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Anthocyanins from Purple Sweet Potato *Ipomoea batatas* Cultivar Ayamurasaki Suppress the Development of Atherosclerotic Lesions and Both Enhancements of Oxidative Stress and Soluble Vascular Cell Adhesion Molecule-1 in Apolipoprotein E-Deficient Mice

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We evaluated the protective potential of anthocyanins from purple sweet potato *Ipomoea batatas* cultivar Ayamurasaki (APSP) against low-density lipoprotein (LDL) oxidation *in vitro* and atherosclerotic lesion development in apolipoprotein E-deficient mice given a cholesterol- and fat-enriched diet with or without 1% APSP for 4 weeks. APSP protected LDL against oxidation more potently than other anthocyanins and L-ascorbic acid *in vitro*. In mice, APSP significantly lowered the atherosclerotic plaque area to about half of the control, the liver level of thiobarbituric acid-reactive substances as an oxidative stress marker, and the plasma level of soluble vascular cell adhesion molecule-1 (sVCAM-1). However, APSP showed no effects on body weight and cholesterol and lipid levels in the plasma. The results suggest that APSP can suppress the development of atherosclerotic lesions and both enhancements of oxidative stress and sVCAM-1 independently of the changes in cholesterol and lipid levels in mice.

KEYWORDS: Dietary antioxidant; LDL oxidation; atherosclerotic plaque; thiobarbituric acid-reactive substances; soluble vascular cell adhesion molecule-1

INTRODUCTION

Recent epidemiological studies have demonstrated that high consumption of vegetables and fruits containing abundant plant polyphenols is associated with a low risk of lifestyle-related diseases, such as cardiovascular disease (*1*−*4*). For example, a "French paradox (*5*)", stating that high-fat consumers show low mortality rates from coronary heart disease in France, has been considered as the basis of the antioxidative activity of polyphenols in red wine (*6*−*8*). It has also been reported that grape polyphenols (*9, 10*) and grape seed proanthocyanidins (*11*) inhibit the development of atherosclerotic lesions in animal models and that grape seed proanthocyanidins show anti-inflammatory and antioxidative activities *ex vivo* (*12*), in animal models (*13, 14*) and human studies (*14, 15*).

Anthocyanins, which are naturally occurring pigments in vegetables, fruits, and flowers, are attractive as functional food materials, owing to their beneficial effects on human health. Many biological activities of anthocyanins have been reported, such as antioxidative activity *in vitro* (*16*) and in animal models (*17, 18*), antithrombotic activity *in vitro* (*19*) and in a human study (*20*), improvement of visual functions *in vitro* (*21*), prevention or improvement of carcinogenesis (*22*), obesity, hyperglycemia (*23*), and asthma (*24*) in animal models, and improvement of endothelial function in human studies (*25*).

Ipomoea batatas Poir., a novel variety of purple sweet potato (PSP) found in Japan in 1985, has been characterized with a high concentration of anthocyanins in its tubers but with a low productivity (*26*). To develop a new variety of PSP with a high concentration of anthocyanins and a high productivity, *I. batatas* cultivar Ayamurasaki in Japan has been crossbred with *I. batatas* Poir. and other varieties of *I. batatas* L. with a high productivity to produce a hybrid variety. These pigments, anthocyanins from PSP (APSP), are characterized by more complex chemical structures (**Figure 1**), higher stabilities, and stronger antioxidative activity than other anthocyanins (non-acylated cyanidin and peonidin 3-*O*-sophoroside-5-*O*-glucosides) (*27*). Components A and D (**Figure 1**) in APSP have been reported to be bioavailable in rats and humans (*28*) and show antioxidative activity in the urine (*29*). Moreover, it has been reported that APSP or beverages containing APSP show several biological

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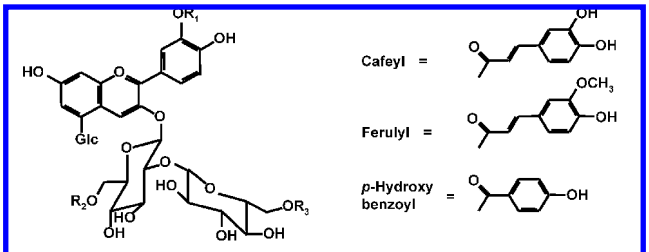

Component	R1	R2	R3	M/Z	DPPH radical scavenging activity (ED_{50} μM) [a]	Bioavailability [b]
A	H	Cafeyl	H	935	19.3±0.2	+
B	H	Cafeyl	Cafeyl	1097	20.0±1.6	—
C	H	Cafeyl	*p*-Hydroxy benzoyl	1055	37.1±1.0	—
D	CH_3	Cafeyl	H	949	20.1±1.6	+
E	H	Cafeyl	Feruloyl	1111	37.3±2.5	—
F	CH_3	Cafeyl	Cafeyl	1111	14.8±1.1	—
G	CH_3	Cafeyl	*p*-Hydroxy benzoyl	1069	30.8±2.8	—
H	CH_3	Cafeyl	Feruloyl	1125	9.9±0.9	—

[a] Fifty percent scavenging dose was calculated 29).

Figure 1. Chemical structure, radical scavenging activity, and bioavailability of anthocyanins in purple sweet potato *I. batatas* cultivar Ayamurasaki (APSP).

activities, such as antimutagenicity *in vitro* (*30*), antihyperglycemia through α-glucosidase inhibition (*31*), and antihypertension (*32*) in animal models, as well as antihepatitis in an animal model (*33*) and a clinical trial (*34*). On the other hand, polyphenol derivatives (caffeoylquinic acid derivatives) from *I. batatas* tubers have been reported to show antioxidative activity (*35*).

However, it remains to be clarified whether APSP have the potential to suppress the development of atherosclerotic lesions. Therefore, to examine the effects of APSP intake on the development of atherosclerotic lesions and on both the increases in oxidation stress and inflammatory responses in an animal model of atherosclerosis, apolipoprotein (apo) E-deficient mice were given a cholesterol- and fat-enriched diet with or without APSP. Moreover, the effects of APSP and other anthocyanins on the resistance of low-density lipoprotein (LDL) against oxidation were evaluated *in vitro*.

MATERIALS AND METHODS

Samples and Reagents. Sanred YM powder (food additive grade containing 43% APSP, 50% dextrin, and 7% citric acid), prepared from *I. batatas* cultivar Ayamurasaki, was purchased from San-Ei Gen F. F. I., Inc. (Osaka, Japan) and used for the animal study. Anthocyanin pigments, Kiriyasured ES and Kiriyasured MT-(Y), were purchased from Kiriya Chemical Co. (Osaka, Japan) as food additive grade. APSP and anthocyanins from elderberry and purple corn were purified from Sanred YM powder and Kiriyasured ES and Kiriyasured MT-(Y), respectively, as follows. After prewashing with 25 mL of methanol and 50 mL of Milli-Q water (Nihon Waters K.K., Tokyo, Japan), an aqueous solution of anthocyanin pigments was applied on a Mega Bond Elute C18 (P/N 12256031, Varian Technologies Japan Ltd., Tokyo, Japan), washed with 250 mL of Milli-Q water, and eluted with 75 mL of methanol. After the evaporation of methanol *in vacuum*, the purified sample was obtained. Purified anthocyanins from red cabbage and grape peel were purchased from Kanto Chemical Co. (Tokyo, Japan) as food analysis grade. All samples of purified anthocyanins (a mixture of some anthocyanin components that have a purity of more than 95%) were used for only *in vitro* study. L-Ascorbic acid was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents and chemicals used were commercial products of analytical grade.

Animals and Diets. A total of 20 male apo E-deficient mice (B6.KOR/Stm Slc-Apoe^{shl}, SPF grade), obtained from Japan S. L. C. Co. (Shizuoka, Japan) at 6 weeks of age, were housed individually in plastic cages in a room with controlled lighting (lights on 8:30 to 20:30), temperature (25 ± 1 °C), and humidity (60 ± 5%) under conventional conditions. A commercial nonpurified solid diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were given free access to the mice, which were maintained and treated in accordance with the guidelines of the Ethical Committee for Animal Experiments of the Yakult Central Institute.

Table 1. Composition of Experimental Diets[a]

	control diet[b] (g/100 g)	1% APSP diet (g/100 g)
α-cornstarch	24.4	23.4
casein	20.0	20.0
sucrose	15.0	15.0
lard	15.0	15.0
dextrine	13.2	12.0
cellulose powder	5.0	5.0
mineral mixture (AIN93G)	3.5	3.5
cholesterol	1.0	1.0
corn oil	1.0	1.0
vitamin mixture (AIN93)	1.0	1.0
L-cystine	0.3	0.3
sodium cholate	0.25	0.25
choline bitartrate	0.25	0.25
citric acid	0.16	
Sanred YM powder[c]		2.33
(APSP)		1.0

[a] Apo E-deficient mice received each diet as a solid diet. [b] An AIN-93G purified diet was modified by supplementation with lard, cholesterol, sodium cholate, and citric acid. [c] Sanred YM powder is composed of 43% APSP, 50% dextrin, and 7% citric acid.

After a 7 day adaptation period, the mice were assigned randomly to 2 groups ($n = 10$), namely, control and APSP groups, and received a control diet (1% cholesterol and 15% lard-enriched basal diet based on AIN-93G purified diet (*36*)) and a 1% APSP-supplemented diet, respectively for 4 weeks. The compositions of the diets are shown in **Table 1**. Body weight was recorded once a week, and diet intake was recorded every 2 or 3 days. After the 4 week period of food intake, the mice received no food but were given only water for 17 h and then anesthetized with diethyl ether.

Blood was collected from the heart into a tube containing ethylenediaminetetraacetic acid (EDTA). Plasma was prepared by centrifugation at 830*g* (4 °C) for 15 min and stored at −80 °C until analysis. The liver was perfused with saline *in situ* (8.5 g of sodium chloride/L), excised, and weighed. Both kidneys were dissected and weighed. The heart and whole aorta were perfused with saline *in situ* to completely exclude blood before removal. Thereafter, each tissue sample was immediately frozen and stored at −80 °C until analysis.

Histochemical Analysis. The average percentage of atherosclerotic plaque area in the whole aorta of apo E-deficient mice was determined by the image analysis method (*37*), with some modifications. Briefly, the whole aorta was excised from the heart, opened longitudinally from the inner side of the aortic arch to the common iliac artery, mounted, fixed in 10% neutral formalin buffer, and stained with oil red O (*38*). An image of the oil red O-stained opened whole aorta was taken using a digital microscope camera (PDMC Ie, Nippon Polaroid K. K., Tokyo, Japan) and analyzed using WINROOF software (Mitani Co., Fukui, Japan).

Fat deposition in the aortic sinus in apo E-deficient mice was evaluated according to the reported methods (*37, 39*), with some modifications. The upper half of the heart was initially dissected, embedded in Tissu Mount (Shiraimatu-kiki, Osaka, Japan), frozen at −80 °C, and then sectioned from top to bottom until three leaflets of the aortic valve appeared. After fixation in 10% neutral formalin buffer, each section was stained with oil red O, counterstained with hematoxylin, and photographed using a microscope equipped with a camera (Optiphot, Nikon Co., Tokyo, Japan). The heart of normal mice (C57BL/6, male, the same age, obtained from Japan S. L. C. Co.), housed under the same conditions as the apo E-deficient mice and fed a commercial nonpurified solid diet (MF, Oriental Yeast Co., Ltd.), was employed as a negative control.

Chemical Analysis. Soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1),

monocyte chemoattractant protein-1 (MCP-1), and adiponectin in plasma were assayed using a mouse sVCAM-1 colorimetric enzyme-linked immunosorbent assay (ELISA) kit (Quantikine M, R&D Systems, Inc., Minneapolis, MN), a mouse MCP-1 colorimetric ELISA kit, a mouse sICAM-1 colorimetric ELISA kit (Pierce Chemical Co., Rockford, IL), and a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceuticals Co., Ltd., Tokyo, Japan), respectively.

Total cholesterol (T-CHL) and HDL-cholesterol (HDL-CHL) in plasma were enzymatically quantified using the HDL-cholesterol E-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) before and after precipitation with phosphotungstic acid and sodium magnesium, respectively. LDL plus very low-density lipoprotein (VLDL)-CHL was calculated from T-CHL and HDL-CHL in plasma. Triglyceride (TG), non-esterified fatty acid (NEFA), and lipid peroxide (LPO) in plasma were determined using triglyceride E-Test Wako (Wako Pure Chemical Industries, Ltd.), the NEFA C-Test Wako (Wako Pure Chemical Industries, Ltd.), and Determiner LPO (Kyowa Medics, Tokyo, Japan) (*40*), respectively. The activities of aspartate transaminase (AST) and alanine transaminase (ALT) in plasma were assayed using the transaminase C II-test Wako (Wako Pure Chemical Industries, Ltd.).

After the extraction of liver lipid (*41*), T-CHL and TG in the liver were measured using the commercial kit as described above. After the homogenization of the liver and kidney, thiobarbituric acid-reactive substances (TBARS) and protein in each tissue were determined according to a reported method (*42*) and with a bicinchoninic acid (BCA) protein assay reagent kit (Pierce Chemical Co.), respectively. The TBARS concentration was calculated using a molecular absorption coefficient of TBARS and was expressed as nmol of TBARS/mg of protein in each tissue.

Resistance against *in Vitro* Oxidation of LDL. The resistance against *in vitro* oxidation of LDL was evaluated according to a previous method (*29, 43*). Briefly, LDL was isolated by density-gradient ultracentrifugation (Hitachi, Tokyo, Japan) at 10 °C for 2 h at 406000*g* from the plasma of 9-week-old male Golden Syrian hamsters (Japan S. L. C. Co.) fed a commercial nonpurified solid diet (MF, Oriental Yeast Co., Ltd.) supplemented with 5% lard and 0.5% cholesterol for 2 weeks. A total of 250 μL of the reaction mixture, which contains LDL (200 μg of protein/mL), each solution of purified anthocyanins (2 μg/mL), and 500 μM 2,2′-azobis(4-methoxy-2,4-dimetylvaleronitrile) as an oxidant in phosphate buffer saline (pH 7.4), was incubated at 37 °C for 7 h in a Costar 96-well UV plate (Corning, Inc., New York). Absorbance at 234 nm was then monitored at 5 min intervals using a microplate reader (SpectraMax pro 190, Nihon Molecular Devices Co., Tokyo, Japan). The lag time was calculated from the monitored curve in the LDL oxidation.

Statistical Analysis. Data are presented as mean ± standard deviation (SD). Statistical significance was determined using Student's *t* test or the Tukey test, and $p < 0.05$ was considered to be statistically significant.

RESULTS

Resistance against *in Vitro* Oxidation of LDL. Absorbance at 234 nm in the reaction mixture containing LDL with an oxidant (control group) increased with incubation time, suggesting that LDL was oxidized by incubation with an oxidant. **Figure 2a** shows the lag time calculated from the LDL oxidation curve. The lag time in the control group was significantly prolonged by all purified anthocyanins. Particularly, purified APSP induced a significantly longer prolongation than other purified anthocyanins from fruits and vegetables, indicating that purified APSP induced the most potent resistance against *in vitro* oxidation among five samples of purified anthocyanins. **Figure 2b** shows the dose dependency of the prolongation of lag time in LDL oxidation by purified APSP compared to L-ascorbic acid. Purified APSP prolonged the lag time dose dependently and more potently than L-ascorbic acid at a higher dose.

General Characteristics of Apo E-Deficient Mice. An abnormal apo E-deficient mouse showing marked inhibition of body weight gain and distinct jaundice with cholecystolithiasis was first identified in the control group and excluded before data analysis. **Table 2** shows the initial and final body weights, body weight gain, diet intake, and weights of the liver and kidney of apo E-deficient mice fed a control diet or 1% APSP-supplemented diet for 4 weeks. There were no significant differences in these general parameters between the control and APSP groups.

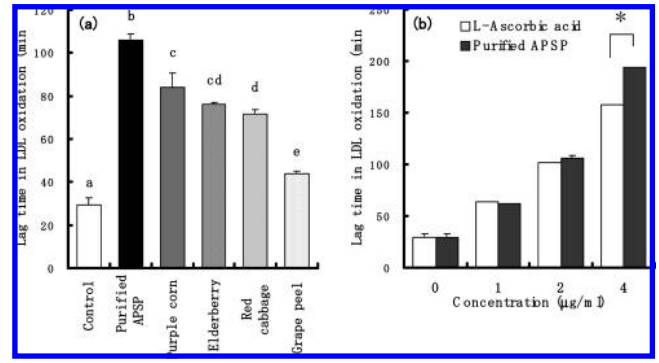

Figure 2. Effects of purified APSP and anthocyanins from fruits and vegetables and L-ascorbic acid on resistance against *in vitro* oxidation of LDL. (a) As described in the Materials and Methods, the lag time was calculated from the monitored curve during LDL oxidation. (b) Prolongation of lag time between APSP and L-ascorbic acid at different doses was compared. The lag time is expressed as mean ± SD ($n = 3$). Mean values within a row without a common letter are significantly different ($p < 0.05$, Tukey test). (∗) $p < 0.05$ indicates a significant difference. (#) $p = 0.05$ (Student's *t* test).

Table 2. General Characteristics of Apo E-Deficient Mice in the Control and APSP Groups[a]

	control ($n = 9$)	APSP ($n = 10$)
initial body weight (g)	24 ± 2	24 ± 1
final body weight (g)	28 ± 2	28 ± 2
body weight gain (g)	4 ± 2	4 ± 2
diet intake (g/day)	2.5 ± 0.2	2.5 ± 0.2
liver weight (dry g)	0.51 ± 0.09	0.50 ± 0.11
kidney weight (wet g)	0.35 ± 0.10	0.36 ± 0.12

[a] Apo E-deficient mice received a control or 1% APSP-supplemented diet (shown in **Table 1**) for 4 weeks.

Evaluation of Atherosclerotic Lesions. An atherosclerotic plaque with fat deposition in the aorta was qualitatively or quantitatively analyzed after oil red O staining to evaluate the development of atherosclerotic lesions in apo E-deficient mice. **Figure 3** shows typical photographs of the oil red O-stained plaque in the whole aorta and aortic arch of apo E-deficient mice given a control or 1% APSP-supplemented diet for 4 weeks. In the apo E-deficient mice given a control diet, staining was not only clear and abundant in the aortic root, three junctions to blood vessels, and an inner area in the aortic arch but was also localized as spots at many junctions to the intercostal artery in the thoracic and abdominal aorta, indicating typical atherosclerotic plaques. The areas of atherosclerotic plaques in the aortic arch and thoracic and abdominal aorta were smaller in the apo E-deficient mice given a 1% APSP-supplemented diet than in the mice receiving a control diet.

Figure 4 shows the average percentage of atherosclerotic plaque area in the whole aorta of apo E-deficient mice given a control or 1% APSP-supplemented diet for 4 weeks. The average percentages of atherosclerotic plaque area in the APSP and control groups were 0.42 ± 0.25 and 0.78 ± 0.41%, respec-

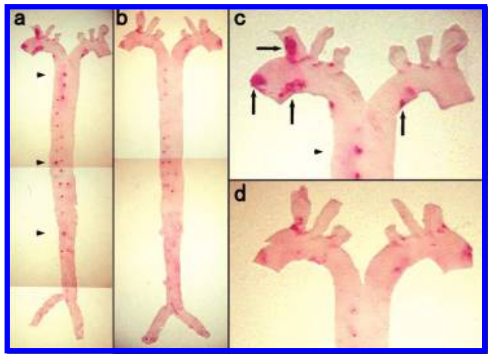

Figure 3. Histochemical detection of fat deposition in whole aorta and aortic arch of apo E-deficient mice given a (a and c) control diet or (b and d) 1% APSP-supplemented diet for 4 weeks. c and d showing the aortic arch are expanded photographs of a and b, respectively, showing the whole aorta. As described in the Materials and Methods, the aortic tree, opened longitudinally from the aortic arch to the common iliac artery, was stained with oil red O after fixation in 10% neutral formalin buffer. The staining of deposited fat was clear and abundant in the aortic arch (arrows) and was localized as spots in the thoracic and abdominal aorta (arrow heads), indicating a typical atherosclerotic plaque in apo E-deficient mice fed with a control diet (a and c). The area and intensity of staining were in the following order: control mice > 1% APSP mice.

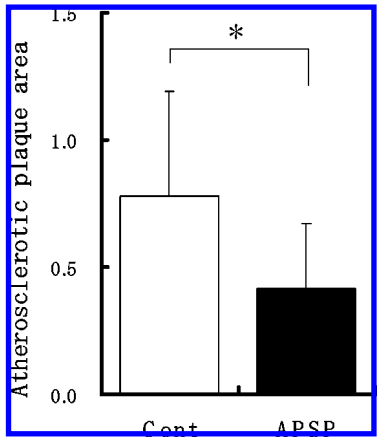

Figure 4. Effects of APSP intake on the average percentage of atherosclerotic plaque area in whole aorta of apo E-deficient mice. As described in the Materials and Methods, sections of the aortic tree were initially prepared from the mice fed with the control and 1% APSP-supplemented diets for 4 weeks. Then, the average percentage of atherosclerotic plaque area was determined by image analysis after staining with oil red O. Values are expressed as mean ± SD (control, $n = 9$; APSP, $n = 10$). (*) $p < 0.05$ indicates a significant difference (Student's t test).

tively. The plaque area was reduced by 46% ($p < 0.05$) in the APSP group compared to the control group.

Figure 5 shows typical photographs of the deposited fat stained with oil red O in the aortic sinus of apo E-deficient mice given a control or 1% APSP-supplemented diet for 4 weeks and in age-matched normal C57BL/6 mice fed a normal diet, serving as a negative control. Staining was localized clearly and abundantly on the vascular wall of the aortic sinus in the apo E-deficient mice given a control diet but not in the normal C57BL/6 mice. On the other hand, the area of staining was smaller in the apo E-deficient mice given a 1% APSP-supplemented diet than in the mice fed with a control diet. These observations showed that APSP intake significantly suppressed the development of atherosclerotic lesions in the aorta of apo E-deficient mice.

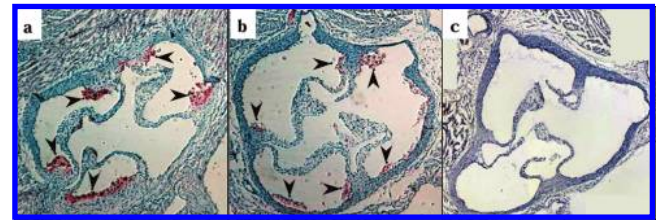

Figure 5. Histochemical detection of fat deposition in aortic sinus of apo E-deficient mice given a (a) control or (b) 1% APSP-supplemented diet for 4 weeks. (c) Normal age-matched C57BL/6 mice fed with a normal diet were employed as a negative control. As described in the Materials and Methods, the section of the heart showing three leaflets of the aortic valve was stained with oil red O and hematoxylin after fixation in 10% neutral formalin buffer. The staining was localized clearly and abundantly on the vascular wall of the aortic sinus (arrow heads) of the apo E-deficient mice given a control diet (a) but not in the normal C57BL/6 mice. The area of staining was in the following order: control mice > 1% APSP mice.

Oxidative Stress Markers. LPO in plasma and TBARS in the liver and kidney were used as oxidative stress markers in this study. Parts **a**−**c** of **Figure 6** show the LPO level in plasma and the TBARS levels in the liver and kidney, respectively, in apo E-deficient mice fed with a control or 1% APSP-supplemented diet for 4 weeks. The TBARS level in the liver was lower ($p < 0.05$) in the APSP group than in the control group. The APSP group also tended to show lower levels of plasma LPO ($p = 0.09$) and TBARS in the kidney ($p = 0.07$) than the control group.

Inflammatory Biomarkers. For inflammatory biomarkers related to the development of atherosclerosis, the plasma levels of sVCAM-1, sICAM-1, and MCP-1 were assayed in this study. Parts **a**−**c** of **Figure 7** show the plasma levels of sVCAM-1, sICAM1, and MCP-1, respectively, in apo E-deficient mice given a control or 1% APSP-supplemented diet for 4 weeks. The APSP group had a lower plasma level of sVCAM-1 ($p < 0.05$) than the control group. However, between both groups, there were no differences in the plasma levels of sICAM-1 and MCP-1.

Lipids and Other Biomarkers in Plasma and Liver. Tables 3 and **4** show lipids and some biomarkers in plasma and the liver, respectively, of the apo E-deficient mice given a control or 1% APSP-supplemented diet for 4 weeks. The mice in both the control and APSP groups were diagnosed as having hypercholesterolemia, showing a markedly high plasma level of T-CHL. However, there were no significant differences in the plasma levels of T-CHL, HDL-CHL, (LDL + VLDL)-CHL, TG, NEFA, adiponectin, AST, and ALT and in the hepatic levels of T-CHL and TG between both groups.

DISCUSSION

Either overproduction of reactive oxygen species (ROS) and/or lowered activity of ROS scavengers *in vivo* induces an increase in oxidative stress, which is associated with hypertension, hyperlipidemia, hyperglycemia, and smoking as risk factors of atherosclerosis. An increase in the oxidative stress in the vascular wall causes LDL oxidation. This oxidation is believed to play a critical role in the initiation and progression of atherosclerosis, producing lesions in the vascular endothelial cells and inducing the development of foam cells and atherosclerotic plaques (*44*−*46*). This theory has been supported by several previous studies that the induction of LDL oxidation *in*

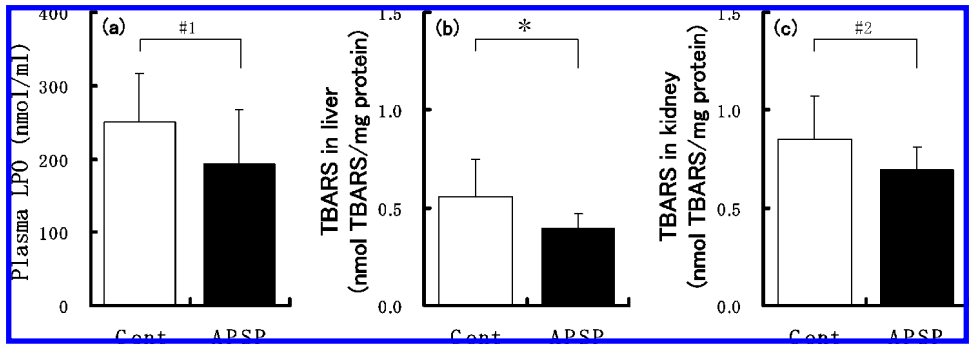

Figure 6. Effects of APSP intake on plasma levels of (a) LPO and TBARS in (b) liver and (c) kidney of apo E-deficient mice. As described in the Materials and Methods, the levels of plasma LPO and tissue TBARS were assayed as biomarkers of oxidative stress in the mice fed with the control and 1% APSP-supplemented diets for 4 weeks. Values are expressed as mean ± SD (control, $n = 9$; APSP, $n = 10$). (*) $p < 0.05$ indicates a significant difference (Student's t test). (#1) and (#2) $p = 0.09$ and 0.07, respectively.

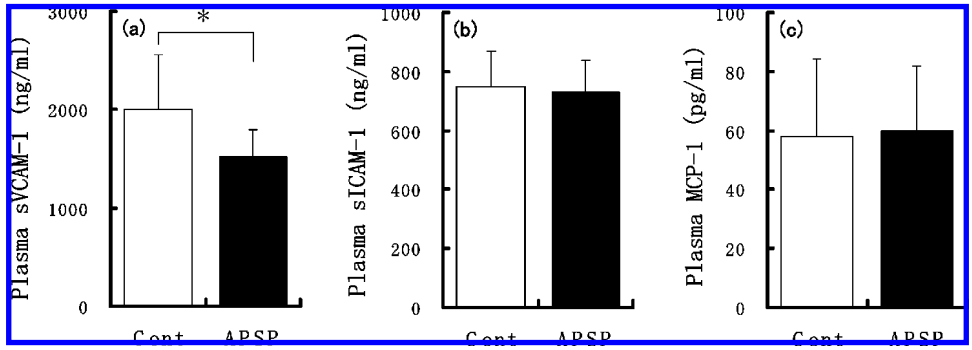

Figure 7. Effects of APSP intake on plasma levels of (a) sVCAM-1, (b) sICAM-1, and (c) MCP-1 in apo E-deficient mice. As described in the Materials and Methods, the plasma levels of sVCAM-1, sICAM-1, and MCP-1 were assayed as biomarkers of inflammation in the mice fed with the control and 1% APSP-supplemented diets for 4 weeks. Values are expressed as mean ± SD (control, $n = 9$; APSP, $n = 10$). (*) $p < 0.05$ indicates a significant difference (Student's t test).

Table 3. Plasma Levels of Cholesterol, Lipids, and Some Biomarkers in Apo E-Deficient Mice in the Control and APSP Groups[a]

	control ($n = 9$)	APSP ($n = 10$)
T-CHL (mg/dL)	930 ± 110	914 ± 155
HDL-CHL (mg/dL)	3.2 ± 1.0	3.3 ± 1.3
(LDL + VLDL)-CHL (mg/dL)	927 ± 110	911 ± 155
TG (mg/dL)	117 ± 31	116 ± 27
NEFA (mEq/L)	1.2 ± 0.3	1.0 ± 0.4
adiponectin (μg/mL)	27 ± 7	24 ± 5
AST (Karmen U)	178 ± 75	146 ± 56
ALT (Karmen U)	47 ± 39	39 ± 32

[a] Apo E-deficient mice received a control or 1% APSP-supplemented diet (shown in **Table 1**) for 4 weeks. Plasma levels of cholesterol, lipids, and some biomarkers were quantified as described in the Materials and Methods.

Table 4. Hepatic Levels of Cholesterol and Triglyceride in Apo E-Deficient Mice in the Control and APSP Groups[a]

	control ($n = 9$)	APSP ($n = 10$)
T-CHL in liver (mg/liver)	61 ± 35	46 ± 31
TG in liver (mg/liver)	118 ± 36	123 ± 52

[a] Apo E-deficient mice received a control or 1% APSP-supplemented diet (shown in **Table 1**) for 4 weeks. Hepatic levels of T-CHL and TG were quantified as described in the Materials and Methods.

vitro and *in vivo* (*6*−*8*) and the development of atherosclerotic lesions in animal models are suppressed by several dietary antioxidants, such as α-tocopheryl acetate (*47*), glutathione (*48*), and antioxidative polyphenols and proanthocyanidin from vegetables and fruits (*3, 9*−*11, 49, 50*).

Our study demonstrated that purified APSP showed a stronger potency in protecting LDL against oxidation than other anthocyanins and L-ascorbic acid *in vitro* (**Figure 2**). Therefore, the main aim in future studies is to determine whether APSP has the potential to suppress the development of atherosclerotic lesions in apo E-deficient mice. Here, it was demonstrated that APSP intake for 4 weeks decreased the atherosclerotic plaque area by 46% ($p < 0.05$) in the whole aorta (**Figure 4**) and induced the formation of a smaller plaque area in the aortic sinus (**Figure 5**) in apo E-deficient mice given a 1% cholesterol and 15% lard-enriched diet. Also, APSP intake decreased the levels of oxidative stress markers, such as both TBARS in the liver ($p < 0.05$) and kidney ($p = 0.07$) and LPO in plasma ($p = 0.09$) (**Figure 6**). These findings indicate that APSP intake has the potential to suppress the development of atherosclerotic lesions induced by the enhancement of antioxidative stress in apo E-deficient mice. Furthermore, components A and D (**Figure 1**) in APSP may be associated with the activity, because they are known to be bioavailable in rats and humans after ingesting APSP (*28*) and they show DPPH radical scavenging activity in rat and human urine and protecting activity for LDL from oxidation at a physiological concentration (*29*).

Oxidized LDL enhances the production of inflammatory cytokines, which in turn induce the production of adhesion molecules (ICAM-1 and VCAM-1) and atherosclerotic chemokines (MCP-1), resulting in the development of atherosclerosis (*46, 51, 52*). It has been shown that some antioxidative polyphenols reduce not only oxidative stress in plasma or tissues but also the production of proinflammatory cytokines (IL-1β and TNF-α) (*13, 24*), sVCAM-1, sICAM-1 (*15*), and MCP-1 (*53*) at the protein or mRNA level in animal models or in a

clinical trial. Also, the decrease in the MCP-1 level has been reported to be involved in the α-tocopheryl acetate-induced attenuation of atherosclerotic lesions in apo E-deficient mice (*47*). These findings suggest that some dietary antioxidants have the potential to suppress the enhancement of inflammatory responses *in vivo*, although it is unclear whether the suppression is a direct or indirect action by these antioxidants.

Our study demonstrated that APSP intake for 4 weeks significantly lowered the plasma level of sVCAM-1 but not sICAM-1 and MCP-1 (**Figure 7**) in apo E-deficient mice, in which the development of atherosclerotic lesions (**Figure 4**) and the enhancement of oxidative stress (**Figure 6**) were suppressed. It is speculated that the decrease in the plasma level of sVCAM-1 in the apo E-deficient mice, regarded as an anti-inflammatory response, resulted from both the anti-LDL oxidation and antioxidative stress activities of APSP. VCAM-1, expressed on activated endothelial cells, mediates the adhesion of leukocytes to endothelial cells and facilitates their transmigration to nascent atheromata. Genetically modified mice with impaired VCAM-1 function have reduced development of early atherosclerotic lesions, and VCAM-1 has been shown to play a pivotal role in the early developmental stage of atherosclerosis in apo E-deficient mice (*54, 55*). Our results suggest that the decrease in sVCAM-1 as an anti-inflammatory response instead of sICAM-1 and MCP-1 may play an important role in the suppression of atherosclerotic lesions in our model using apo E-deficient mice.

Apo E-deficient mice have been frequently used as a convenient atherosclerotic model for developing spontaneously atherosclerotic lesions and hypercholesterolemia, despite the existence of some differences between experimental and human lesions, such as remarkable hypercholesterolemia and severe atherosclerotic lesions with less plaque disruption in mice. In apo E-deficient mice fed a normal diet, more than 16 weeks of feeding is required to develop a sufficiently analyzable atherosclerotic plaque. In contrast, the feeding of a cholesterol- and fat-enriched diet accelerates the development of an atherosclerotic plaque in apo E-deficient mice, and this can shorten the feeding period from more than 16 to 4−8 weeks because of the induction of marked hypercholesterolemia. It has also been reported that some dietary antioxidants show both activities to suppress the development of atheroscrelotic lesions and to lower the plasma cholesterol level (*9, 50*). This complicates the understanding of mechanisms in the antiatheroscrelotic activity, because each antioxidative activity and plasma cholesterol-lowering activity makes it possible to suppress the development of atherosclerotic lesions in apo E-deficient mice with hypercholesterolemia. Furthermore, APSP shows various biological activities, which include not only antioxidative activity (*29*) but also antihyperglycemia (*31*) and antihypertension (*32*) activities; however, their lowering activity for plasma cholesterol and/or lipids has not yet been proven.

In our preliminary study, the sufficiently analyzable atherosclerotic plaque was developed by feeding of a 1% cholesterol- and 15% lard-enriched diet for 4 weeks in apo E-deficient mice. Therefore, we used the 4 week feeding period for this diet to investigate early events in the plaque formation in apo E-deficient mice. As a result, it was demonstrated that there were no significant differences in the plasma and liver levels of cholesterol and lipids between the control and APSP groups, in which marked hypercholesterolemia was observed (**Tables 3** and **4**). Our results clearly indicate that APSP intake suppresses the development of atherosclerotic plaque and both enhacements of oxidative stress and sVCAM-1 in apo E-deficient mice, independent of the decrease in the plasma levels of cholesterol and lipids.

In conclusion, APSP is a potent dietary antioxidant against LDL oxidation *in vitro* and has the potential to suppress the development of atherosclerotic lesions and both enhancements of oxidative stress and sVCAM-1, independent of the decrease in the plasma levels of cholesterol and lipid, in apo E-deficient mice. Also, both activities of antioxidative stress and anti-inflammatory response induced by APSP may be involved in the suppression of the development of atherosclerotic lesions. Moreover, APSP has some biological activities (*29*−*34*) and is generally taken as food in Japan. The results suggest the hypothesis that the habitual intake of APSP as food prevents lifestyle-associated diseases and reduces the risk of atherosclerosis in humans. Further studies are necessary to prove this hypothesis.

ABBREVIATIONS USED

PSP, purple sweet potato *I. batatas* cultivar Ayamurasaki; APSP, anthocyanin(s) from PSP; apo E, apolipoprotein E; MCP-1, monocyte chemoattractant protein-1; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; NEFA, non-esterified fatty acid; LPO, lipid peroxide; AST, aspartate transaminase; ALT, alanine transaminase; TBARS, thiobarbituric acid-reactive substances; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; ROS, reactive oxygen species.

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