These results suggest that Japanese yam is more effective than diosgenin in reducing fat accumulation and improving cholesterol metabolism during chronic consumption of a high-cholesterol diet.

Key Words Japanese yam, lipid metabolism, diosgenin, dietary supplements, cholesterol

Japanese yam (Dioscorea japonica Thunb., Jin) belongs to Dioscoreaceae and is a traditional Japanese food that lacks toxic components, even when consumed raw. The characteristic stickiness of Japanese yam is an important factor in assessments of quality. In particular, Jin has high viscosity and has been used as a nutritional supplement since ancient times.

The viscous components of Jin essentially include polysaccharide protein complexes and viscosity has been correlated with intramolecular interactions of mucopolysaccharide contents (1). Its complexes comprise 53–55% carbohydrate and 2.0–2.3% protein, and differ little between varieties of Dioscoreaceae (2). The molecular structure of these complexes is characterized by protein bound to the main chain of acetylated mannose (2). Moreover, Jin reportedly includes other proteins in addition to the major protein component dioscorin (3), but lacks homology to well-known mannose-binding proteins (4).

Dioscoreaceae contain the steroidal sapogenin diosgenin (Dio), which is a biologically active phytochemical that has been used for the treatment of various disorders including inflammation, and cancer (5). Recently, 1,25D3-membrane-associated, rapid response steroid-binding protein (1,25D3-MARRS) was identified as a target protein for Dio. Moreover, Tohda et al. reported that Dio may function as an exogenous stimulator of 1,25D3-MARRS that induces axonal growth and regrowth of neurons (6). Hence, Dio may act on critical signaling pathways as a therapy for Alzheimer’s disease.

Among wild species of Dioscoreaceae, native strains of Jin are cultivated and collected in fields and mountains all over Japan, and its constituents and shapes vary between production areas and growers (7, 8). In previous studies, we investigated a Jin variety known as Inabu-2-gou from the Mountainous Agricultural Institute, Aichi Agricultural Research Center, and showed that its high viscosity reflects the presence of a sulphated acidic glycoprotein, and that resistant starch comprises about 2% of the fresh weight (8). We also demonstrated the presence of Dio in Inabu-2-gou (9) and showed high levels of high-density lipoprotein-cholesterol (HDL-Chol) in the blood following consumption of a high-Chol diet supplemented with freeze-dried Jin or Dio, with consequent decreases in fat accumulation in the liver and intra-abdominal viscera (9). Because high fat westernized diets are conducive to visceral fat accumulation,
components of Jin and Dio may ameliorate the related lifestyle diseases. Accordingly, in the present study, we investigated the effects of dietary Jin and Dio on lipid metabolism in chronically high-Chol diet-fed rats.

MATERIALS AND METHODS

Animals and diets. Six-week-old male Wistar rats (n=24) weighing 170–190 g were purchased from Japan SLC, Inc. Rats were housed in individual cages in an animal holding room with a 12 h light/12 h dark cycle at 22±2°C. On arrival, rats were acclimatized for 1 wk and provided a standard rodent chow diet (CE-2, CLEA Japan, Inc., Japan) and water ad libitum. Following acclimatization, rats were randomly divided into four groups of 6 animals, including a control group (CRL), a high-Chol diet group (C10), a high-Chol Jin-supplemented diet group (C6-J4), and a high-Chol Dio-supplemented diet group (C6-D4). Rats in the CRL group were fed standard rodent chow and rats in C10, C6-J4, and C6-D4 groups were fed high-Chol diets containing 0.5% Chol and 0.125% sodium cholate for 6 wk (Table 1). Rats in the C6-J4 group were fed a high-Chol diet supplemented with 26% of Jin (freeze-dried powder of Inabu-2-gou) and rats in the C6-D4 group were fed high-Chol diet supplemented with 0.5% of Dio (Tokyo Chemical Industry Co., Ltd., Japan) for weeks 7–10 of the experimental period. During this period, rats in the CRL and C10 groups were continuously fed standard and high-Chol diets, respectively. The total energy of each diet was 372 kcal/100 g.

Rats were weighed weekly and uneaten food was weighed to calculate food intake. Rat feces were collected during the last 4 d of the treatment period and were dried and weighed. After fasting for 10 h, rats were sacrificed under pentobarbital anesthesia (Somnopentyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan). Blood was obtained and wet liver, left quadriceps, and intraperitoneal visceral fat (kidney fat, and fat around the abdominal wall) were weighed.

Animal experiments were performed in accordance with the guidelines for animal experimentation from the Ministry of Environment of Japan and were approved by the Animal Experimental Committee of Nagoya Women’s University (authorization number: No. 24-5).

Sample preparation. Blood was collected when dissected and centrifuged at 1,700 ×g for 10 min (KUBOTA 5200, Kubota Corporation, Tokyo, Japan) after being placed in room temperature (RT) for 30 min. Subsequently, supernatants were collected. Liver lipids were extracted as previously described by Folch et al. (10). Briefly, liver tissues were filtered after homogenization in a 2:1 chloroform-methanol mixture. The chloroform phase containing lipids was then evaporated under a nitrogen stream at 70°C. Liver samples were then resuspended in isopropl alcohol containing 10% Triton X.

Skeletal muscle was homogenized in a 7:1:11:0.05 chloroform-isopropyl-alcohol-NP40 mixture and suspensions were centrifuged at 15,000 ×g for 10 min at RT. Supernatants were then incubated for 45 min at 50°C, vacuum dried in a rotary evaporator for 30 min, and resuspended in isopropl alcohol. Fecal samples were processed as described by Eaton and Klaassen (11). Briefly, dried feces were crushed using a mill and incubated in 99% ethanol at 70°C for 1 h. The extraction process was repeated three times.

Measurements of lipid components in blood and tissues. Total Chol (T-Chol), HDL-Chol, triglyceride (TG), and bile acid contents of tissues, feces, and blood samples were determined using the Chol E Test Wako, HDL-Chol E Test Wako, TG E Test Wako, and Total bile acids Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively. Using these measured values, low-density lipoprotein (LDL)+very low-density lipoprotein (VLDL)-Chol was calculated according to the following equation:

\[
\text{LDL+VLDL-Chol}=\text{T-Chol}-\text{HDL-Chol}
\]

Observations of skeletal muscles and liver tissues. Left quadriceps and liver tissues were fixed in 4% paraformaldehyde phosphate buffer solution and histological sections were examined and photographed using a microscope after staining with hematoxylin and eosin reagent (H&E staining) or Oil Red O reagent. Image analyses were performed using Image J software. Fiber sizes of skeletal muscles were analyzed by measuring the lesser diameter (minimal Feret’s diameter) (12) and the number of muscle fibers per unit area were determined.

Determinations of mitochondrial DNA copy numbers.
Mitochondria DNA copy numbers were quantified using polymerase chain reaction methods. Specifically, DNA was extracted using a blood and tissue kit (QIAGEN, Tokyo, Japan), and primers were designed to detect mitochondrial DNA-encoded cytochrome b (Cyt b; forward, 5′-TAT CGA CCT CCC CGC CCC ATC T-3′; reverse, 5′-AGC CGT AGT TTA CGT CTC GGC A-3′) and cytochrome oxidase subunit II (COII; forward, 5′-TGA GCC GTC CCT TCA CTA G-3′; reverse, 5′-TGA GCC GCA GAT TTC AGA G-3′). Separate primers were designed to detect nuclear DNA-encoded β-actin (forward, 5′-AGC GAG CCG GAG CCA ATC AG-3′; reverse, 5′-TGC GCC GCC GGT TTT TAT AGG-3′) (13). Reaction end products were separated using 1% agarose gel electrophoresis and ethidium bromide staining.

Determination of micellar solubility of Chol. Micellar solubility of Chol was measured using the method described by Nagaoka et al. (14). Briefly, Chol and Jin were added to micellar solutions containing 6.6 mM sodium taurocholate, 0.5 mM Chol, 1 mM oleic acid, 0.5 mM monolein, 0.6 mM phosphatidylcholine, 132 mM NaCl, and 15 mM sodium phosphate (pH 7.4). Chol and Jin concentrations were 5.2 mM and 0–2.0% (w/v), respectively. Subsequently, mixtures were incubated at 37°C for 24 h and ultracentrifuged at 100,000 × g for 60 min at 37°C. Supernatants were then collected and the concentrations of Chol in the supernatants were determined using Wako Chol E Test.

**RESULTS**

**Jin inhibited body weight gain without decreasing skeletal muscle size in rats fed a high-Chol diet**

Rat body weights during the experimental period are presented in Fig. 1A. Although body weights increased throughout the experimental period in the CRL, C10 (high-Chol diet), and C6-D4 groups (0.5% Dio-supplemented high-Chol diet), increases in body weight in the C6-J4 group (26% Jin-supplemented high-Chol diet) were gradual after 7 wk and ceased after 10 wk. To confirm this inhibiting effect, we calculated weight gains for the first 6 wk and last 4 wk. The body weight gains for the last 4 wk in the C6-J4 group were significantly suppressed to approximately 60% compared with those in the other groups (Table 2). Dietary intake did not differ significantly between groups (Fig. 1B), and total energy contents were 372 kcal/100 g in all animals, showing that Jin supplementation resulted in inhibited body weight gain. According to analyses by Japan Food Research Laboratories, the present Japanese yam contained dietary fibers as well as Dio (6.3 g and 0.07 mg/100 g of freeze-dried, respectively). Using a Megazyme Resistant Starch Assay Kit (Megazyme International, Ltd., Wicklow, Ireland), we determined that it also contained 45.4 g of resistant starch in 100 g of

<table>
<thead>
<tr>
<th>Body weight gain (g)</th>
<th>Until the 6th week1</th>
<th>After the 7th week2</th>
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<tr>
<td>CRL 168.8 ± 20.45</td>
<td>40.3 ± 6.28*</td>
<td></td>
</tr>
<tr>
<td>C10 159.8 ± 14.88</td>
<td>46.1 ± 8.95*</td>
<td></td>
</tr>
<tr>
<td>C6-J4 164.6 ± 15.99</td>
<td>24.6 ± 5.89b</td>
<td></td>
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<tr>
<td>C6-D4 174.1 ± 11.82</td>
<td>41.0 ± 10.03a</td>
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</table>

Data are presented as means ± standard errors of six rats. Different letters indicate significant differences between groups; Turkey-Kramer test, p < 0.05.

1 Initial body weights were deduced from weights measured in the 6th week after the start of the experiment.

2 Body weights measured in the 6th week were deduced from weights before fasting.
freeze-dried product. Taken together, these data showed that Jin inhibited body weight gains due to different compositions from Dio.

To determine whether inhibition of weight gains affected skeletal muscle tissues, H&E images of quadriceps cross-sections after the experimental period were examined. The staining patterns were similar between the CRL and C10 groups and between the C6-J4 and C6-D4 groups, revealing that connective tissues between muscle fibers were decreased in rats fed Jin- or Dio-supplemented diets (Fig. 2A). Moreover, significant increases in muscle fiber diameters and areas were observed in the C6-J4 group, showing that Jin supplementation resulted in inhibition of weight gain with increasing rather than decreasing skeletal muscle fiber size (Fig. 2B). Interestingly, in the quadriceps cross-sections of the C6-D4 group, muscle fiber diameters and areas did not significantly differ compared with the CRL and C10 groups, but the numbers of muscle fibers were significantly more than those of the other groups (Fig. 2B). These results suggested that Dio supplementation may contribute to the density enhancement of skeletal muscle fibers, leading to decreased connective tissues between muscle fibers.

Decreases in fat accumulation in livers were accompanied by the Jin-supplemented diet.

Shapes and sizes of rat livers differed significantly between the present experimental groups (Fig. 3A). Specifically, whereas livers in the C10 group showed hypertrophy with uneven surfaces and white regions, these indicators of liver damage were significantly ameliorated in the C6-D4 group, and more so in the C6-J4 group. Although the ratios of liver weight to body weight were high in the C10, C6-J4, and C6-D4 groups (Fig. 3B), these were significantly lower in the C6-J4 than in the C10 and C6-D4 groups, suggesting that liver hypertrophy was ameliorated by Jin supplementation.

Liver Chol and TG contents in the C10 group were significantly higher than those in the CRL group, reflecting enhanced accumulation of Chol and TG in liver tissues following feeding on the present high-Chol diet (Figs. 3C and D). However, no significant differences in liver Chol contents were observed among the C10, C6-J4, and C6-D4 groups, indicating no effects of Jin or Dio supplementation on liver Chol contents (Fig. 3C). In contrast, liver TG contents were significantly lower in the C6-J4 group than in the C10 and C6-D4 groups and were decreased almost to CRL levels (Fig. 3D). This indicated that Jin supplementation prevents TG accumulation in liver.

H&E image analyses of liver tissues revealed large unstained vesicles in the C10 group, and smaller unstained vesicles in the C6-D4 group, which decreased in the C6-J4 group to sizes similar to those observed in the CRL group (Fig. 4A). Moreover, vesicles that were identified as oil droplets indicated that liver fat accumulation differed among groups.

In further experiments, localization of lipids from portal to central veins was investigated using Oil Red O reagent. Although liver lipids were clearly observed around portal veins and were comparatively less apparent around central veins (Fig. 4B, a–c), fat accumulation was ubiquitously increased by the high-Chol diet (Fig. 4B, d–f). However, fat accumulation in the C6-J4 group was decreased, and Oil Red O images were similar to those from CRL animals (Fig. 4B, g–i). Oil droplets were smaller in the C6-D4 group than in the C10 group (Fig. 4B, j–l) but were not as marked as those in the C6-J4 group. These results verified that Jin significantly inhibited liver lipid accumulation and that Dio also has beneficial effects.

Decreased body fat following Jin supplementation

Intra-abdominal visceral fat contents (kidneys, around testicles, and posterior wall of stomach) after the experimental period were calculated as intra-abdominal visceral fat weights per body weight. In these experiments, intra-abdominal visceral fat contents did not differ significantly among the CRL, C10, and C6-D4 groups (Fig. 5A), indicating that intra-abdominal visceral fat was not affected by the present high-Chol diet and was not inhibited by Dio supplementation. However, intra-abdominal visceral fat contents in the C6-J4 group significantly decreased compared with those in the other groups, leading to suppressed weight gains in Jin-fed rats.

In further experiments, TG contents in quadriceps were significantly greater in the C10 group than in the CRL group, indicating that fat accumulation in quadriceps was affected by the high-Chol diet. In addition, TG contents in the C6-J4 and C6-D4 groups were increased in comparison with those in the CRL group, but tended to decrease in comparison with those in the C10 group. Moreover, inhibition of fat accumulation in the liver and intra-abdominal viscera differed between C6-J4 and C6-D4 groups (Fig. 3D), whereas TG contents in quadriceps were similar (Fig. 5B). These data suggested that Dio might enhance energy metabolism in skeletal muscles. Accordingly, we measured mitochondrial DNA (mtDNA) copy number per nuclear genome to examine the effects of Jin and Dio on aerobic metabolism in skeletal muscles. The copy number significantly increased in the C10, C6-J4, and C6-D4 groups compared with that in the CRL group, but did not differ among the three groups, showing that the high-Chol diet resulted in increased copy numbers of mtDNA (Fig. 5C). Meanwhile, as shown in Fig. 2B, Dio supplementation resulted in a significant increase in the numbers of muscle fibers in the quadriceps. Taken together, it was assumed that Dio may contribute to the increased fat metabolism in skeletal muscles at a tissue level. Chol contents in quadriceps tended to increase in the C10 group, but did not differ significantly between any of the present study groups (Fig. 5D).

Effects of Jin supplementation on blood lipids

Blood TGs significantly increased at the end of the experiment in the CRL group compared with those in the other three groups (Table 3), suggesting accumulation in the blood rather than incorporation into visceral tissues in the CRL group. In addition, blood TGs concentrations in the C6-J4 and C6-D4 groups tended to
Fig. 2. Effects of Jin or Dio on skeletal muscle tissues in chronically high-Chol-fed rats. (A) Representative H&E staining sections of rat femoral muscles; Scale bar, 100 μm. (B) Minimal Feret’s diameters (Fiber Diameter), areas (Fiber Area), and numbers (Fiber Number) were determined from >200 measurements of five sections per 4–5 femoral muscles from each group. Data are presented relative to CRL values. Different letters indicate significant differences between groups; Turkey-Kramer test, \( p<0.05 \).

Fig. 3. Effects of Jin or Dio on lipid accumulation in livers of chronically high-Chol diet-fed rats. (A) Macroscopic appearance of livers at laparotomy after the treatment period. (B) Liver-to-body weight ratios. (C) Liver Chol contents are shown as mg per g of liver. (D) Liver TG contents are presented as mg per g of liver. Data are presented as means±SE of six rats. Different letters indicate significant differences between groups; Turkey-Kramer test, \( p<0.05 \).
Fig. 4. Localization of lipids in livers from chronically high-Chol diet-fed rats. (A) Representative H&E staining sections of rat livers after the treatment period. (B) Representative Oil Red O staining sections of rat livers in CRL (a–c), C10 (d–f), C6-J4 (g–i), and C6-D4 (j–l) groups. Lipid distributions were examined by dividing liver lobules into periportal (a, d, g, j), portal vein to central venule (b, e, h, k), and central venule areas (c, f, i, l); Scale bar, 25 μm.
increase compared with those in the C10 group.

Blood T-Chol levels were higher in the C10 and C6-D4 groups than in the CRL group, and were significantly higher in the C6-D4 group than in the C10 group. In previous studies, Xu et al. (15) and Cayen et al. (16) identified Dio as a phytosterol in the blood after absorption from the intestinal tract. Accordingly, the present data suggest that T-Chol levels were affected by Dio supplementation in the C6-D4 group. The blood T-Chol levels in the C6-J4 group were not significantly higher than those in the CRL group.

Blood HDL-Chol concentrations were lower in the C10 group than in the CRL group, but did not differ significantly among the CRL, C6-J4, and C6-D4 groups. This suggested that transport of Chol to the liver was accelerated by supplementation with Jin or Dio. These data confirmed the observations of decreased Chol in skeletal muscles in the C6-J4 and C6-D4 groups (Fig. 5D). In addition, the data indicated that Jin or Dio supplementation resulted in reduced Chol absorption in the intestine and/or increased Chol excretion from the liver. Bile acid and Chol excretions were significantly increased by Jin.

Bile acid and Chol contents were determined in the feces at the end of the treatment period, and daily bile acid and Chol excretion were calculated. In these experiments, bile acid and Chol excretions were significantly higher in the C10 group than in the CRL group (Figs. 6A and B), reflecting high Chol in the diet. However, bile acid and Chol excretions were significantly higher in the C6-J4 group than in the C10 group (Figs. 6A and B). In particular, both Chol excretion and fecal weight in the C6-J4 group were increased to approximately 3-fold compared with the C10 and C6-D4 groups (Figs. 6B and C), suggesting that Chol absorption was affected by dietary fiber in Jin.

Fig. 5. Lipid contents of abdomen and skeletal muscle tissues from chronically high-Chol diet-fed rats. (A) Ratios of intra-abdominal fat to body weights; Intra-abdominal fat weights included dissected kidney leaf fat, epididymal fat, and mesenteric fat, and are presented relative to body weights. (B) TG contents in skeletal muscles are shown as mg per g of femoral muscle. (C) Mitochondrial DNA copy numbers were determined in femoral muscles of rats according to ratios of mitochondrial DNA-encoded Cyt b and COII DNA to nuclear β-actin DNA. (D) Chol contents in skeletal muscles are shown as mg per g of femoral muscle. Data are presented as means±SE of six rats. Different letters indicate significant differences between groups; Turkey-Kramer test, p<0.05.
Japanese Yam Consumption Improves Lipid Metabolism

Table 3. Serum lipid levels.

<table>
<thead>
<tr>
<th></th>
<th>CRL</th>
<th>C10</th>
<th>C6-J4</th>
<th>C6-D4</th>
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<tr>
<td>TG (mg/dL)</td>
<td>92.5 ± 20.41a</td>
<td>54.1 ± 6.03b</td>
<td>61.4 ± 8.81b</td>
<td>64.2 ± 12.52b</td>
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<td>T-Chol (mg/dL)</td>
<td>69.8 ± 10.41a</td>
<td>109 ± 13.05b</td>
<td>91.7 ± 25.24ab</td>
<td>149 ± 7.61c</td>
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<tr>
<td>HDL-Chol (mg/dL)</td>
<td>50.8 ± 20.59a</td>
<td>24.3 ± 13.00b</td>
<td>47.3 ± 11.68ab</td>
<td>35.4 ± 5.11ab</td>
</tr>
<tr>
<td>LDL+VLDL-Chol (mg/dL)</td>
<td>16.0 ± 10.22a</td>
<td>66.4 ± 18.43b</td>
<td>44.3 ± 28.26ab</td>
<td>114 ± 8.40c</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE of six rats. Different letters indicate significant differences between groups (Tukey-Kramer test, p < 0.05).

Micellar solubility of Chol was significantly inhibited by dietary intake of Jin

The micellar solubility of Chol with 0–2.0% Jin was determined as described previously by Nagaoka et al. (14). Micellar solubility of Chol was significantly decreased in the presence of Jin in a concentration-dependent manner (Fig. 7), revealing that Jin included the component which inhibited the micellar solubility of Chol. This result suggested that the micellar solubility of lipids was decreased in the C6-J4 group, thus inhibiting the absorption of both Chol and dietary lipids. Consequently, decreases in the supply of Chol and TG to the liver likely led to decreased Chol and fat accumulation in liver and peripheral tissues, and decreased body weight gains.

DISCUSSION

In the present study, we demonstrated the lipid-lowering effects of Jin supplementation and prevention of weight gain without reduction of skeletal muscle fiber size in chronically high-Chol fed rats. Although the Dio-supplemented diet did not produce significant differences in body weight gains, it decreased lipid accumulation in the liver and skeletal muscle and increased the number of skeletal muscle fibers. On the other hand, while quadriceps Chol contents decreased concomitantly with increased serum HDL-Chol in the C6-J4 and C6-D4 groups, fecal bile acid and fecal Chol contents were higher in the C6-J4 group than in the C6-D4 group. These data show that Jin is more effective than Dio in reducing fat accumulation and improving Chol metabolism in chronically high-Chol diet-fed rats. In addition, Jin inhibited micellar Chol solubility in vitro in a concentration-dependent manner. Taken together, it is suggested that the effects of dietary Jin on lipid metabolism during chronic consumption of a high-Chol diet may be controlled by Dio as well as other constituents.

High-Chol diet promotes lipid storage

After 10 wk of high-Chol feeding, both TG and Chol contents were increased in livers from rats (Figs. 3C and D) and in muscular quadriceps femoris tissues (Figs. 5B and D). In particular, lipid accumulation was observed in both venae centrales hepatis and in periportal sites, resulting in external morphological changes (Fig. 4). Although chronic consumption of diets containing high levels of saturated fat reportedly induces markers of intramyocellular lipid accumulation in the soleus of mice (17), lipids in the muscular quadriceps femoris were localized to connective tissues in the present animals (data not shown). Furthermore, high fat diet-fed rats reportedly showed increased mitochondrial biogenesis and fatty acid oxidation capacity in skeletal muscles (18, 19). In the present study, increases in mitochondrial biogenesis in skeletal muscles of high-Chol diet-fed rats (Fig. 5C) may have been induced by liver and skeletal muscle lipid stores.

Dio blocks chronic high Chol diet-induced lipid accumulation

Rats of the present C6-D4 group were fed a 0.5% Dio-supplemented high-Chol diet for 4 wk after high-Chol feeding for 6 wk. In this study, we demonstrated that Dio supplementation resulted in significant increase in the numbers of muscle fibers (Fig. 2B) and inhibition of lipid accumulation in the liver (Fig. 4) and in skeletal muscles (Fig. 5B). Dio is a component of the steroid sapogenin and was previously detected in the plasma of rats and humans after oral administration (15, 16), showing that the orally taken Dio may be adsorbed and act directly on the tissues in the body. Meanwhile, in previous studies, Dio increased serum dehydroepiandrosterone (DHEA) levels (20) and DHEA is believed to act as a precursor of testosterone, that induces skeletal muscle differentiation (21–23). Thus, the adsorbed Dio may also act indirectly on skeletal muscles, leading to increased the numbers of muscle fibers. Furthermore, we also demonstrated that the high-Chol diet resulted in increased copy numbers of mtDNA (Fig. 5C). These results suggest that the Dio-supplemented high-Chol diet may contribute to the increased lipid metabolism in skeletal muscle at the tissue level. However, Dio-supplementation did not markedly elevate the blood TG concentrations (Table 3) and reduce the accumulation of fat in the liver and intra-abdominal viscera (Figs. 3D and 5A). Taken together, it is suggested that the Dio-supplemented high-Chol diet may facilitate alterations in skeletal muscles, but not trigger fat metabolism.

It is widely accepted that Dio promotes fecal Chol excretion by stimulating biliary Chol secretion and decreasing intestinal Chol absorption. Accordingly, Chol synthesis was significantly increased in liver homogenates, isolated hepatocytes, and in whole livers from Dio-fed rats (24–26). Although liver (Fig. 3C) and fecal (Fig. 6B) Chol contents did not change significantly in the present Dio-supplemented rats, plasma HDL-Chol (Table 3) and fecal bile acid (Fig. 6A) levels were slightly increased in the C6-D4 group, potentially reflecting synthesis of bile acids from Chol.
Jin blocks lipid absorption in the intestines and decreases lipid accumulation following chronic consumption of a high-Chol diet

Although the analysis of the present Japanese yam by Japan Food Research Laboratories indicated Dio contents of 0.07 mg/100 g of freeze-dried product, quantities of the Dio glucoside dioscin were not reported. Orally administered dioscin is reportedly absorbed from the intestines, distributed to the liver, plasma, and other tissues, and is metabolized to Dio in the liver (27–30). Since Jin contains both Dio and dioscin, the effects of Jin supplementation in rats may reflect both molecules. However, Jin led to greater improvements in lipid metabolism than Dio, suggesting the presence of other active ingredients. Accordingly, in the analyses by Japan Food Research Laboratories, 6.3 g of dietary fibers were found to be contained in 100 g of the present freeze-dried Jin. Therefore, the present Jin-supplemented diet contained 1.6 g of dietary fiber and resulted in significant 3-fold increases in lipid excretion (Fig. 6C), suggesting that dietary fiber may also affect lipid metabolism in rats.

Although Chol contents in livers of the C6-J4 group did not differ significantly from those in the C10 group (Fig. 3C), plasma HDL-Chol concentrations in the C6-J4 group were similar to those in the CRL group (Table 3). Hence, Jin supplementation may alter Chol homeostasis in the liver. Moreover, feces from the C6-J4 group had considerably higher bile acid and Chol contents than those in the other group (Figs. 6A and B). In particular, daily Chol excretion was 116±20.8 mg and potentially exceeded daily Chol intake (Fig. 6B). Furthermore, we demonstrated that dietary Jin inhibited micellar Chol solubility in vitro in a concentration-dependent manner (Fig. 7). Therefore, inhibition of lipid digestion and absorption may follow the failure of micellar formation in the presence of dietary fiber in Jin preparations, leading to increased bile acid and Chol excretion and decreased Chol and TG levels in rats.

Jin contains mucin that primarily comprises the soluble dietary fiber (SDF) mannan (2, 8). Nishimura et al. has reported that Chinese yam contains 1.9% (w/w) of the SDF (31). Proposed biological mechanisms for the Chol-lowering effects of SDF include (1) increased barrier properties of the unstirred layer between micelles and intestinal absorptive cells, (2) molecular associations of SDF and bile salt, and (3) formation of a local SDF matrix that entraps bile salt micelles (32–35).
addition, we also confirmed using a Megazyme Resistant Starch Assay Kit (Megazyme International Ltd.) that the present freeze-dried Jin contained 45.4% (w/w) resistant starch. The short-chain fatty acids that are produced from these dietary fibers by anaerobic intestinal microbiota have been shown to exert direct and/or indirect effects on mammalian energy metabolism (36). Raw Chinese yam has been reported to be especially effective as a source of resistant starch and facilitates the production of short-chain fatty acids and to have a plasma-Chol-lowering effect due to the inhibition of VLDL release from the liver (31). Jin supplementation resulted in the reduction of blood LDL+VLDL-Chol concentrations (Table 3), suggesting that Jin may contain the component(s) that control VLDL release from the liver. Meanwhile, it has been reported that short-chain fatty acids are produced by symbiotic relationships with the cecal flora, indicating that the balance of the intestinal flora is also important for the serum and liver Chol concentrations (37). Thus, the effects of Jin on the intestinal lumen may control the lipid and Chol metabolism. However, these mechanisms remain controversial and warrant further studies.

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