Beneficial Effects of Grape Seed Extract on Malondialdehyde-Modified LDL

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Summary Following consecutive 12-wk administration of tablets containing 0, 200 or 400 mg grape seed extract (calculated as proanthocyanidin) to 61 healthy subjects with LDL cholesterol (LDL-C) levels of 100 to 180 mg/dL, effects of such treatment compared to administration of placebo tablets on malondialdehyde-modified LDL (MDA-LDL), representing one oxidized type of LDL, were investigated by a single blind method. MDA-LDL level in the 200 mg (calculated as proanthocyanidin) group was significantly ($p=0.008$) reduced compared to the basal level, 12 wk after the start of administration. In the 400 mg (calculated as proanthocyanidin) group, a significant decrease in MDA-LDL level compared to the basal level was found 6 and 12 wk after the start of administration (6 wk: $p=0.015$, 12 wk: $p=0.009$). Subjects with high levels of MDA-LDL/ApoB (MDA-LDL/ApoB ≥ 100 mU/mL) in the 200 mg group showed significantly ($p=0.011$) reduced MDA-LDL levels at 12 wk after the start of administration. In the 400 mg group, significant decreases in MDA-LDL level compared to the basal level were seen 6 and 12 wk after the start of administration (6 wk: $p=0.001$, 12 wk: $p<0.001$); and at week 6, significantly ($p=0.048$) lower values were observed compared to those in patients who took placebo tablets (0 mg proanthocyanidin). In subjects demonstrating the least body weight changes during the test period (less than ± 1.0 kg) in the 400 mg group, there was an increasing trend ($p=0.088$) in adiponectin levels 12 wk after the start of treatment. These results suggested that tablets containing grape seed extract exerted reducing effects on oxidized LDL, and might be useful in preventing lifestyle-related diseases such as arteriosclerosis.

Key Words grape seed extract, malondialdehyde-modified LDL, adiponectin, LDL oxidation

Previous epidemiologic studies showed that mortality due to arteriosclerosis-induced myocardial infarction was low in the French population despite their dietary habits characterized with a high intake of animal fat (the French paradox). It is suggested that the antioxidant effect of polyphenols contained in red wine which is frequently consumed by French people may account for such a paradox (1). So far, it has been shown that polyphenols in red wine demonstrates *in vitro* inhibition of oxidation of LDL-cholesterol (LDL-C) (2), and plasma LDL-C in subjects taking red wine through antioxidant effects (3). Among all polyphenols in red wine, proanthocyanidin is contained at the highest ratio, followed by catechin (4). It is also known that proanthocyanidin possesses the strongest antioxidant effect among polyphenols contained in red wine (5).

Oxidized LDL plays a central role in the occurrence of arteriosclerosis, and its progress in the body (6–9). In the present study, tablets containing proanthocyanidin

which was highly purified from grape seeds as the main ingredient, were orally administered to adults of both sexes for 12 wk, in order to investigate effects on oxidized LDL and their safety (Study 1).

Recently, possible relationships between low concentrations of adiponectin and obesity (10), diabetes mellitus (11), and arteriosclerosis (12) have been disclosed. However, no study has reported on effects of grape seed extract on adiponectin. We investigated relationships between the intake of grape seed extract and adiponectin level (Study 2).

MATERIALS AND METHODS

Test foods. Table 1 shows compositions of the test tablets. Tablets including grape seed extract were prepared so that they were indistinguishable by their outer appearance, and either contained no proanthocyanidin (control), 200 mg proanthocyanidin (200 mg group), or 400 mg proanthocyanidin (400 mg group).

Subjects. Experimental protocols were approved by the Aiwa Clinic Institutional Review Board (KKM-001–

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Table 1. Composition of test tablets.

Component	Group		
	Placebo	200 mg/d (50 mg×4 tablets)	400 mg/d (100 mg×4 tablets)
Grape seed extract (mg)	—	69.375	138.750
(inside) Proanthocyanidin 72% containing	—	(50)	(100)
Caramel (mg) (staining)	0.025	—	—
Microcrystalline cellulose (mg)	187.500	123.125	87.500
Deduced maltose starch syrup (mg)	54.975	50.000	16.250
Sucrose fatty acid ester (mg)	7.500	7.500	7.500
Shellac	trace amounts	trace amounts	trace amounts

Table 2. Clinical data.

Item	Unit	Placebo	200 mg	400 mg
Sex	M/F	10/10	10/11	9/11
Age	Year	53.2±2.1	51.0±2.4	52.9±2.0
Height	cm	161±1.8	161±2.0	160±1.8
Body weight	kg	63.1±2.18	63.5±2.68	62.3±2.47
BMI		24.4±0.59	24.2±0.66	24.1±0.62
Systolic blood pressure	mmHg	126.4±4.5	127.0±3.2	125.2±3.1
Diastolic blood pressure	mmHg	78.6±2.5	76.5±1.8	79.1±2.0
Pulse	bpm	65.3±1.3	64.9±2.4	67.8±1.9
White blood cell count	/μL	5725±273	6100±366	5975±419
Red blood cell count	×10 ⁴ /μL	442±12	448±9	449±11
Hemoglobin	/μL	13.7±0.31	13.9±0.31	13.9±0.31
Hematocrit	%	40.8±1.01	41.4±0.93	41.5±1.03
Platelet count	×10 ⁴ /μL	25.3±0.87	24.5±0.99	25.1±1.38
LDL-Cholesterol	mg/dL	133±5.0	134±3.7	133±3.5
HDL-Cholesterol	mg/dL	58±2.6	63±3.2	65±2.9
Total cholesterol	mg/dL	224±5.7	225±5.3	225±4.4
Triglycerides	mg/dL	135±19.3	116±11.9	119±14.0
Total protein	mg/dL	7.3±0.10	7.3±0.10	7.3±0.09
Albumin	mg/dL	4.4±0.05	4.4±0.05	4.6±0.05
A/G	mg/dL	1.6±0.03	1.6±0.05	1.7±0.04
Total bilirubin	mg/dL	0.77±0.05	0.68±0.04	0.73±0.04
AST (GOT)	IU/L/37°C	22.0±1.97	20.2±1.14	23.1±1.54
ALT (GPT)	IU/L/37°C	25.3±2.84	25.8±3.45	27.9±2.41
ALP	IU/L/37°C	214±12.3	230±14.3	212±13.2
LDH	IU/L/37°C	171±5.4	179±4.5	172±6.4
γ-GTP	IU/L/37°C	38.1±7.33	29.3±4.87	33.6±4.59
CPK	IU/L/37°C	108±7.6	108±8.7	115±9.3
Uric acid	mg/dL	5.6±0.31	5.2±0.26	5.2±0.33
Blood urea nitrogen	mg/dL	13.5±0.62	14.5±0.77	12.7±0.50
Creatinine	mg/dL	0.76±0.03	0.73±0.03	0.69±0.03
Glucose	mg/dL	101±2.9	96±1.7	98±1.7
HbA _{1c}	%	4.94±0.10	5.00±0.07	5.03±0.08

Data represent means±SE.

01). In compliance with the fundamental principles stipulated in the Helsinki Declaration, adequate explanations of the objectives and methods of this study were given to participants by attending physicians, and written consent forms were obtained from all enrolled subjects. The study period was from August to December 2003.

Subjects were selected to participate in the study if:

1) they were aged ≥30 y and <70 y; 2) their LDL-C

levels at baseline were ≥100 mg/dL and <180 mg/dL; 3) they discontinued drinking alcoholic beverages 2 d prior to the test; 4) they were given adequate explanations of the objectives and contents of the study, and provided written consent and voluntary wishes to participate in this study; and 5) they were attended by registered physicians.

Subjects were excluded if:

1) they were taking medicines that could affect lipid

metabolism such as anti-hyperlipidemic drugs or anti-hypertensive drugs; 2) they were taking health foods suspected of affecting lipids or blood pressure; 3) they were commonly taking foods derived from grape seeds known to exert strong antioxidant effects (e.g., seed oil), health foods, or vitamin preparations (C, E) (including persons drinking lots of healthy tea); 4) they usually drank red wine (more than 1 half-bottle a week); 5) they had diabetes mellitus or remarkable hepatic dysfunctions; 6) they suffered from familial hyperlipidemia; 7) they were or were suspected of being pregnant, or were nursing mothers; 8) they were judged inappropriate by attending physicians.

ate by attending physicians.

Study methods. Following screening of 130 candidates, only 61 subjects were eligible for the study. Eligible subjects were randomly classified into 3 groups by the controller (Yoko Shoji, Associate Professor, St. Marianna University School of Medicine). A pre-observation period of 2 wk preceded the 12-wk ingestion period, and the follow-up period was of 2 wk. Subjects ingested 4 tablets daily before breakfast every day for 12 wk. In cases with no breakfast, tablets were taken after lunch. Choice of tablet contents was left to the discretion of the subjects themselves. Both efficacy and

Table 3. Effects of grape seed extract supplementation on serum lipid concentration.

Item	Group	n	Before	Week 6	Week 12
Total cholesterol (mg/dL)	Placebo	18	219±4.9	231±6.0 [#]	226±5.1 [#]
	200 mg	18	226±7.1	228±7.9	225±7.7
	400 mg	17	222±4.9	221±6.0	229±7.4
LDL-Cholesterol (mg/dL)	Placebo	18	132±4.6	143±5.3 [#]	138±4.5
	200 mg	18	134±4.9	138±5.7	134±5.4
	400 mg	17	132±4.4	132±5.0	138±6.1
HDL-Cholesterol (mg/dL)	Placebo	18	58.3±3.08	61.4±2.92	64.2±3.27 [#]
	200 mg	18	63.1±4.10	64.9±4.79	68.7±4.86 [#]
	400 mg	17	61.4±2.98	63.6±3.18	67.1±3.51 [#]
Triglycerides (mg/dL)	Placebo	18	117±14.4	132±18.1	122±14.1
	200 mg	18	116±10.7	148±22.2	111±14.5
	400 mg	17	137±20.9	120±14.7	129±19.5
RLP-Cholesterol (mg/dL)	Placebo	18	6.4±1.24	7.2±1.19	6.0±1.00
	200 mg	18	6.0±0.45	6.9±0.82	5.2±0.63 [#]
	400 mg	17	6.7±1.18	5.7±0.66	6.2±1.21
Apolipoprotein A1 (mg/dL)	Placebo	18	145±4.3	147±4.5	147±3.9
	200 mg	18	153±5.9	154±6.9	154±6.0
	400 mg	17	151±4.2	152±4.7	153±5.3
Apolipoprotein B (mg/dL)	Placebo	18	113±3.8	115±4.4	111±3.7
	200 mg	18	116±3.5	113±3.5	111±3.3 [#]
	400 mg	17	115±3.1	111±3.4 [#]	112±3.9
Apolipoprotein E (mg/dL)	Placebo	18	4.4±0.38	4.7±0.45 [#]	4.6±0.35
	200 mg	18	4.3±0.20	4.4±0.22	4.3±0.22
	400 mg	17	4.1±0.17	4.2±0.21	4.3±0.17
Apolipoprotein B/A1	Placebo	18	0.80±0.04	0.80±0.04	0.77±0.03
	200 mg	18	0.78±0.04	0.76±0.04	0.74±0.04 [#]
	400 mg	17	0.77±0.03	0.74±0.03	0.75±0.03
MDA-LDL (U/L)	Placebo	18	122±7.0	129±8.6	113±7.4
	200 mg	18	122±6.4	130±6.8	107±5.3 [#]
	400 mg	17	122±7.9	108±6.3 [#]	105±6.5 [#]
MDA-LDL/Apo B (mU/mg)	Placebo	18	107±3.7	111±4.6	102±5.8
	200 mg	18	105±4.5	115±5.1	97±4.1
	400 mg	17	105±5.0	97±3.8 [#]	93±4.3 [#]

Data represent means±SE.

[#] Compared to before ($p<0.05$).

safety were evaluated 6 and 12 wk after the commencement of administration.

In Study 1, antioxidant effects and safety induced by grape seed extract were assessed, while in Study 2, effects of grape seed extract on adiponectin were investigated.

Items for determination. Height·body weight·body mass index (BMI), blood pressure·pulse rate, medical diagnosis findings. For blood biochemistry tests, total cholesterol, LDL-C, HDL-C, triglycerides, white blood cell counts, hematocrit, hemoglobin, platelet counts, total protein, albumin, A/G, total bilirubin, AST (GOT), ALT (GPT), ALP, LDH, γ -GTP, CPK, uric acid, BUN, creatinin, fasting blood glucose level, hemoglobin_{A1c} (HbA_{1c}), malondialdehyde modified LDL (MDA-LDL or oxidized LDL) as the oxidized LDL, remnant-like particles-cholesterol

(RLP-C), apoprotein (immunoturbidimetric method), and adiponectin (ELISA method) were assessed. Urine tests were used to assess protein, glucose, occult blood reaction, and urine sediments. MDA-LDL levels were determined by an EIA method (13) while RLP-C was measured by immunoadsorption.

Statistical analysis. For Study 1, a paired *t*-test was used for within-group comparisons of antioxidant effects of grape seed extract and its safety evaluation while a one-way analysis of variance followed by a multiple comparison test (Dunnett test) was used for inter-group comparisons. With regards to MDA-LDL/ApoB, Kondo et al. (14) demonstrated that MDA-LDL/ApoB in subjects with low HDL (TG $\leq$ 1.69 mM, HDL $\leq$ 0.91 mM) was 99 ± 49 mU/mL; therefore, we called the group with more than 100 mU/mL MDA-LDL/ApoB the high MDA-LDL/ApoB group.

Fig. 1. Effects of grape seed extract supplementation on serum MDA-LDL in high MDA-LDL/ApoB subjects. Data represent means $\pm$ SE. * p <0.05 compared to placebo using the Dunnett test. # p <0.05 compared to before treatment using a paired *t*-test.

Fig. 2. Effects of grape seed extract supplementation on MDA-LDL/ApoB in high MDA-LDL/ApoB subjects. Data represent means $\pm$ SE. # p <0.05 compared to before treatment using a paired *t*-test.

Table 4. Changes in body weight, BMI, blood pressure, pulse of subjects given grape seed extracts.

Item	Group	n	Before	Week 6	Week 12
Body weight (kg)	Placebo	18	62.1 $\pm$ 2.22	62.0 $\pm$ 2.19	62.5 $\pm$ 2.24
	200 mg	18	63.5 $\pm$ 2.71	64.0 $\pm$ 2.71	64.1 $\pm$ 2.70
	400 mg	17	62.3 $\pm$ 2.57	62.8 $\pm$ 2.56	62.4 $\pm$ 2.59
BMI	Placebo	18	24.2 $\pm$ 0.63	24.2 $\pm$ 0.61	24.3 $\pm$ 0.64
	200 mg	18	24.4 $\pm$ 0.66	24.6 $\pm$ 0.66	24.6 $\pm$ 0.65
	400 mg	17	24.0 $\pm$ 0.65	24.2 $\pm$ 0.65	24.1 $\pm$ 0.64
Systolic blood pressure (mmHg)	Placebo	18	122.7 $\pm$ 4.39	124.2 $\pm$ 4.99	127.8 $\pm$ 4.84
	200 mg	18	126.4 $\pm$ 2.97	127.8 $\pm$ 3.22	129.2 $\pm$ 3.22
	400 mg	17	126.2 $\pm$ 3.98	126.9 $\pm$ 3.40	127.7 $\pm$ 3.00
Diastolic blood pressure (mmHg)	Placebo	18	77.1 $\pm$ 2.60	77.0 $\pm$ 2.87	81.1 $\pm$ 2.80
	200 mg	18	77.9 $\pm$ 1.66	80.1 $\pm$ 2.08	79.6 $\pm$ 2.00
	400 mg	17	78.0 $\pm$ 2.46	79.1 $\pm$ 2.39	79.5 $\pm$ 1.98
Pulse (bpm)	Placebo	18	67.9 $\pm$ 2.15	66.5 $\pm$ 2.44	68.8 $\pm$ 2.33
	200 mg	18	71.1 $\pm$ 2.40	70.6 $\pm$ 2.62	70.0 $\pm$ 2.40
	400 mg	17	72.8 $\pm$ 2.28	70.1 $\pm$ 1.83	73.0 $\pm$ 2.54

Data represent means $\pm$ SE.

Table 5. Changes in biochemical conditions of subjects given grape seed extracts.

Item	Standard value	Group	n	Before	Week 6	Week 12
White blood cell count	M 3,900–9,800/ μ L	Placebo	18	5389 $\pm$ 217	5350 $\pm$ 225	5444 $\pm$ 211
	F 3,500–9,100/ μ L	200 mg	18	5989 $\pm$ 385	5833 $\pm$ 409	5839 $\pm$ 320
		400 mg	17	5882 $\pm$ 439	5718 $\pm$ 592	5782 $\pm$ 452
Red blood cell count	M 427–570 $\times 10^4$ / μ L	Placebo	18	435 $\pm$ 12	447 $\pm$ 12	438 $\pm$ 13
	F 376–500 $\times 10^4$ / μ L	200 mg	18	443 $\pm$ 11	453 $\pm$ 11	453 $\pm$ 10
		400 mg	17	449 $\pm$ 13	450 $\pm$ 11	454 $\pm$ 11
Hemoglobin	M 13.5–17.6 g/dL	Placebo	18	13.6 $\pm$ 0.33	14.1 $\pm$ 0.35	13.8 $\pm$ 0.35
	F 11.3–15.2 g/dL	200 mg	18	13.7 $\pm$ 0.38	14.0 $\pm$ 0.36	13.9 $\pm$ 0.34
		400 mg	17	13.9 $\pm$ 0.36	14.0 $\pm$ 0.31	14.1 $\pm$ 0.31
Hematocrit	M 39.8–51.8%	Placebo	18	40.6 $\pm$ 1.06	41.9 $\pm$ 1.10	41.1 $\pm$ 1.20
	F 33.4–44.9%	200 mg	18	40.8 $\pm$ 1.13	41.8 $\pm$ 1.09	41.8 $\pm$ 1.04
		400 mg	17	41.5 $\pm$ 1.20	41.7 $\pm$ 1.06	42.0 $\pm$ 1.07
Platelet count	M 13.1–36.2 $\times 10^4$ / μ L	Placebo	18	24.1 $\pm$ 0.98	24.9 $\pm$ 0.75	24.8 $\pm$ 0.93
	F 13.0–36.9 $\times 10^4$ / μ L	200 mg	18	24.5 $\pm$ 0.99	25.8 $\pm$ 1.13	26.0 $\pm$ 1.15
		400 mg	17	25.4 $\pm$ 1.19	25.1 $\pm$ 1.30	25.8 $\pm$ 1.19
Total protein	6.7–8.3 g/dL	Placebo	18	7.2 $\pm$ 0.07	7.4 $\pm$ 0.09	7.3 $\pm$ 0.11
		200 mg	18	7.3 $\pm$ 0.07	7.4 $\pm$ 0.07	7.5 $\pm$ 0.07
		400 mg	17	7.2 $\pm$ 0.09	7.2 $\pm$ 0.08	7.4 $\pm$ 0.10
Albumin	4.0–5.0 g/dL	Placebo	18	4.3 $\pm$ 0.04	4.4 $\pm$ 0.04	4.4 $\pm$ 0.06
		200 mg	18	4.4 $\pm$ 0.05	4.4 $\pm$ 0.06	4.5 $\pm$ 0.04
		400 mg	17	4.4 $\pm$ 0.05	4.4 $\pm$ 0.05	4.5 $\pm$ 0.05
A/G	1.2–2.0	Placebo	18	1.5 $\pm$ 0.04	1.5 $\pm$ 0.03	1.5 $\pm$ 0.04
		200 mg	18	1.5 $\pm$ 0.05	1.5 $\pm$ 0.05	1.5 $\pm$ 0.05
		400 mg	17	1.6 $\pm$ 0.05	1.6 $\pm$ 0.04	1.6 $\pm$ 0.05
Total bilirubin	0.2–1.0 mg/dL	Placebo	18	0.8 $\pm$ 0.05	0.8 $\pm$ 0.05	0.8 $\pm$ 0.06
		200 mg	18	0.7 $\pm$ 0.04	0.7 $\pm$ 0.05	0.7 $\pm$ 0.06
		400 mg	17	0.7 $\pm$ 0.06	0.7 $\pm$ 0.06	0.6 $\pm$ 0.05
AST (GOT)	10–40 IU/L/37°C	Placebo	18	24.4 $\pm$ 2.94	23.1 $\pm$ 2.02	27.1 $\pm$ 3.21
		200 mg	18	22.2 $\pm$ 1.37	23.3 $\pm$ 1.02	25.2 $\pm$ 0.95
		400 mg	17	23.5 $\pm$ 1.41	22.7 $\pm$ 1.02	22.1 $\pm$ 1.07
ALT (GPT)	5–40 IU/L/37°C	Placebo	18	24.9 $\pm$ 3.34	24.9 $\pm$ 3.56	31.2 $\pm$ 5.34
		200 mg	18	25.7 $\pm$ 3.72	27.7 $\pm$ 3.32	28.8 $\pm$ 2.86
		400 mg	17	26.5 $\pm$ 2.57	25.6 $\pm$ 2.34	25.0 $\pm$ 1.45
ALP	115–359 IU/L/37°C	Placebo	18	205 $\pm$ 13	221 $\pm$ 13	224 $\pm$ 13
		200 mg	18	231 $\pm$ 12	243 $\pm$ 13	243 $\pm$ 15
		400 mg	17	207 $\pm$ 15	214 $\pm$ 16	213 $\pm$ 16
LDH	115–245 IU/L/37°C	Placebo	18	171 $\pm$ 8	170 $\pm$ 6	170 $\pm$ 7
		200 mg	18	179 $\pm$ 4	182 $\pm$ 5	181 $\pm$ 5
		400 mg	17	164 $\pm$ 7	162 $\pm$ 7	160 $\pm$ 6
g-GTP	M $\leq$ 70 IU/L/37°C	Placebo	18	34.9 $\pm$ 7.22	40.1 $\pm$ 8.63	41.5 $\pm$ 8.53
	F $\leq$ 30 IU/L/37°C	200 mg	18	27.8 $\pm$ 4.58	30.2 $\pm$ 4.54	30.6 $\pm$ 4.65
		400 mg	17	33.8 $\pm$ 4.71	33.6 $\pm$ 5.17	33.4 $\pm$ 5.07
CPK	M 57–197 IU/L/37°C	Placebo	18	261 $\pm$ 152	102 $\pm$ 6	99 $\pm$ 11
	F 32–180 IU/L/37°C	200 mg	18	116 $\pm$ 9	123 $\pm$ 10	120 $\pm$ 13
		400 mg	17	124 $\pm$ 16	116 $\pm$ 11	107 $\pm$ 9
Uric acid	M 3.7–7.0 mg/dL	Placebo	18	5.5 $\pm$ 0.37	5.7 $\pm$ 0.39	5.4 $\pm$ 0.37
	F 2.5–7.0 mg/dL	200 mg	18	5.3 $\pm$ 0.23	5.4 $\pm$ 0.24	5.4 $\pm$ 0.24
		400 mg	17	5.2 $\pm$ 0.28	5.0 $\pm$ 0.30	4.9 $\pm$ 0.29
Blood urea nitrogen	6–20 mg/dL	Placebo	18	12.6 $\pm$ 0.80	12.5 $\pm$ 0.54	13.0 $\pm$ 0.73
		200 mg	18	14.1 $\pm$ 0.89	13.8 $\pm$ 0.73	13.1 $\pm$ 0.78
		400 mg	17	12.8 $\pm$ 0.69	11.9 $\pm$ 0.85	12.6 $\pm$ 0.75
Creatinine	M 0.61–1.04 mg/dL	Placebo	18	0.8 $\pm$ 0.04	0.8 $\pm$ 0.05	0.7 $\pm$ 0.04
	F 0.47–0.79 mg/dL	200 mg	18	0.8 $\pm$ 0.06	0.7 $\pm$ 0.04	0.7 $\pm$ 0.03
		400 mg	17	0.7 $\pm$ 0.04	0.7 $\pm$ 0.03	0.7 $\pm$ 0.03
Glucose	70–109 mg/dL	Placebo	18	97.8 $\pm$ 3.05	98.0 $\pm$ 2.71	100.3 $\pm$ 3.32
		200 mg	18	93.5 $\pm$ 1.96	96.1 $\pm$ 3.04	95.8 $\pm$ 2.38
		400 mg	17	94.2 $\pm$ 1.39	95.5 $\pm$ 2.61	96.9 $\pm$ 2.01
HbA _{1c}	4.3–5.8%	Placebo	18	4.9 $\pm$ 0.11	4.9 $\pm$ 0.11	4.9 $\pm$ 0.11
		200 mg	18	5.1 $\pm$ 0.08	5.0 $\pm$ 0.08	5.0 $\pm$ 0.08
		400 mg	17	5.1 $\pm$ 0.08	5.0 $\pm$ 0.08	5.0 $\pm$ 0.09

Data represent means $\pm$ SE.

For Study 2, a paired *t*-test was used to assess effects of grape seed extract on adiponectin while a one-way analysis of variance followed by a multiple comparison test (Dunnnett test) was used for inter-group comparisons. Furthermore, values indicating a correlation between adiponectin and various measurements were standardized, and subjected to a single regression analysis. The standardization of the data was calculated by the ratio of the standard deviation for the difference in mean value and each value. Including the age, the mean value was adjusted to 0 in all items, and the standard deviation was adjusted to 1. Therefore, it is considered that the effect of the unit can be avoided, when the correlation coefficient is calculated.

All measured values were shown as means $\pm$ SE. Measured values were analyzed with SPSS ver 13.0J (SPSS Japan Inc.). Significant level was less than 5% in the two-tail test.

RESULTS

Table 2 shows baseline values (at the time of screening) of eligible subjects. No test item was significantly different among the 3 groups. Investigations were performed for 53 subjects following exclusion of 4 subjects including 2 drop-out cases due to their own voluntary discretion during the test period, 1 case who caught the common cold accompanied by diarrhea and headaches (no correlation with the tablets), 1 case who experienced asthmatic attacks (no correlation with the tablets) and another 4 subjects whose compliance with instructions was poor.

Study 1 (Antioxidant effect of grape seed extract)

Table 3 shows temporal changes in serum lipid after intake of grape seed extract. Although total cholesterol and LDL-C levels significantly increased compared to basal levels in the control group, there was no significant difference among the groups. HDL-C levels significantly increased compared to those at baseline in the control, 200 mg, and 400 mg groups 12 wk after the start of administration. However, no significant difference was found among groups. During the study period, triglyceride levels didn't significantly change. RLP-C level in the 200 mg group significantly decreased compared to values prior to the 12th week; however, there was no significant difference in RLP-C level with that in the control group. ApoA1 level did not show any significant changes during the study period. While ApoB level in the 200 mg and 400 mg groups significantly decreased compared to baseline values, no significant change in the control group was found. Despite a significant increase in ApoE level at the 6th week in the control group, no significant difference was observed among other groups. ApoB/A1 level in the 200 mg group significantly decreased at week 12, but there was no significant difference compared to the control group.

MDA-LDL level 6 wk after the start of administration was significantly decreased compared to previous levels in the 400 mg group, and was significantly reduced from previous levels 12 wk after the start of administration both in the 200 mg and 400 mg groups. There was

Table 6. Correlations between each measured value and adiponectin

Item	<i>r</i>	<i>p</i>
Age	0.352	0.010
Body weight	-0.597	<0.001
BMI	-0.470	<0.001
LDL-Cholesterol	0.052	0.714
MDA-LDL	-0.152	0.276
HDL-Cholesterol	0.426	0.001
Total cholesterol	0.154	0.270
Triglycerides	-0.368	0.007
RLP-Cholesterol	-0.303	0.027
Apolipoprotein A1	0.371	0.006
Apolipoprotein B	-0.241	0.083
Apolipoprotein E	-0.106	0.449
Apolipoprotein B/A1	-0.395	0.003

n = 53.

a significant decrease in MDA-LDL/ApoB level from values prior to weeks 6 and 12 in the 400 mg group.

Figure 1 illustrates changes in serum MDA-LDL levels of subjects with a high MDA-LDL/ApoB level (11 cases in the Placebo group, 12 cases in the 200 mg group, and 10 cases in the 400 mg group) demonstrating more than 100 mU/mL MDA-LDL/ApoB. Figure 2 shows temporal changes in MDA-LDL/ApoB levels. Serum MDA-LDL levels at week 6 in the 400 mg group were significantly lower than previous values in the control group while they were significantly lower than previous values at the 12th week both in the 200 mg and 400 mg groups. In addition, a multiple comparison test revealed a decreasing trend in the 200 and 400 mg groups compared to the control group. MDA-LDL/ApoB levels were significantly decreased at the 6th and 12th wk in the 400 mg group compared to previous values, indicating a decreasing trend relative to that in the control group.

Safety of tablets

Tables 4 and 5 show results obtained for physiological and clinical laboratory tests after commencement of administration in the control group, and the 200 mg and 400 mg groups. From physiological and hematological tests, neither abnormal values nor abnormal changes were observed, and no abnormalities in subjective or objective symptoms were noted. Even in urinary sedimentation, no problematic findings were found (data are not shown).

Study 2 (Correlation between grape seed extract and adiponectin)

There were no significant changes in adiponectin levels attributable to ingestion of grape seed extract. To correlate adiponectin level with various parameters before ingestion of the tablets, the observed values were standardized and subjected to a single regression analysis. Results obtained are shown in Table 6. Adiponectin level showed a significant positive correlation with age ($r=0.352$), HDL-C ($r=0.426$), and ApoA1 ($r=0.371$), but showed a significant negative correlation with body

Table 7. Effects of grape seed extract supplementation on serum adiponectin (change in body weight $< \pm 1.0$ kg).

Item	Group	n	Before	Week 6	Week 12
Adiponectin ($\mu\text{g/mL}$)	Placebo	10	9.98 ± 1.30	$10.91 \pm 1.39^{\#}$	10.77 ± 1.39
	200 mg	11	8.83 ± 0.87	9.13 ± 0.80	8.89 ± 0.86
	400 mg	14	8.70 ± 1.06	8.93 ± 1.01	$9.44 \pm 1.06^{\S}$

Data represent means $\pm$ SE.

[#] Compared to before ($p < 0.05$).

[§] Compared to before ($p = 0.088$).

weight ($r = -0.597$), BMI ($r = -0.470$), triglycerides ($r = -0.368$), RLP-C ($r = -0.303$), and ApoB/A1 ($r = -0.395$). Among these parameters, adiponectin demonstrated the highest correlation with body weight. Accordingly, an analysis was conducted to investigate effects of grape seed extract on adiponectin level using subjects with the least alterations in body weight during the study period (less than ± 1 kg). Results obtained are shown in Table 7. Intake of grape seed extract tended to increase adiponectin level ($p = 0.088$) at the 12th week in the 400 mg group compared to previous values.

DISCUSSION

In the present study, a parallel inter-group test using a single blind method for 53 subjects presenting LDL-C values ≥ 100 mg/dL and < 180 mg/dL was performed following administration of grape seed extract for 12 wk. In Study 1, we investigated both the effects and safety of grape seed extract ingestion on oxidized LDL. In Study 2, effects of grape seed extract on adiponectin level were evaluated.

It is well recognized that oxidized LDL plays a role in the onset of arteriosclerosis. The present study showed that administration of grape seed extract lowered MDA-LDL levels and MDA-LDL/ApoB values, indicating MDA modification of ApoB which is the major apolipoprotein in LDL-C. In particular, remarkable decreases in both MDA-LDL and MDA-LDL/ApoB levels were observed in subjects with high MDA-LDL/ApoB. According to a previous report (14) showing that MDA-LDL/ApoB was 99 ± 49 mU/mL in subjects with low levels of HDL, we defined subjects with MDA-LDL/ApoB values of more than 100 mU/mL as high MDA-LDL/ApoB subjects, who were considered to be higher risk persons predisposed to arteriosclerosis. Results showed that grape seed extract significantly decreased the MDA-LDL level 6 wk after the start of tablet administration in subjects with high MDA-LDL/ApoB in the 400 mg group compared to previous values and the control group. There was a significant reduction in the MDA-LDL level 12 wk after tablet administration in the 200 mg and 400 mg groups compared to previous values. The MDA-LDL/ApoB level in subjects with high MDA-LDL/ApoB was significantly reduced at weeks 6 and 12 in the 400 mg group compared to previous values. Although a decreasing trend was observed in the 200 mg group compared to previous values, this was not statistically

significant. Such a decreasing effect on oxidized LDL by grape seed extract demonstrated dose-dependency.

It has been reported (15) that intake of red wine is associated with a reduction of mortality, and with a decrease in morbidity from dementia. These effects may be due to effects of polyphenol contained in grape seeds on various lifestyle-related diseases. The main phenolic components of red wine are anthocyanins, catechins, resveratrol, phenolic acids, and the flavonols quercetin and myricetin (16, 17). The concentrations of these components in red wine can vary widely (18–20). Several *in vitro* studies have shown an antioxidant effect of red wine and fractionated phenolic compounds on LDL oxidation (21–24). The results of the present study show that supplementation of human subjects with grape seed extract can inhibit oxidation of LDL.

Proanthocyanidin, adopted as the major ingredient of the grape seed extract tablets, is contained in various plants, besides being contained in small amounts in fruits like grapes, in barley, in beans, and in their processed foods. By ingesting these foods, most of us intake proanthocyanidin daily. Proanthocyanidin is known to exert remarkable antioxidant effects in aqueous systems (25). Proanthocyanidin is specifically accumulated in blood vessel walls, and captures active oxygen in the interstitial fluid (aqueous system) of blood vessel walls, whereby generation of oxidized LDL is inhibited to reduce foam cells, leading to inhibition of the onset of arteriosclerosis (26). In fact, it is known that proanthocyanidin contained in grape seeds exhibits arteriosclerosis-inhibiting effects in rabbits fed with cholesterol-loaded food (27). It was also reported that meals with concomitant ingestion of grape seed extract increased antioxidant substance levels in plasma, thereby reducing oxidized substances, resulting in decreasing postprandial oxidation-related stress, and increasing resistance of LDL toward oxidative modification (28–30). Preuss et al. (31) evaluated oxidized LDL autoantibodies as an index of reduction of other oxidized LDL. As a result, they reported that administration of grape seed extract to hyperlipidemic patients for 8 wk decreased oxidized LDL autoantibody levels. No report is available on the possible effects of ingestion of grape seed extract for more than 12 wk on oxidized LDL levels. Although there is no report on effects of grape seed extract on serum lipids following administration of grape seed extract for more than 12 wk, it was documented that administration of grape seed extract for 8 wk was not

associated with any effects on total cholesterol, LDL-C, or triglycerides (31). The same was true in the present study, as there was no effect on these lipids. In the present study, indices for evaluation of reducing effects of grape seed extract on oxidized LDL levels were MDA-LDL and MDA-LDL/ApoB in which MDA represents the most notable lipid peroxidized product bound to the lysine residue in ApoB in the form of Schiff's base. As a result, it was confirmed that grape seed extract had oxidized LDL reducing effects, and grape seed extract might be useful in preventing lifestyle-related diseases such as arteriosclerosis.

In addition, a possible correlation of adiponectin with lifestyle-related diseases has been recently reported. It has been reported that adiponectin levels in human blood are decreased in obese persons (type 2 diabetics), and in adult patients with coronary artery diseases, indicating the importance of anti-arteriosclerotic effects (32). Cnop et al. (33) revealed a positive correlation between adiponectin and HDL-C with age, and negative correlations between adiponectin and BMI, waist/hip, and triglycerides. It is known that red wine intake and moderate alcohol consumption increase the adiponectin level (34–36). The present study failed to clearly show effects of grape seed extract on adiponectin in all subjects included in the analysis. However, it was postulated that adiponectin had the highest correlation with body weight, as it was affected by changes in body weight. Accordingly, analysis was performed for subjects showing less alteration in body weight during the study period as eligible subjects, resulting in observation of an increase in adiponectin level due to administration of grape seed extract. However, this increase was not statistically significant.

Recently, it has been reported (37) that oolong tea increases plasma adiponectin level in patients with coronary artery diseases, and similar effects could be expected for grape seed extract. Altogether, increasing expectations exist for future research taking into account the age of subjects for evaluation. Furthermore, it has been verified that blood flow improves the effects of grape seed extract (38); therefore, grape seed extract could be useful in preventing lifestyle-related diseases.

During the study period, no harmful event attributable to the tablets containing grape seed extract was observed. Even in clinical laboratory tests, any alterations including minor changes fell within basal level ranges, indicating the safety of tablets containing grape seed extracts.

In conclusion, tablets containing grape seed extract are safe, and tend to particularly lower oxidized LDL and elevate adiponectin level in subjects with higher levels of oxidized LDL, indicating that grape seed extract might be a useful food in preventing the occurrence of lifestyle-related diseases including arteriosclerosis.

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