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Effect of Dietary Wine Pomace Extract and Oleanolic Acid on Plasma Lipids in Rats Fed High-Fat Diet and Its DNA Microarray Analysis

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The aim of this study was to evaluate the concentration of oleanolic acids (OA) in pomace, a winemaking byproduct, and its influence on the levels of plasma lipids in rats fed a high-fat diet and on hepatic gene expression using DNA microarray analysis in vivo. HPLC analyses of pomace ethanol extract (PEE) revealed a high amount of OA ranging from 4 to 11 g/100 g. Male Sprague−Dawley rats were fed a normal-fat diet (NF group), a high-fat diet with 21% lard (HF group), a high-fat diet with 0.05% OA (OA group, 50 mg/kg/day), or a high-fat diet with 0.45% PEE (PEE group, 450 mg/kg/day). Plasma triacylglycerol and phospholipid concentrations were significantly lower in the OA and PEE groups than in the HF group. The microarray analysis of hepatic mRNA revealed reduced expression levels of lipogenic genes including acetyl-CoA carboxylase and glycerol-3-phosphate acyltransferase, probably resulting from the suppression of transcription factor Srebf1 expression. Gene expression of gluconeogensis and inflammatory cytokines was also down-regulated in the OA and PEE groups, suggesting that administration of OA or PEE could ameliorate obesity-induced insulin resistance, as well as prevent hyperlipidemia.

KEYWORDS: DNA microarray; oleanolic acid; triterpene

INTRODUCTION

Grape production has developed into the most important fresh fruit crop in the world, of which 70% is used for wine production (*1*). Harvested grapes are first destemmed and crushed, which produces approximately 4 wt % stem despite differences due to species. White cultivars are immediately pressed, which produces 20 wt % of pomace. As for red cultivars, must with skin and seed is fermented for 7 days before being pressed, which produces 16 wt % of pomace. These winemaking byproducts, called pomace or marc, are produced in vast amounts across the world, and they have been tested for use as compost, as a supply source of polyphenol, and as a raw material of grape seed oil (*2*−*6*). Procyanidin is already commercially available as a supplement because of its high antioxidant potency (*7*).

As for functional components of grape skin, 3β-hydroxyolean-12-en-28-oic acid, commonly called oleanolic acid (OA) (**Figure 1**), has been studied as have other hydrophilic polyphenol components (*8*−*10*). Triterpenoic acids such as OA, together with its analogue and glycoside as well as free form, are widely distributed in plants (*11, 12*). These triterpenoids have been known to exhibit multiple biological properties, such as hepatoprotection (*13*−*17*), anti-inflammation (*10, 11, 18*), and antitumor activity (*19*). All of the pharmacological properties of OA were reviewed by Liu (*20*). In addition, it is clear that slight structural differences in triterpenoids induce different physiological activities (*12, 17, 19, 20*). In the in vivo animal experiments, because plant triterpenes have always been given by the methods of sc or ip, the effect of their oral administration (po) was unclear. However, it was reported that dietary OA and its analogue maslinic acid possess therapeutic effects on high cholesterol diet-induced hyperlipidemia in rat (*21*). This suggests that dietary triterpenes have some impacts on lipid metabolisms in vivo.

During the winemaking process, it is assumed that OA could not shift from skin to the must and remains intact in pomace

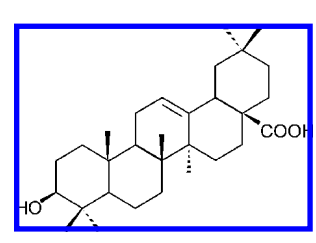

Figure 1. Chemical structure of oleanolic acid.

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because of its lipophilic property. The aim of this study was to investigate the concentrations of OA in the pomace of different cultivars and from different production methods, the effects of pomace ethanol extract (PEE) and its content of OA on plasma lipid levels in rats fed high-fat diets, and their mechanism by a nutrigenomic analysis using DNA microarray.

MATERIALS AND METHODS

Experimental Samples. Five red cultivars (Kiyomi, Wild Grape, Yamasachi, Kiyomai, and Zweigeltrebe) and three white cultivars (Kerner, Bacchus, and Morio Muskat) were used for wine production at Tokachi-Ikeda Research Institute for Viticulture and Enology, Ikeda, Hokkaido, Japan, in 2005. The harvested red cultivars were destemmed and crushed prior to alcoholic fermentation. Alcoholic fermentation was carried out using a commercial yeast (Lalvin EC1118, Lallemand Inc.) at 25 °C for 7 days. Then, each must ferment was pressed to yield fresh pomace. The harvested white cultivars were destemmed and crushed before being pressed to yield fresh pomace. Fresh pomace was dried in the sun for 2 days and then dried at 60 °C for 24 h. To investigate the composition of pomace ingredients, 7 g of each dried pomace was divided into skin, seed, and stem and then weighed.

Preparation of Pomace Ethanol Extracts and Their Component Analyses. *Extraction.* Twenty grams of dried pomace was milled and agitated with 100 mL of ethanol for 1 h and then filtered using filter paper (Advantec no. 2, Toyo Roshi Kaisha). After three repetitions of a similar operation, the combined extracts were concentrated to dryness using a rotary evaporator, and the dry matter was weighed to yield the pomace ethanol extract (PEE).

Determination of Polyphenol Contents. PEE was partitioned with hexane/ethanol/water (2:1:1, v/v/v), and the upper phase (lipophilic fraction) was removed after centrifugation. Total polyphenols were quantified in the lower phase using Folin−Ciocalteu's reagent and gallic acid as a standard (*22*).

Determination of Lipid Contents. Lipid components were separated from PEE according to Folch's method (*23*). Briefly, PEE was partitioned with chloroform/methanol/water (8:4:3, v/v/v). The lower phase was concentrated to dryness, and the lipids were weighed to yield the total lipids.

Determination of Sugar Contents. The sugar contents of PEE were determined using anthrone/sulfuric acid reagent according to the method of Radin et al. (*24*).

HPLC Analysis. The concentration of OA in the total lipids prepared from each pomace was determined by normal phase HPLC analysis as follows: instrument, Shimadzu LC-9A; column, LiChrospher Si60 (250 × 4 mm, Merck); detection, refractive index detector (ERC-7520, ERMA); solvent, hexane/2-propanol (97.5:2.5, v/v); flow rate, 1.0 mL/min; column temperature, 40 °C. The amounts of OA (identified by cochromatography with commercial OA) in each sample were calculated from the calibration curve of standard OA (Wako). The representative calibration curve was $y = 25365x - 5526$ (y = area; x = mg of OA). The correlation coefficient was 0.9970, from 0.04 to 0.4 mg of OA was injected, and the detection limit of OA was approximately 0.025 mg.

Animals. Male Sprague−Dawley rats (4 weeks old) were obtained from Japan Clair. Twenty-four rats were fed AIN-93 (Japan Clair) for 1 week (*25*) and then divided into four groups of six animals each. Each group was fed experimental diets for 4 weeks. The animal room was kept at 23 ± 1 °C, and the relative humidity was kept at 65 ± 5%. Room lighting consisted of 12 h periods of light and dark. The diets were given by the pair-feeding method (vs HF, as described below), and water was given ad libitum. All rats were treated according to the guidelines for experimental animals of the Obihiro University of Agriculture and Veterinary Medicine, Japan. Food intake and body weight were monitored daily throughout the experimental period.

Diet. Two kilograms of pomace (Kiyomi) was milled and extracted with 10 L of ethanol to prepare PEE for the animal experiments. The composition of the PEE obtained was consistent with that of PEE prepared from 20 g of pomace, as described above, which included 11% OA. A normal-fat diet based on the AIN-93 (NF group), a high-fat diet containing lard and sucrose (HF group), a HF diet containing 0.05% OA (Wako) (OA group, 50 mg/kg/day), and a HF diet containing 0.45% PEE (PEE group, 450 mg/kg/day) were used as experimental diets. The compositions of the diets are given in **Table 1**.

Table 1. Composition of the Diets (Percent)

component	dietary group			
	NF[a]	HF[b]	OA[c]	PEE[d]
cornstarch	34.8			
casein	23.6	23.6	23.6	23.6
α-cornstarch	13.2	8.4	8.4	8.4
sucrose	10.0	28.8	28.8	28.8
soybean oil	7.0	7.0	7.0	7.0
lard	-	20.8	20.8	20.8
cellulose	6.4	6.4	6.4	6.4
mineral mix (AIN93)	3.5	3.5	3.5	3.5
vitamin mix (AIN93)	1.0	1.0	1.0	1.0
L-cystine	0.3	0.3	0.3	0.3
choline bitartrate	0.2	0.2	0.2	0.2
tert-butylhydroquinone	0.001	0.001	0.001	0.001
OA			0.05	
PEE				0.45

[a] NF:, normal-fat diet group. [b] HF, high-fat diet group. [c] OA, HF diet containing 0.05% OA (OA group). [d] PEE, HF diet containing 0.45% PEE (PEE group).

Preparation of Plasma and Liver Samples. At the end of feeding trials, all rats were fasted for 24 h before being anesthetized with diethyl ether. After the abdominal cavity had been opened, blood was collected from the heart with a heparinized syringe and put into ice-cold tubes. Then, the livers were perfused with saline before being removed and weighed. The livers were cut finely in an RNA later (Takara Bio) at 4 °C and frozen at −80 °C. The plasma was separated by centrifugation at 1000*g* for 20 min at 4 °C and stored at −80 °C until required for analysis.

Analysis of Plasma Lipids. The total cholesterol, triacylglycerol, and phospholipid concentrations in the plasma samples were determined using commercial test kits (Cholesterol E-, Triglyceride E-, and Phospholipid C-test Wakos, respectively, Wako) according to the enzymatic methods of Allain et al. (*26*), Spayd et al. (*27*), and Takayama et al. (*28*), respectively.

DNA Microarray Analysis. DNA microarray analysis was performed according to the CodeLink Bioarray System Appendix (GE Healthcare). Total RNA was extracted from each liver using the SV total RNA Isolation System (Promega), and its quality was checked by its absorbance at 260 and 280 nm and by electrophoresis. Equal amounts of the RNA from six rats of each experiment group were pooled to normalize individual differences. Two micrograms of total RNA was used to synthesize double-strand cDNA by reverse transcription, which was then transcribed in vitro in the presence of biotinylated UTP (Perkin-Elmer). Biotinylated target cRNA was fragmented and hybridized to CodeLink Rat Whole Genome Bioarray (GE Healthcare) at 37 °C for 18 h. All hybridizations of this experiment were performed with arrays from the same manufacturing lot. After hybridization, the arrays were washed and stained with Streptavidin-Cy5 (GE Healthcare). After staining, microarray slides were scanned by an arrayWorx scanner (Applied Precision). The collected fluorescence signals were background corrected and converted into numeric data with the CodeLink System Software (GE Healthcare). These data were normalized and analyzed using GeneSpring 7.2 software (Silicon Genetics). At first, we chose probes with a "present flag" for further analysis. Next, we chose the probes for which the signal level was up- or down-regulated more than 1.5-fold compared to the control group. For biological interpretation of the differentially expressed genes, the GO ontology browser of GeneSpring was used. GO biological processes with $p < 0.01$ were considered to be significantly changed. Moreover, differentially expressed genes were categorized and mapped onto pathway using KEGG tools (http://www.genome.jp/kegg/tool/color_pathway.html).

Statistical Analysis. All results were expressed as the mean. The data from the animal experiments were expressed as the mean plus error bars. Statistical significance was evaluated by ANOVA followed by the Scheffe test. A p value of <0.05 was regarded as statistically significant.

Table 2. Ingredients of Pomace and Chemical Properties of the PEE

	pomace ingredients[b]				g/100 g of PEE[d]			
variety	skin	seed	stem	yield[c]	lipid[e]	OA	PP[f]	other
Kiyomi	65	24	11	8	82	11	2	16
Wild Grape	30	60	10	10	63	4	2	35
Yamasachi	43	41	15	7	59	9	1	40
Kiyomai	36	46	19	9	62	5	1	37
Zweigeltrebe	65	19	15	11	63	7	6	31
Kerner[a]	46	40	13	37	19	2	3	78
Bacchus[a]	63	20	18	25	25	1	3	72
Morio Muskat[a]	50	38	11	21	40	6	2	58

[a] White cultivers, the others red cultivers. [b] Dry weight percent. Values are means. [c] Ethanol extract (g/100 g of dry matter). Values are means. [d] Values are means. [e] Total lipids including OA. [f] Polyphenol.

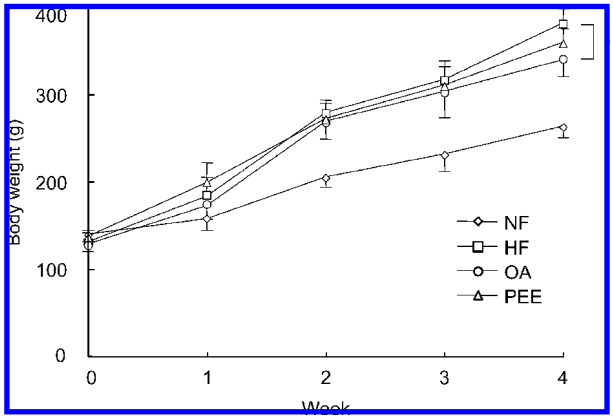

Figure 2. Time courses of changes in body weights of rats ($n = 6$/group). NF, normal-fat diet group; HF, high-fat diet group; OA, high-fat diet containing OA; PEE, high-fat diet containing PEE. ∗, significantly different in the OA group versus the HF group in the rat final body weight ($p < 0.05$).

RESULTS

Oleanolic Acid Contents of Pomace. Dried pomace obtained from eight cultivars contained 30−65% skin, 19−60% seed, and 10−19% stem (**Table 2**). These differences are due to the greatly different seed sizes among the cultivars. The yields of PEE were 7−11% for the red cultivars and 21−37% for the white cultivars. The lipid contents of PEE were 59−77% for the red cultivars and 10−40% for the white cultivars. Because the pomace derived from the white cultivars was produced ahead of fermentation, the PEE contained a high amount of sugars (approximately 50%) (data not shown). The polyphenol concentration was high only in Zweigeltrebe at 6%. The OA contents of PEE were different among the varieties and ranged from 1 to 11%, but were particularly high in Kiyomi at 11%.

Food Intake and Body Weight. Average food consumption (mean ± SEM) was lower for the HF group (17.9 ± 0.5 g/day) than for the NF group (19.8 ± 0.3 g/day), but the OA (18.0 ± 1.0 g/day) and PEE (17.4 ± 0.5 g/day) groups were identical with the HF group due to the pair-feeding method. The time courses for changes in body weights are shown in **Figure 2**. Body weights were significantly greater in the three HF groups than in the NF group at 1 week or later. The body weights of the OA and PEE groups were less than that of the HF group at 2 weeks or later. The final body weights were as follows: NF group, 262.8 ± 13.4 g; HF group, 381.3 ± 16.8 g; OA group, 341.9 ± 21.0 g; PEE group, 358.8 ± 23.9 g. Dietary OA significantly suppressed the HF diet-induced increase in the rat final body weight.

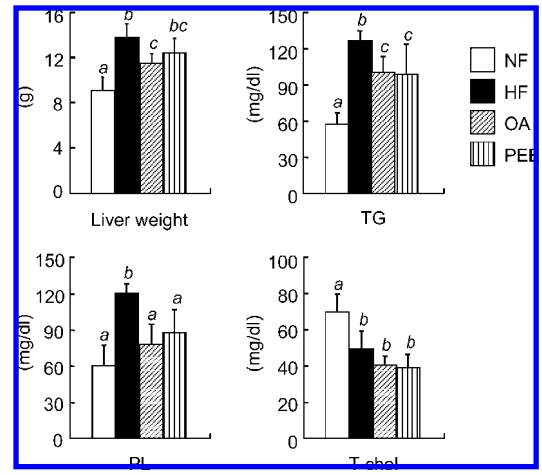

Figure 3. Liver weights and plasma lipid levels of rats fed experimental diets for 4 weeks. Values for each sample with different italic letters in the same lipid class were significantly different at $p < 0.05$. TG, triacylglycerol; PL, phospholipids; T-chol, total cholesterol.

Liver Weight and Plasma Lipid Level. Rat liver weight and plasma triacylglycerol, phospholipid, and total cholesterol concentrations are shown in **Figure 3**. The liver weights of the OA and PEE groups tended to be lower than those of the HF groups and were significantly different in the OA group. Although plasma triacylglycerol and phospholipids levels were significantly higher in the HF group than in the NF group, these lipid levels were significantly decreased by dietary OA and PEE. Moreover, the total cholesterol levels of the OA and PEE groups tended to be lower than those of the HF group, although they were not statistically significant.

Gene Expression Analyses. *Function Category.* Expression changes of the rat hepatic genes were analyzed using DNA microarray. Genes for which the expression intensity changed by >1.5-fold by dietary OA and PEE compared to the HF group were classified by function categories using the KEGG pathway (**Table 3**). The classification indicated that there are a lot of genes for which the expression intensity changed in relation to insulin signaling, adipocytokine, and diabetes. It was found that these pathways had a lot of genes for which expressions were down-regulated. In addition, the number of genes for which expression changed was slightly higher in the PEE than in the OA group. Cancer-related genes were also down-regulated, although it was unrelated to lipid metabolism.

Lipid Metabolism-Related Genes. Lipid metabolism-related genes for which expression intensity changed by >1.5-fold compared to the HF group are shown in **Table 4**. As for fatty acid biosynthesis-related genes, acetyl-CoA carboxylase (Acacb), the rate-limiting enzyme of fatty acid synthesis, was down-regulated 0.49-fold (OA) and 0.11-fold (PEE). Stearoyl-CoA desaturase (Scd) 2 in the OA group and Scd1 in the PEE group were down-regulated 0.66- and 0.65-fold, respectively. Triacylglycerol synthesis-related genes such as glycerol-3-phosphate dehydrogenase (Gpd2), which synthesizes glycerol-3-phosphate, in the PEE group (0.58-fold) and glycerol-3-phosphate acyltransferase (Gpam), which synthesizes glycerolipids from glycerol-3-phosphate, in the OA (0.56-fold) and PEE groups (0.16-fold) were down-regulated, respectively. The expression of lipolytic genes and PPARα were hardly changed (data not shown). Although dietary OA and PEE did not significantly lower plasma cholesterol levels (**Figure 3**), some key genes relevant to the biosynthesis, transport, and catabolism of cholesterol were down-regulated, particularly in the PEE group. Sterol regulatory element binding factor 1 [Srebf1 (commonly

Table 3. Numbers of Genes for Which Expression Changed by >1.5-Fold in the Liver[a]

		OA		PEE	
path code	function	up	down	up	down
rno04910	insulin signaling pathway	10	18	10	23
rno04920	adipocytokine signaling pathway	6	15	6	18
rno03320	PPAR signaling pathway	3	7	3	9
rno04940	type I diabetes mellitus	8	15	8	15
rno04930	type II diabetes mellitus	10	6	11	7
rno00010	glycolysis/gluconeogenesis	5	7	5	6
rno00620	pyruvate metabolism	4	2	3	3
rno00020	citrate cycle (TCA cycle)	2	0	2	1
rno00071	fatty acid metabolism	3	2	4	3
rno00561	glycerolipid metabolism	4	5	5	7
rno00564	glycerophospholipid metabolism	4	6	6	3
rno00100	biosynthesis of steroids	1	2	1	3
rno00120	bile acid biosynthesis	2	2	3	5
rno02010	ABC transporters, general	4	8	3	7
rno00480	glutathione metabolism	1	1	2	0
rno00980	metabolism of xenobiotics by CYP	3	2	2	1
rno04110	cell cycle	5	8	7	11
rno05212	pancreatic cancer	5	16	4	19
rno05210	colorectal cancer	7	13	7	18
rno05216	thyroid cancer	4	5	4	4

[a] Differentially expressed genes were categorized and mapped onto pathway using KEGG tools (http://www.genome.jp/kegg/tool/color_pathway.html). Mean up- and down-regulation of mRNA expression.

Table 4. Expression Change (vs High-Fat Group) of Genes in the Liver Relevant to Lipid Metabolism[a]

			fold change	
Genbank	gene symbol	description	OA	PEE
		Fatty Acid Synthesis		
NM_053922	Acacb	acetyl-coenzyme A carboxylase β	0.49	0.11
NM_139192	Scd1	stearoyl-coenzyme A desaturase 1	0.81	0.65
NM_031841	Scd2	stearoyl-coenzyme A desaturase 2	0.66	0.95
		Lipogenesis		
AI176852	Gyk	glycerol kinase	0.46	0.37
U83880	Gpd2	glycerol-3-phosphate dehydrogenase 2	0.78	0.58
NM_017274	Gpam	glycerol-3-phosphate acyltransferase	0.42	0.16
NM_017006	G6pdx	glucose-6-phosphate dehydrogenase	0.56	0.42
		Lipolysis		
BG373440	Dci	dodecenoyl-coenzyme A δ isomerase	2.14	1.34
		Sterol Metabolism		
NM_013134	Hmgcr	3-hydroxy-3-methylglutaryl-coenzyme A reductase	0.78	0.60
U36992	Cyp7b1	cytochrome P450, subfamily 7B, polypeptide 1	0.77	0.65
		Transcription Factor		
BQ190242	Srebf1	sterol regulatory element binding factor 1	0.62	0.56

[a] A p value of <0.01 was considered to be significantly changed.

called SREBP-1)], which is a key transcription factor for hepatic lipogenic enzyme genes (*29*), was down-regulated 0.62-fold (OA) and 0.56-fold (PEE).

Insulin Signaling-Related Genes. Because there were a lot of genes for which expression intensity changed in the insulin signaling pathway (**Table 3**), insulin signaling-related genes for which the expression intensity changed by >1.5-fold, compared to the HF group, are shown in **Table 5**. As for inflammatory cytokine-related genes, tumor necrosis factor (Tnf) and its receptor (Tnfrsf1a), and interleukin 1 (Il1b and Il1a) were down-regulated, particularly in the PEE group. Moreover, insulin receptor substrates (Irs2 and IRS-2), leptin (Lep), and the AMP-activated protein kinase β-2 regulatory subunit (Prkab2) were acutely up-regulated. As for the gluconeogenesis-related genes, glucose-6-phosphatase (G6pc) in the OA and PEE groups and

Table 5. Expression Change (vs High-Fat Group) of Genes in the Liver Relevant to Glucose Metabolism and Insulin Signaling[a]

			fold change	
Genbank	gene symbol	description	OA	PEE
		Inflammatory Cytokine		
AJ002278	Tnf	tumor necrosis factor (TNF superfamily, member 2)	0.66	0.22
NM_013091	Tnfrsf1a	tumor necrosis factor receptor superfamily, member 1a	0.71	0.59
NM_031512	Il1b	interleukin 1 β	0.35	0.31
NM_017019	Il1a	interleukin 1 α	0.72	0.66
		Insulin Signaling		
AF087674	Irs2	insulin receptor substrate 2	2.22	1.75
AF083418	IRS-2	insulin receptor substrate-2 (IRS-2)	1.73	1.39
NM_013076	Lep	leptin	2.40	2.97
NM_022627	Prkab2	AMP-activated protein kinase β-2 regulatory subunit	6.57	5.98
		Gluconeogenesis		
NM_013098	G6pc	glucose-6-phosphatase, catalytic	0.49	0.52
NM_012558	Fbp1	fructose-1,6-biphosphatase 1	0.75	0.61
		Transcription Factor		
BE106518	Foxo1a	forkhead box O1A	0.52	0.59

[a] A p value of <0.01 was considered to be significantly changed.

fructose-1,6-biphosphatase 1 (Fbp1) in the PEE group were down-regulated. Forkhead box O1A (Foxo1a), which regulates the transcription of gluconeogenic genes in the liver (*30*), was down-regulated 0.52-fold (OA) and 0.59-fold (PEE).

DISCUSSION

It is well-known that the polyphenols in grape skin can move into wine during alcoholic fermentation and contribute to the various physiological functions of red wine. Moreover, oleanolic acid, a lipophilic triterpene, is also one of the characteristic components of grape skin. The wax component of grape skin was found to be made of OA long ago (*31*). It was reported that the OA contents of the skin hardly changed before and after experimental fermentation of white cultivars (*8*). In this study, it was shown that considerable amounts of OA are present in pomace after alcoholic fermentation of red cultivars. Although we previously analyzed lipid components of grape musts and wines with various production stages (*32, 33*), OA could not be detected in any samples at any stage by TLC analysis, indicating that the OA that exists in the skin cuticle is hardly eluted into wine during the alcoholic fermentation process. Therefore, it was shown that OA remains intact in the skin tissue and is concentrated in the pomace. Because OA is derived from the skin, red cultivars with a high skin proportion were rich in OA (**Table 2**). However, in white cultivars, the skin proportions are unrelated to OA contents, suggesting that the OA contents largely vary due to differences between varieties.

Dietary PEE and its inclusion OA significantly lowered the plasma lipid levels of rats fed a high-fat and high-sucrose diet. Although PEE contains 2% polyphenol as well as 11% OA and polyphenol was expected to have a synergistic effect with OA, there were no statistically significant differences in the plasma lipid levels between OA and PEE groups in this experiment system. However, from the results of the comparative analysis of hepatic gene expression using DNA microarray (**Table 3**), the numbers of genes for which expression intensity changed by >1.5-fold were higher in the PEE group than in the OA group, suggesting that some components other than OA may influence hepatic gene expression (*10*). Although OA is distributed worldwide besides in grape skin, these microarray data suggest that PEE may have more nutraceutical activities

and become a possible source for supplement use. Comprehensive gene analysis revealed that suppression of lipid synthesis-related gene expression could cause the plasma lipid lowering effects in rats (**Table 4**). This is possibly mediated by the suppression of Srebf1, which enhances the transcription of genes encoding enzymes of fatty acid biosynthesis in the liver (*29*). Srebf1 (SREBP-1c), an SREBP subtype, is strongly involved in the regulation of transcription of fatty acid and triacylglycerol biosynthesis systems rather than that for cholesterol (*34*). Srebf1 responds to sugar or insulin, and its expression rises at the transcription level, thereby inducing accumulation of fat from food ingredients (*35*). Because Srebf1 does not form a feedback loop involving its end-product, its enhanced signal promotes fat accumulation. Therefore, in excess energy conditions, expression of Srebf1 is up-regulated, and fatty accumulation is promoted, causing metabolic syndrome (*36*). In fact, expression of Srebf1 in the HF group was up-regulated 1.8-fold compared to that of the NF group (data not shown in the table), whereas dietary OA and PEE suppressed the expression to the level of the NF group. Because it was suggested that OA, a plant triterpene, was poorly absorbed by aural administration (*37*), the OA that is slightly absorbed may reach the liver and exhibit a special effect there. Liu et al. also reported that dietary OA therapeutically inhibited hyperlipidemia in rats induced by the high-cholesterol diet (*21*). It can be concluded that naturally occurring triterpenes possess preventive and therapeutic effects on diet-induced hyperlipidemia.

In this study, the PEE group strongly suppressed fatty acid and sterol biosyntheses and Srebf1 expression compared to the OA group at the mRNA expression level. It is known that polyunsaturated fatty acids suppress Srebf1 expression (*38*) and that PEE is rich in linoleic acid derived from grape seeds (*39*). However, because the soybean oil used in the HF groups contains approximately 50% linoleic acid, the polyphenol components of PEE may be a responsible factor as well as OA (*40*). Dietary PEE significantly lowered Scd1 expression. It is reported that this gene is related to the plasma triacylglycerol level of rodents (*41, 42*). Moreover, G6pdx, which encodes an NADPH-producing enzyme, was down-regulated, which lowered the supply of NADPH, which is required for fatty acid synthesis, resulting in suppression of lipid synthesis.

The plasma cholesterol levels were lowered in the HF groups compared to the NF group. Because 3-hydroxy-3-methylglutaryl-coenzyme A lyase (Hmgcl, NM_024386) and 3-hydroxybutyrate dehydrogenase (Bdh, BF564876) were up-regulated 7.5- and 1.5-fold, respectively (data not shown in the table), it was assumed that metabolism via HMG-CoA altered ketone body synthesis but not sterol synthesis in the HF groups.

Promotion of obesity due to the high-fat diet generally induced insulin resistance. In these experimental diet groups, gene expression relevant to gluconeogenesis and inflammatory cytokine was impaired. Moreover, Irs and Lep were up-regulated, whereas forkhead transcription factor (Foxo1a) expression was impaired. These suggest that dietary OA and PEE may promote insulin sensitivity in rats fed a high-fat diet. Foxo1a, a transcription factor regulated by phosphorylation, is located downstream of Lep. Although leptin is known to be a hormone secreted from adipocytes, leptin expression in the liver is related to promotion of insulin sensitivity and improvement of glucose and lipid metabolic disorders (*43, 44*). It is known that Foxo1a induces expression of the G6pc and phosphoenolpyruvate carboxykinase (Pck1) genes, which are responsible for the promotion of gluconeogenesis in the liver (*31*). In this study, expression of Pck1 was slightly suppressed (0.71- and 0.79-fold for OA and PEE, respectively), but that of G6pc was significantly impaired. This suggests that dietary OA and PEE prevented abnormal glucose tolerance above the level of normal conditions. Prkab is also known to prevent the activities of acetyl-CoA carboxylase (Acac) and gluconeogenic G6pc by phosphorylation (*45, 46*) and to be activated by polyphenol (*47*). Although Prkab is a phosphorylated enzyme, up-regulation of Prkab2, which is located downstream of Irs, at the mRNA level may be also related to the hepatic lipid metabolisms.

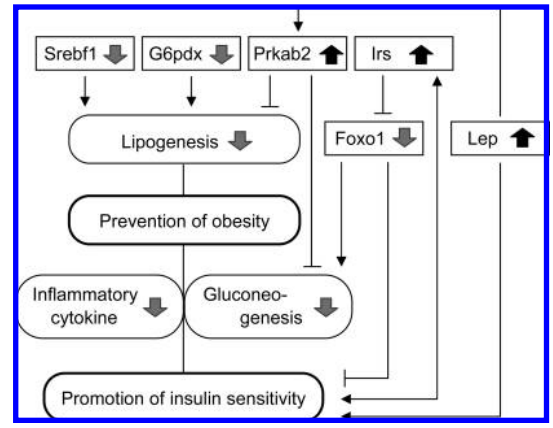

Figure 4. Summary of the speculated mechanisms of the plasma lipid-lowering effect as determined by rat hepatic gene expression analysis. Black up arrows and gray down bold arrows indicate the up- and down-regulation of mRNA expression, respectively. Narrow arrows and inverted T-bar indicate activation and inhibition, respectively.

In conclusion, we found that dietary OA and PEE could lower plasma triacylglycerol and phospholipids in rats fed a high-fat diet (antihyperlipidemia effect). The rat hepatic gene expression was analyzed using DNA microarray, and the speculated mechanism of the plasma lipid lowering effects by dietary OA and PEE is summarized in **Figure 4**. The suppression of lipogenic gene expression is particularly due to the down-regulation of Srebf1, which could prevent obesity. Moreover, down-regulation of gluconeogenic genes and their transcription regulation factor, Foxo1a, as well as inflammatory cytokines, and up-regulation of Irs and Lep were observed in the liver, suggesting the promotion of insulin sensitivity in obese rats. Oleanolic acid, a key component in pomace, could be useful for maintaining human health, especially with regard to metabolic syndrome. In the future, on the basis of the nutrigenomic information obtained in this study by using DNA microarray, further accumulation of biochemical data such as insulin, leptin, and adiponectin levels in the blood will be needed.

ABBREVIATIONS USED

OA, oleanolic acid; PEE, pomace ethanol extract; NF, normal-fat diet; HF, high-fat diet.

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