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# Persimmon *(Diospyros Kaki* Thunb 'Saijo') Peel Improved Dyslipidemia and its Related Production of Atherogenic Autoantigen Complexes in Low-Density Lipoprotein Receptor-Deficient Mice

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**Abstract:** Role of persimmon (*Diospyros kaki*, Thunb 'Saijo') peel was investigated on developing atherosclerosis in low-density lipoprotein receptor (LDLR)-deficient mice with emphasis on lipid metabolism, physio-biological oxidation, production of related atherogenic autoantigens, and production of anti-atherogenic natural antibodies. Male LDLR-deficient mice were fed high fat diet or high fat diet supplemented with 10% dried and powdered persimmon peel (PP) for 12 weeks. PP supplementation significantly prevented the increment of plasma cholesterol and triglyceride levels. High fat diet feeding increased plasma level of oxidized LDL/β2-glycoprotein I (oxLDL/β2GPI) complexes regarded as an atherogenic autoantigen, while PP supplementation significantly blocked the increment ($p<0.05$). After a 12-week feeding, atherosclerotic plaques in mice fed with diet supplemented with PP decreased by 70% as compared to that of mice fed the high fat diet ($p<0.005$). PP feeding also reduced urinary 11-dehydro-thromboxane $B_2$, a stable metabolite of the platelet activation marker thromboxane $A_2$, but level of IgM anti-oxLDL antibodies was not changed. Thus, these results obviously demonstrate that persimmon peel may have an anti-atherogenic property through normalization of lipid metabolism and reduced production of the atherogenic complexes.

**Keywords:** Persimmon peel, oxidized low-density, lipoprotein, β2-glycoprotein I, atherosclerosis, II-dehydro-thromboxane $B_2$.

## 1. INTRODUCTION

The fruit persimmon (*Diospryros kaki*) contains several bioactive compounds, such as polyphenols, flavonoids, terpenoids, steroids, dietary fiber, carotenoids and minerals [1]. Though persimmon peel (PP) is rich in carotenoids and polyphenols rather than persimmon pulp [2], the peel is discarded during production of dried fruit. It has been reported that PP components have beneficial effects such as antioxidant activity [3-5], and tyrosinase inhibiting activity (whitening activity) [6]. It has also been reported that young persimmon fruit has hypolipidemic effect [7,8]. In the present study we examined the effect of PP on dyslipidemia, atherogenesis, and production of immune-regulated components in hypercholesterolemic mouse model to evaluate the possibility that PP is a food supplement against atherogenesis.

Atherosclerosis is a chronic inflammatory disease that results from disturbed lipoprotein metabolism, the formation of pro-inflammatory lipid peroxidation products, and host's immune responses. *In vitro* observations suggest that native low-density lipoprotein (LDL) itself does not induce any features associated with atherosclerosis (e.g. formation of lipid-laden form cells from macrophages) but oxidatively modified LDL (oxLDL) and its byproducts are highly pro-inflammatory and pro-atherogenic [9, 10].

OxLDL accumulation in macrophage-derived foam cells in atherosclerotic lesions has been detected using anti-oxLDL antibodies [11, 12]. OxLDL in human circulation has also been detected using anti-oxLDL antibodies and anti-apolipoprotein B (apoB) antibodies [13-15]. OxLDL contains a wide variety of oxidation-specific epitopes that makes it an excellent immunogen. These oxidation specific epitopes lead to profound immune responses including autoantibody production that modulate lesion formation. Natural antibodies, mainly IgM responses to oxLDL have been found and some of them cloned [16-18]. Some autoantibodies to oxLDL derived from 'naive' atherosclerotic mice share complete genetic and structural identity with antibodies from the classic anti-phosphorylcholine B-cell clone T15, which protects against common infectious pathogens including pneumococci [19].

We demonstrated that oxLDL/β2GPI complex is a major atherogenic and thrombogenic autoantigen in patients with

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antiphospholipid syndrome (APS), a systemic autoimmune disease [20-24], and that elevated level of oxLDL/β2GPI complexes was detected in patients with systemic autoimmune diseases, diabetes mellitus, and chronic renal disease [25-28]. OxLDL/β2GPI induced β2GPI specific autoreactive T cells while soluble β2GPI alone did not [29, 30].

Mice lacking LDL receptor (LDLR) have less overt disease than apolipoprotein E (ApoE)-deficient mice [31]. Very low-density lipoprotein (VLDL) formed in the liver is partially metabolized by lipoprotein lipase, generating plasma intermediate density lipoprotein (IDL) particles. Usually, IDL is cleared by the liver *via* LDLR. When LDLR is defective, IDL remains in the circulation where they are converted to LDL. LDLR-deficient mice have a modest 2 fold-higher plasma cholesterol level (than normal C57BL/6 mice) when maintained on a normal chow diet, and they only develop atherosclerotic plaques slowly [32]. However, in response to a high-fat, high-cholesterol diet, LDLR-deficient mice exhibit massive elevations in plasma cholesterol and rapidly develop atherosclerotic lesions throughout the aorta. In contrast, ApoE deficient mice develop lesions while being fed a chow diet. We considered it would be convenient to use LDLR-deficient mice to examine the dietary effect, as most part of their lesions would be generated by the effect of diet.

In this study, we measured the level of oxLDL/β2GPI complexes in LDLR-deficient mice fed a high fat diet and the PP-supplemented high fat diet. The objectives of the current study were to determine whether a diet containing PP could alter the progression of atherosclerosis in the LDLR-deficient mice, influence blood lipid levels, markers of oxidative stress such as 8-OHdG, atherogenic autoantigen complexes, and natural antibody production against oxLDL.

## 2. MATERIALS AND METHODS

### 2.1. Diets

In this study, we used three types of diet. The first was a normal chow diet (MF, Oriental Yeast, Co. Ltd. Tokyo, Japan) containing 0.08% cholesterol and a high fat diet. The second was a high fat diet (HF) containing 0.2% cholesterol and 21% milk fat (Oriental Yeast). The third diet was HF containing 10% dried persimmon *(Diospyros Kaki* Thunb. 'Saijo') peel (PP) powder, which was designated as HF + PP. PP was sun-dried for 30 days and further dried with vacuum dryer (BCD-2000U; Yahiro Industries, Minokamo, Japan) at 40°C for 14 h, followed by milling with pinmill (Sogo-Sangyo, Tokyo, Japan). The resulting powder was added at 10% in HF. The macronutrient composition of the dried persimmon peel powder and experimental diets are shown in Table **1**. Total calories were calculated according to Atwater energy equivalent.

### 2.2. Animals and Experimental Protocol

LDLR deficient mice (B6, 129S7-Ldlr$^{tm1Her}$/J) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Three to five animals were housed per cage in a temperature-controlled animal facility with a daily photoperiod of 12 h of light at the Department of Animal Resources, Advanced Science Research Center, Okayama University. Mice fed normal chow diet (MF) until the experiment started. At 12 weeks of age, male LDLR deficient mice were randomly assigned to three groups and fed HF (n=11), HF + PP (n=7), or normal chow (n=10) diet for 12 weeks. Besides, eight 12 weeks old male LDLR deficient mice were fed on HF and received acetylsalicyclic acid (aspirin) (30 mg/L) in their drinking water, which was replaced with fresh water every other day, for 12 weeks. Considering that each animal drinks in average 3 to 4 mL of water per day, this would be equal to 90 to 120 μg aspirin per day for a mouse of 30-g of weight. On a body scale-adjusted scale, this amount would be equal to 180 to 240 mg/day if the animals weighed 60 kg [33]. Body weight was measured at the beginning of the study and following every 4 weeks. On the same day for body weight measurement, each mouse was set in a metabolism cage with water but without any foods for 5-6 h. Then urine sample dropped into the container at the bottom was gathered. Right after the urine collection, blood samples were obtained from animals by retro-orbital bleeding with EDTA as an anticoagulant. All animal experiments were performed according to the guidelines of Okayama University and the study protocol was approved by the Committee on Animal Experimentation of Okayama University.

### 2.3. Plasma Lipids

Plasma cholesterol and triglyceride were determined enzymatically using commercial kits (Cholesterol E-test and Triglyceride E-Test, respectively; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Table 1. Nutritional Data for the Persimmon Peel and Experimental Diets**

| Constituents | ND | HF | PP | HF + PP |
|---|---|---|---|---|
| Protein (%) | 27.8 | 17.9 | 4.1 | 16.5 |
| Fat (%) | 4.9 | 21.0 | 1.4 | 19.0 |
| Carbohydrates (%) | 48.4 | 49.1 | 71.3 | 51.3 |
| Fiber (%) | 4.4 | 3.0 | 12.7 | 4.0 |
| Ash (%) | 7.7 | 2.0 | 2.5 | 2.1 |
| Moisture (%) | 6.8 | 7.0 | 8.0 | 7.1 |
| Energy (kcal/100g) | 349 | 457 | 314 | 442 |

ND, Normal mouse chow diet (MF, Oriental Yeast); HF, a high fat diet containing 21% milk fat, 0.2% cholesterol; PP, dried and powdered persimmon peel; HF + PP, HF added with 10% PP.

### 2.4. Preparation of Mouse Aortas and Quantification of Atherosclerosis

Mice were euthanized after the final blood collection at 24 weeks of age. The aortic tree was perfused with phosphate buffered saline (PBS) containing 20 μM/L butylated hydroxytoluene (BHT) and 2 μM EDTA, pH7.4, by inserting a cannula into the left ventricle and allowing free efflux from an incision in the vena cava. After removal of the surrounding adventitial fat tissue, the aorta was opened longitudinally from the aortic root to the iliac bifurcation, fixed with PBS containing 4% formaldehyde, and stained with Sudan IV. The extent of atherosclerosis was determined by the "en face" method [34]. Quantification was performed by capturing the images of aortas with Penguin 150 CL camera (Pixera Corp. San Jose, CA) connected to SZX12 dissection microscope (Olympus Corp. Tokyo, Japan). The lesion percent of aorta was estimated by Scion Image analysis.

### 2.5. Preparation and Oxidation of Mouse LDL

LDL fraction was collected from another group of LDLR-deficient mice to prepare oxLDL/β2GPI complexes for an ELISA. Twelve LDLR-deficient mice fed HF for more than 4 weeks were anesthetized with ethyl ether and blood was drawn from the right ventricle using EDTA as an anticoagulant. The plasma was separated by centrifugation and pooled. LDL was separated by sequential ultracentrifugation at 550,000 x g adjusting the density with potassium bromide as described previously [13], using TL-100 ultracentrifuge (Beckman Coulter) and TLA100.3 rotor. After LDL fraction was dialyzed against PBS to remove EDTA and potassium bromide, it was catalytically oxidized with 5 μM $CuSO_4$ for 16 hours at 37°C as described before [24]. The oxidation was terminated by adding EDTA at final concentration of 1 mM. Aliquots were taken and subjected to assay for thiobarbituric acid-reactive substance (TBARS) and agarose gel electrophoresis. OxLDL/β2GPI complex as a calibrator was prepared by incubating mouse oxLDL and human β2GPI at the ratio of 2:1 (w/w) for 12 h at 37°C. The oxLDL and oxLDL/β2GPI complexes were stored at -80°C until use.

### 2.6. Antibodies

Mouse anti-β2GPI monoclonal antibody WB-CAL-1 was prepared as described previously [35]. Rabbit anti-mouse LDL polyclonal antibodies were prepared as follows. Briefly, Japanese white rabbits were immunized with the LDL obtained from LDLR-deficient mice together with Freund's Incomplete Adjuvant (Sigma-Aldrich, St. Louis, MO, USA) and boosted four times following every two weeks. The antibody titer of rabbit sera was checked with an ELISA against mouse native LDL and mouse oxLDL. IgG of mouse LDL antibody was purified by protein A-Sepharose CL-4B (GE Healthcare UK Ltd. Buckinghamshire, UK), and labeled with horseradish peroxidase (HRP) (Seikagaku Co., Tokyo, Japan).

### 2.7. ELISA for Mouse oxLDL/β2GPI Complexes

The ELISA for mouse plasma oxLDL/β2GPI complex was performed as follows, with slight modification of human assay system [25]. Anti-β2GPI monoclonal antibody WB-CAL-1 (8 μg/ml) was adsorbed on the wells of 96-well microtiter plate (Maxisorp, Nunc) by overnight incubation at 4°C. Then the wells were blocked with BSA for 2 h. Mouse plasma (100-fold diluted) or oxLDL/β2GPI complexes as a calibrator (8–800 ng/ml apoB equivalent) were incubated at 4°C overnight. The wells were subsequently incubated with HRP-labeled rabbit anti-mouse LDL antibodies for 3 h at room temperature. Color was developed with adding TMBUS substrate (Moss Inc, Pasadena MD, USA). The reaction was terminated by adding 2 N sulfuric acid and the OD at 450 nm was measured. Between each step, the wells were extensively washed with TBS containing 0.05% Tween 20. The intra-assay precision (CV) was less than 10%.

### 2.8. ELISA for 11-Dehydrothromboxane $B_2$ (11-dhTX$B_2$)

Urinary 11-dhTX$B_2$ was determined using 11-dhTX$B_2$ test kit (Corgenix, Broomfield CO, USA). The 10-fold diluted urine samples, 11-dhTX$B_2$ conjugated to alkaline phosphatase (AP), and purified mouse monoclonal antibody directed to 11-dhTX$B_2$ were incubated together in wells coated with a polyclonal anti-mouse antibody. Incubation allowed the endogenous 11-dhTX$B_2$ present in the samples to compete with the purified AP-conjugated 11-dhTX$B_2$ to the bound anti-11-dhTX$B_2$ antibody. After washing, paranitrophenylphosphate (pNPP) chromogenic substrate was added and color developed in the wells at an intensity inversely proportional to the urine sample concentration of 11-dhTX$B_2$. The concentration of 11-dhTX$B_2$ was adjusted by urinaly creatinine. Creatinine concentration was determined with Creatinine Assay Kit (Cayman Chemical Company Ann Arbor, MI, USA).

### 2.9. 8-Hydroxy-2'-Deoxyguanosine (8-OHdG) and Adiponectin Measurement

Urinary 8-OHdG levels were determined using an ELISA kit according to the manufacturer's instructions (Japan Institute for the Control of Aging, Fukuroi, Japan). Mouse plasma adiponectin levels were measured using an ELISA kit according to the manufacturer's instructions (Otsuka Pharmaceutical Co Ltd, Tokyo, Japan).

### 2.10. ELISA for IgM Anti-oxLDL Antibody

The ELISA to measure mouse IgM anti-oxLDL was performed as follows. Mouse oxLDL (2 μg/ml) was adsorbed on the wells of a 96-well microtiter plate (Immulon 1B, Dynex Technologies, Chantilly, VA, USA) by overnight incubation at 4°C. Then the wells were blocked with BSA for 2 h. Mouse plasma (100-fold diluted) was added and incubated at 37°C for 1 h at room temperature. The wells were subsequently incubated with HRP-labeled goat anti-mouse IgM (American Qualex, San Clemente, CA, USA) for 1 h at room temperature. Further steps were performed in the same way as the ELISA for oxLDL/β2GPI complexes.

### 2.11. Statistical Analysis

Data are presented as means +/- SD. One way analysis of variance (ANOVA) and Dunnet analysis as post hoc were used to determine significant differences at $p<0.05$ among 3 groups or more with KaleidaGraph software Version 4.0 (Synergy Software, Reading, PA, USA). Unpaired *t* test was used to determine significant differences at $p<0.05$ between two groups.

## 3. RESULTS

### 3.1. Body Weight and Blood Lipids

Changes in the body weight of LDLR-deficient mice are shown in Fig. (**1A**). In HF and HF + PP groups, body weight significantly increased compared to that of the MF group ($p<0.05$, at 24 weeks of age). There was no significant difference in either the initial or the final body weight between HF and HF + PP groups. After 4 weeks and 8 weeks of feeding, plasma cholesterol level in the HF + PP group was significantly lower than that in the HF group (Fig. **1B**, $p<0.005$). Plasma triglyceride level of HF + PP group was significantly lower than that in the HF group at 20 weeks of age ($p<0.005$).

### 3.2. Plasma oxLDL/β2GPI Level

Plasma oxLDL/β2GPI complex levels in LDLR-deficient mice fed HF and HF + PP significantly increased after high fat loading, but the complexes in LDLR-mice fed MF stayed in the level similar to the beginning of the experiment (Fig. **1D**). HF feeding led to significant increment in plasma oxLDL/β2GPI complexes ($p<0.0005$), while HF + PP feeding resulted in significant decrease ($p<0.05$) at 24 weeks of age.

### 3.3. Atherosclerotic Lesions

Mice were euthanized at the end of the experiment. And their aortas were analyzed for the extent of atherosclerosis. Atherosclerotic lesions in the entire arterial tree and in each section of aorta were quantified by the en face method with Sudan IV stain. Moderate atherosclerosis lesions were observed in LDLR-deficient mice fed HF. PP supplementation reduced the lipid-deposit lesion areas in the entire aortic tree by 70% compared with mice fed HF (Fig. **2B**). Atherosclerotic lesions were also significantly smaller in the aortic arch and abdominal aorta than those in the HF group (Fig. **2C-E**).

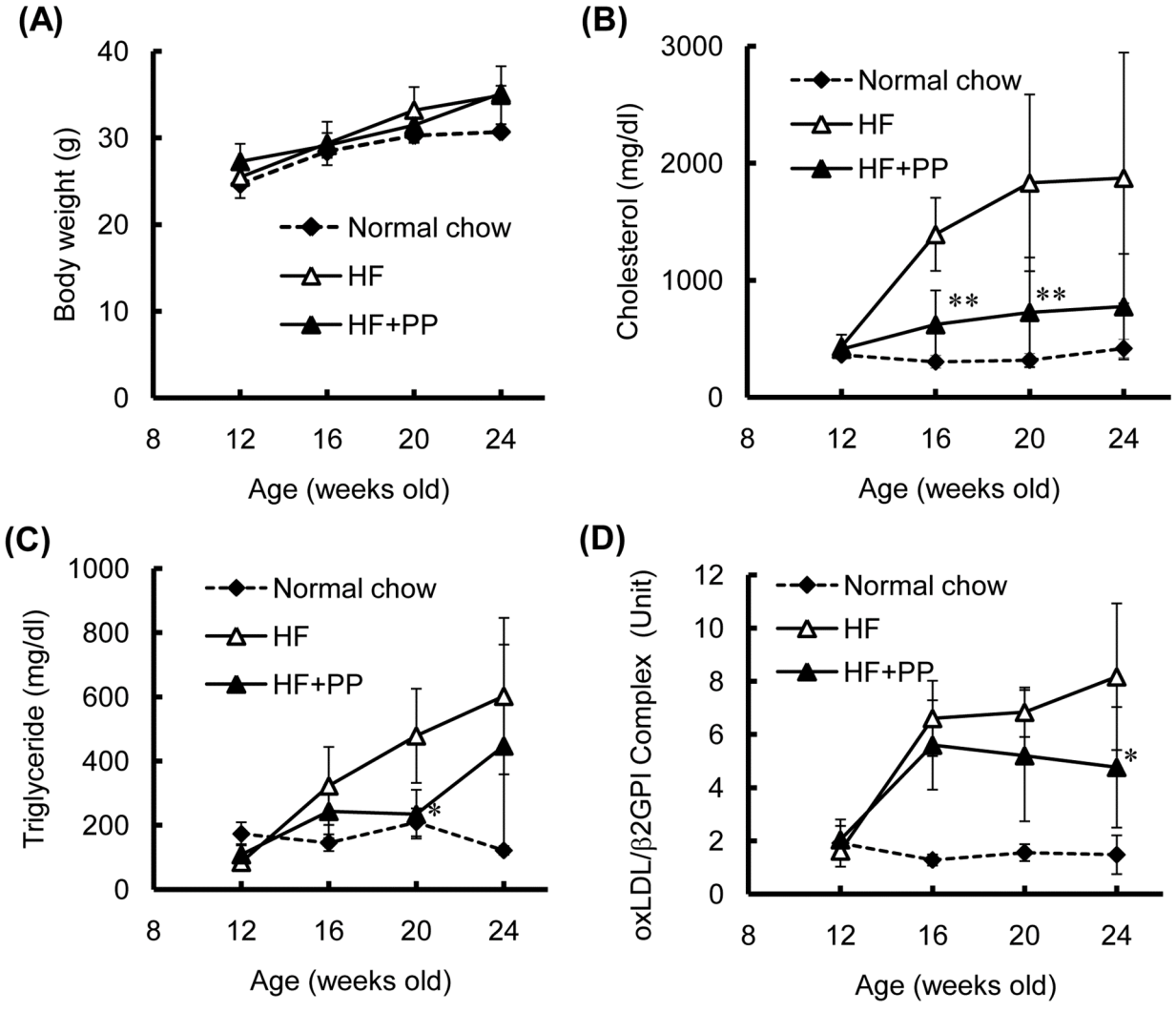


**Fig. (1).** Change in body weight and plasma parameters of LDLR-deficient mice.

(**A**) The body weight of LDLR-deficient mice fed HF, HF + persimmon peel (PP), and normal chow diet during the study. (**B**) Plasma cholesterol level of mice fed HF, HF+PP, and normal chow diet during the study. (**C**) Plasma triglyceride level of mice fed HF, HF+PP, and normal chow diet during the study. (**D**) Plasma oxLDL/β2GPI complex levels of mice fed HF, HF+PP, and normal chow during the study. Statistical analysis was performed between HF group and HF + PP group of the same age. * $p<0.05$, ** $p<0.005$. Four to eleven mice were applied for each group.

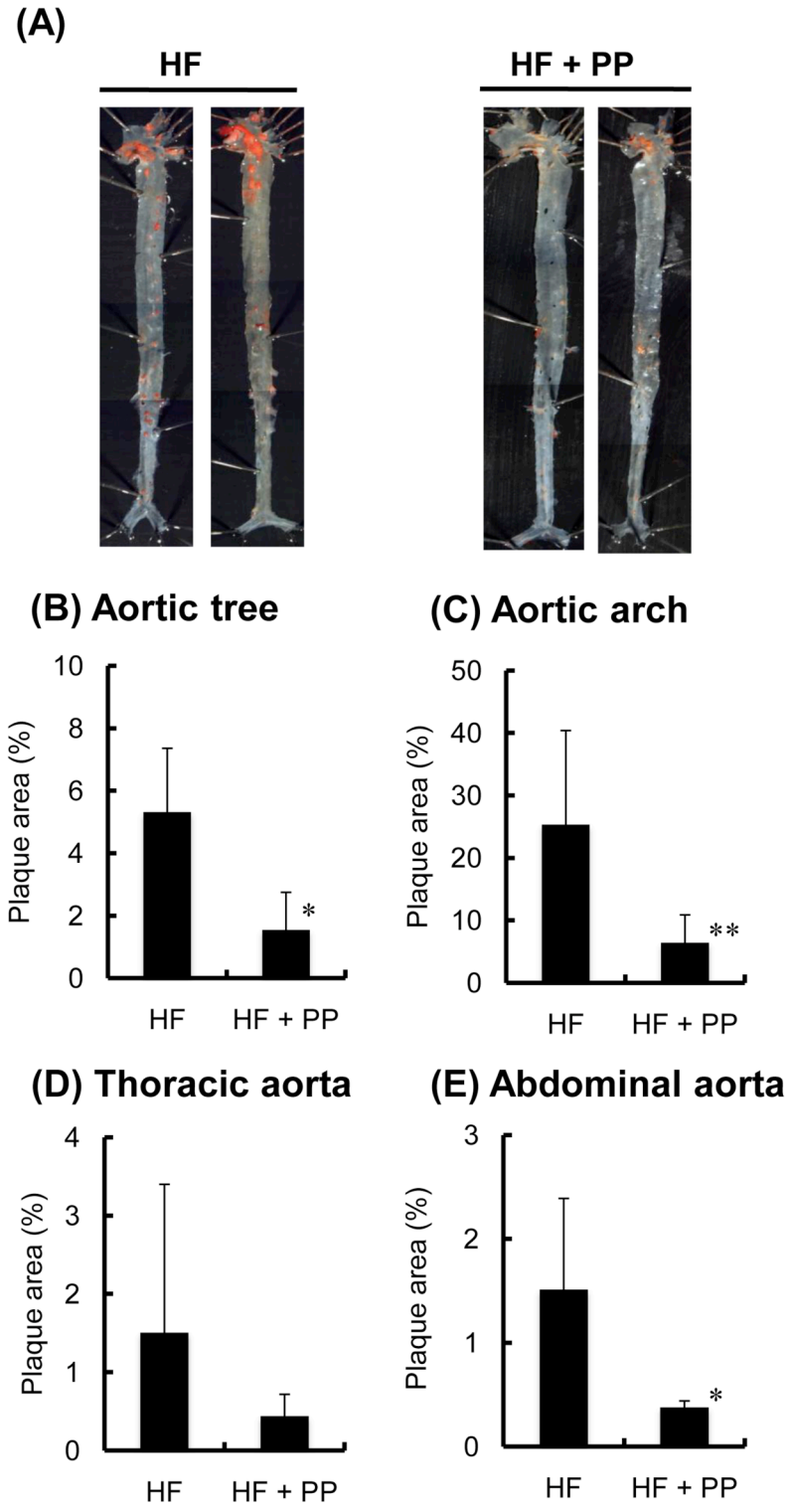


**Fig. (2).** Atherosclerotic plaque formation in LDLR-deficient mice. (**A**) Representative atherosclerotic plaques in aortic trees stained with Sudan IV of LDLR-deficient mice fed HF and HF+ persimmon peel (PP). Atherosclerotic lesion size was quantified en face as described in materials and methods and indicated as plaque area in percent of total vessel area (**B**), aortic arch (**C**), thoracic aorta (**D**), and abdominal aorta (**E**). Statistical analysis was performed between HF group and HF + PP group of the same weeks of age. * $p<0.05$, ** $p<0.005$

### 3.4. Urinary 8-OHdG and Plasma Adiponectin

Urinary 8-OHdG and plasma adiponectin were measured in LDLR-deficient mice fed HF and HF + PP at 12 weeks of age (baseline) and after 12 weeks of treatment (24 weeks of age), respectively (Fig. **3**). There was no significant change of urinary 8-OHdG levels and plasma adiponectin levels between control and persimmon supplementation in the beginning, and those between baseline and post-treatment.

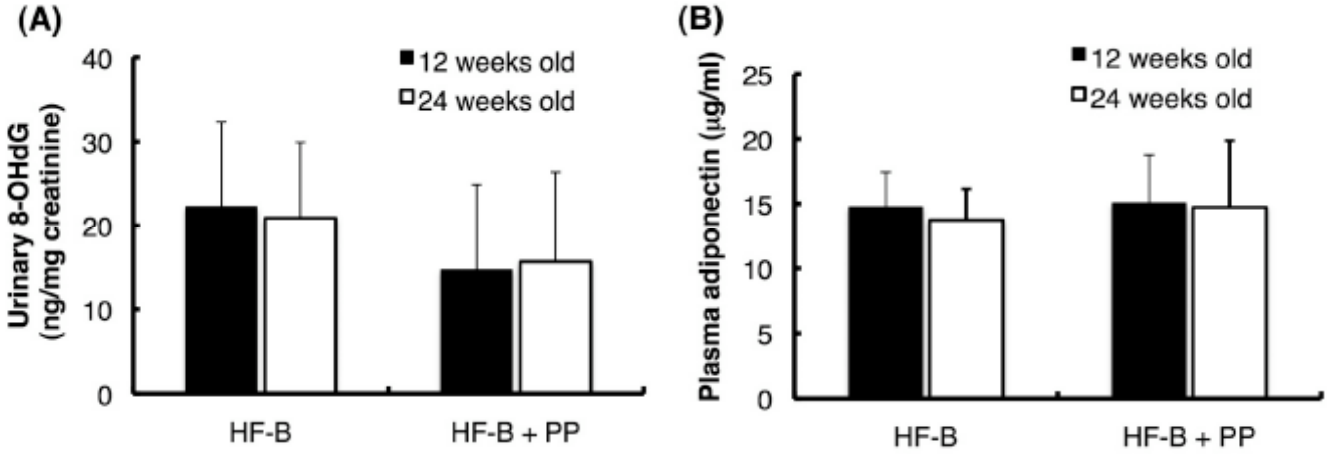


**Fig. (3).** Oxidation marker and plasma adiponectin in LDLR-deficient mice. (**A**) Urinary 8-OHdG level in LDLR-deficient mice fed HF and HF + persimmon peel (PP) at 12 and 24 weeks of age. (**B**) Plasma adiponectin level in mice fed HF and HF + PP at 12 and 24 weeks of age.

### 3.5. Urinary 11-Dehydrothromboxane $B_2$

Mice fed HF showed significant increase in urinary 11-dhTXB$_2$ (Fig. **4**) at 20 weeks of age. Mice fed HF and took aspirin containing water ad libitum (which approximately corresponds to 5 mg/kg /day) kept the low level of urinary 11-dhTXB$_2$ throughout the experimental period. After 8 weeks of feeding, persimmon peel supplementation showed significant inhibition of 11-dhTXB$_2$ ($p$<0.05, Fig. **4**) when compared with HF.

### 3.6. Antibody Against oxLDL

We analyzed IgM antibodies against oxLDL in the plasma of LDLR-deficient mice. The level of antibodies against oxLDL significantly increased over time in mice fed normal chow diet. At 24 weeks of age, antibody levels in mice fed HF were significantly higher than those in mice fed normal chow diet. In LDLR-deficient mice fed HF + PP, the level of antibodies against oxLDL at 20 weeks of age was lower than that in HF, but it was not significant. At 24 weeks of age, antibody levels in HF+ PP largely varied and difference between HF and HF + PP resulted in no statistical significance.

## 4. DISCUSSION

In the present study, we showed that persimmon peel supplementation inhibited the expected increment of plasma cholesterol, triglyceride, and oxLDL/β2GPI complexes and decreased the area of atherosclerotic lesions in LDLR-deficient mice. To the best of our knowledge, this is the first report showing significant improvement of atherosclerotic lesion with dietary persimmon peel.

Persimmon peel supplementation prevented the increment of plasma cholesterol and triglyceride. It is necessary to elucidate the mechanism and functional component of lipid-

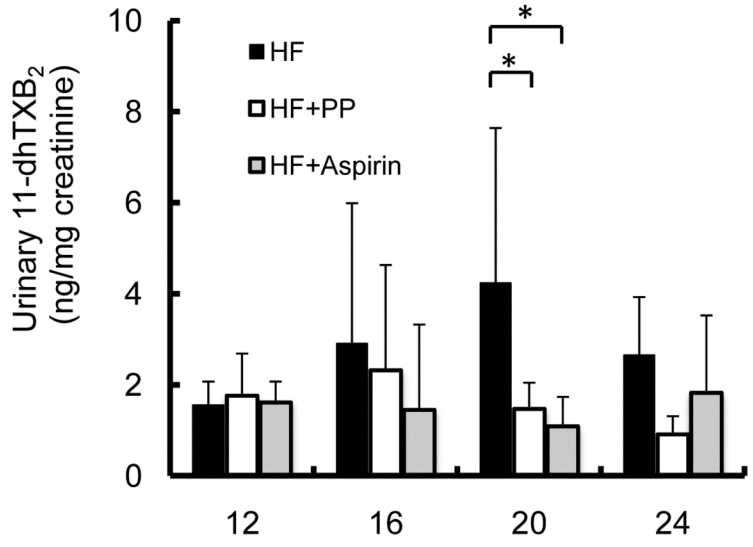


**Fig. (4).** Urinary 11-dhTXB$_2$ levels in LDLR-deficient mice.

LDLR-deficient mice were fed HF or HF+ persimmon peel (PP) from 12 weeks of age to 24 weeks of age. Mice in HF + Asp group were fed HF and received aspirin from 12 weeks of age to 24 weeks of age. Urine samples were collected at designated weeks of age and stored at -80$^o$C until use. Urinary 11-dhTXB$_2$ level was assayed by ELISA as described in Materials and Methods. *$p$<0.05 compared to HF diet group.

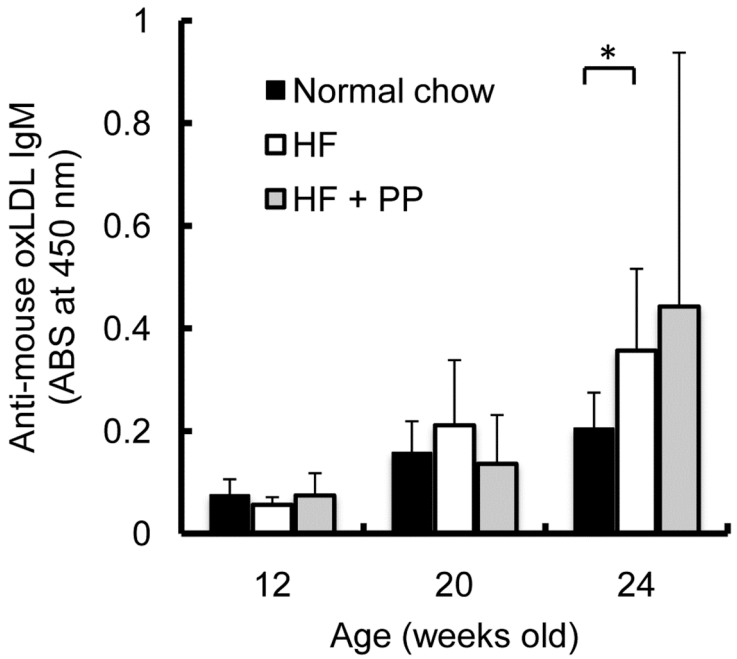


**Fig. (5).** Plasma anti-mouse oxLDL level in LDLR-deficient mice. IgM anti-oxLDL antibody titer was determined by an ELISA as described in material and methods. *$p<0.05$ compared to normal chow diet group.

lowering effect of persimmon peel. However, some reports about the lipid-lowering effect of persimmon fruits may suggest the mechanism [7, 36]. The intake of young persimmon fruit reduced the cholesterol level and accelerated fecal bile acid excretion in ApoE-deficient mice [8] and with C57BL/6.Cr mice [37]. They observed increased expression of the hepatic gene for cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid biosynthesis. Besides, young persimmon had higher bile-acid binding ability than grown persimmon fruit. The excretion of bile acid into feces is one of the major pathways for the removal of body cholesterol. Fecal bile acids increased significantly with young persimmon treatment, suggesting that enhanced excretion of fecal bile acid prevented hepatic steatosis and the rise in plasma cholesterol levels.

Unlike native LDL, oxLDL binds to β2GPI to form immunogenic and pro-atherogenic oxLDL/β2GPI complexes. We consider that the interaction of oxLDL with β2GPI is an anti-oxidant mechanism aimed at neutralizing the effect of oxLDL. OxLDL/β2GPI complexes and autoantibodies to these complexes have been demonstrated in patients with systemic autoimmune disease such as SLE and APS, as well as in patients with non-autoimmune chronic inflammatory conditions such as diabetes mellitus, renal diseases and acute coronary syndrome. In this study, we observed a significant increase in plasma oxLDL/β2GPI complexes in LDLR-deficient mice fed HF compared to those fed normal chow diet, at 4 weeks of feeding. This elevation persisted through to the end of the experiment. Persimmon peel supplementation significantly lowered the complex level compared to HF ($p<0.05$). Kato *et al.* [38] reported that plasma oxLDL in ApoE-deficient mice fed on a chow diet increased 3 fold at 20 weeks of age and then decreased to the basal level by 40 weeks of age. For oxLDL/β2GPI complexes, we did not observe such a transient increase.

Natural autoantibodies are usually defined as antibodies that are formed in normal individuals in the complete absence of any exogenous antigenic stimulation. They have an important role in providing a first line of defense against invading pathogens and as such represent a nonredundant component of the humoral immune system. Natural antibodies are predominantly IgM [39]. ApoE-deficient mice fed cholesterol have very high autoantibody titers, particularly IgM, to a wide variety of oxidation-specific epitopes [16]. The monoclonal autoantibodies secreted by hybridomas cloned from ApoE-deficient spleens were all IgM and localized by immunostaining of atherosclerotic lesions of mice and humans [19]. In our study, LDLR-deficient mice fed high fat diet showed significantly high titer of IgM anti-oxLDL antibodies compared with mice fed normal chow diet. PP supplementation reduced plasma cholesterol and oxLDL/β2GPI complexes which contain the target of these antibodies, however, it did not significantly reduce anti-oxLDL antibodies.

The effect of persimmon peel on the production of natural antibody should be further investigated. However, we have noticed from a previous line of our studies that β2GPI is complexed with LDL once LDL is oxidized at arterial vessels and that the oxLDL/β2GPI complexes are proportionally leaking out to the circulation. So, size/severity of atherosclerotic plaques can be roughly predicted by measuring plasma oxLDL/β2GPI complexes. Our data in the present study suggested that persimmon peel intake most probably prevents development of atherosclerosis. Beside, we also expect that minimal requirement of oxLDL present in atherosclerotic plaques to induce IgM natural autoantibodies must be relatively so small amount and that enough amount of antigenicity remains in mice fed with the HF-PP diet.

8-OHdG is one of the most abundant oxidative DNA adducts and it has been used as an indicator of oxidative

DNA damage associated with aging. Lee *et al.* [4] reported that pretreatment with persimmon peel proanthocyanidin showed protective effect against oxidative damage under $H_2O_2$-induced cellular senescence in human fibroblasts. In this study, we didn't observe significant change in urinary 8-OHdG level by persimmon peel feeding.

Adiponectin is an anti-inflammatory cytokine that is specifically and abundantly produced by adipocytes as a secretory protein and plays a key role in metabolic syndrome. The serum adiponectin concentration after 12 weeks of HF + PP feeding did not change significantly when compared with HF feeding. There was a report that ApoE-deficient mice fed high fat diet had a marked atherosclerotic lesion formation, but did not show significant difference in adipocytokine levels including adiponectin [40].

We also monitored urinary 11-dh$TXB_2$ during this study to clarify the effect of persimmon peel on the platelet activation. Thromboxiane $A_2$ ($TXA_2$) is the major cyclooxygenase product of arachidonic acid in platelets and a potent platelet aggregator. The biosynthesis of $TXA_2$ increases in diseases associated with platelet activation. Since $TXA_2$ is a very labile compound, its hydrolysis product, $TXB_2$ has been assayed as a quantitative index of platelet activation. 11-dh$TXB_2$ was identified as the most abundant enzymatic metabolite of infused $TXB_2$ [41] and is now considered to be the most appropriate parameter to follow the endogenous synthesis of $TXA_2$ [42]. Aspirin is a potent inhibitor of cyclooxygenase. In LDLR-deficient mice administered low-dose aspirin, significant decrease of vascular inflammation accompanied with the decrease of urinary 2, 3-dinor-$TXB_2$ was reported [31].

Diets rich in cholesterol and chlolate such as Paigen diet have been used to study atherogenesis and proinflammatory changes in microvasculature [43]. This diet has been shown to induce cholesterol gallstone disease in inbred mouse strains and chronic hepatic inflammation and fibrosis in rats [44], increase production of reactive oxygen species [45], and increase platelet-leukocyte interaction [46]. It has been reported that urinary 11-dh$TXB_2$ significantly increased in the cirrhotic patients in comparison with the controls [47]. In this study, LDLR-deficient mice fed HF showed modest elevation of urinary 11-dh$TXB_2$, and that was decreased by persimmon peel supplementation. There are several reports that plant polyphenol, such as green tea catechins and olive oil polyphenol, showed antiplatelet effect due to the inhibition of $TXA_2$ formation [48, 49]. The precise mechanism of antiplatelet activity by PP remains to be elucidated.

In conclusion, our study demonstrated that persimmon peel prevented the increment of blood cholesterol, triglyceride, and oxLDL/β2GPI atherogenic autoantigen levels, and prevented the progression of atherosclerosis in the LDLR-deficient mouse, but did not influence natural antibody induction against oxidized LDL. Though the precise mechanism and functional constituents of preventing atherosclerosis are not identified, persimmon peel would be beneficial in the development of preventive food supplement against dyslipidemia and atherosclerosis.

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