Grape Polyphenols Exert a Cardioprotective Effect in Pre- and Postmenopausal Women by Lowering Plasma Lipids and Reducing Oxidative Stress[1]

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ABSTRACT To evaluate the effects of grape polyphenols on plasma lipids, inflammatory cytokines, and oxidative stress, 24 pre- and 20 postmenopausal women were randomly assigned to consume 36 g of a lyophilized grape powder (LGP) or a placebo for 4 wk. The LGP consisted of 92% carbohydrate and was rich in flavans, anthocyanins, quercetin, myricetin, kaempferol, and resveratrol. After a 3-wk washout period, subjects were assigned to the alternate treatment for an additional 4 wk. The placebo consisted of an equal ratio of fructose and dextrose and was similar in appearance and energy content (554 kJ) to LGP. Plasma triglyceride concentrations were reduced by 15 and 6% in pre- and postmenopausal women, respectively ($P < 0.01$) after LGP supplementation. In addition, plasma LDL cholesterol and apolipoproteins B and E were lower due to LGP treatment ($P < 0.05$). Further, cholesterol ester transfer protein activity was decreased by ~15% with intake of LGP ($P < 0.05$). In contrast to these beneficial effects on plasma lipids, LDL oxidation was not modified by LGP treatment. However, whole-body oxidative stress as measured by urinary F_2-isoprostanes was significantly reduced after LGP supplementation. LGP also decreased the levels of plasma tumor necrosis factor-α, which plays a major role in the inflammation process. Through alterations in lipoprotein metabolism, oxidative stress, and inflammatory markers, LGP intake beneficially affected key risk factors for coronary heart disease in both pre- and postmenopausal women. J. Nutr. 135: 1911–1917, 2005.

KEY WORDS: • *grape polyphenols* • *coronary heart disease* • *whole body oxidative stress* • *postmenopausal women* • *triglycerides*

Well-known coronary heart disease (CHD)[3] risk factors include elevated total cholesterol (TC), triglyceride (TG), and LDL cholesterol (LDL-C) concentrations (1). The loss of estrogen has profound effects in increasing plasma lipids and apolipoproteins associated with CHD (2,3). In addition to common risk factors, inflammation has emerged as another key risk factor for CHD. Increases in body weight exacerbate both hypercholesterolemia and the inflammation associated with an increased risk for CHD. Furthermore, adipose tissue was shown to secrete and produce the inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), as well as plasminogen activator inhibitor-1 (4).

To control for the increased risk of CHD associated with these factors, postmenopausal women could make a variety of lifestyle modifications. Weight loss and increased physical activity are the recommended treatments to lower plasma lipids. However, normal BMI and regular exercise might not be sufficient to prevent hyperlipidemias. Dietary modifications such as a higher intake of fruits rich in polyphenols could be a feasible alternative for individuals with elevated levels of plasma cholesterol and TG.

The French Paradox (low incidence of coronary artery disease despite a diet high in saturated fat) is a clear indication that polyphenols may have a protective role in CHD. The most noted role of grape polyphenols is their ability to decrease LDL oxidation in both humans (5) and numerous animal models (6). Furthermore, several animal studies showed a decrease in the development of atherosclerosis with polyphenol intake (6–8). Oxidized LDL (ox-LDL) was shown to promote inflammatory responses including the production and secretion of TNF-α, a proinflammatory cytokine (4,9). Through the inflammatory cascade, TNF-α stimulates the production of adhesion molecules on the endothelium of the aorta, thereby promoting monocyte uptake. Once within the endothelium, monocytes differentiate into macrophages,

[1] Supported by funds provided by the California Table Grape Commission, a USDA Hatch Project grant to M.L.F., and National Institutes of Health grant HL-70006 to N.S.S.

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[3] Abbreviations used: apo, apolipoprotein; CETP, cholesterol ester transfer protein; CHD, coronary heart disease; CRP, C-reactive protein; HDL-C, HDL cholesterol; IL-6, interleukin-6; LCAT, lecithin cholesteryl acyltransferase; LDL-C, LDL cholesterol; LGP, lyophilized grape powder; LPL, lipoprotein lipase; MTP, microsomal transfer protein; ox-LDL, oxidized LDL; PMSF, phenylmethylsulfonyl fluoride; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor α; WC, waist circumference.

0022-3166/05 $8.00 © 2005 American Society for Nutritional Sciences.

Manuscript received 20 April 2005. Initial review completed 12 May 2005. Revision accepted 23 May 2005.

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which readily take up ox-LDL and eventually promote the development of atherosclerotic plaques.

Although grape juice was shown to increase plasma TG due to its high carbohydrate content (5), several studies using polyphenols as a dietary treatment showed TG metabolism was affected. Narigenin, a grapefruit polyphenol, was shown to reduce microsomal transport protein (MTP) activity, thereby decreasing apolipoprotein (apo) B and TG secretion (10,11). Decreases in apo B concentrations in golden Syrian hamsters fed red wine polyphenols were also reported (12). Similarly, our laboratory reported previously that lyophilized grape powder (LGP) decreased TG, apo B concentrations, and the progression of atherosclerosis in ovariectomized guinea pigs, a model for menopause (7). Similar to the previous reports of the actions of naringenin (10), LGP may alter TG metabolism by affecting the overall secretion of TG-rich lipoproteins, thereby affecting the development of atherosclerosis.

Due to our previous findings, the objectives of this study were to investigate further the effects of LGP on plasma lipids, lipoprotein metabolism, LDL oxidation, inflammation, and oxidative stress in both pre- and postmenopausal women. Our hypothesis was that LGP treatment would have an overall protective effect against CHD by reducing plasma lipids through alterations in lipoprotein remodeling in the plasma compartment, by decreasing inflammation, and by acting as a free radical scavenger to decrease in vivo lipid peroxidation.

SUBJECTS AND METHODS

Materials. Enzymatic kits for the measurement of plasma lipid concentrations were purchased from Boehringer Mannheim and Roche Diagnostics. Apo B kits were purchased from Wako Diagnostics. EDTA, aprotinin, sodium azide, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma Chemical. Quick-seal ultracentrifuge tubes were from Beckman. TNF-α and IL-6 kits were purchased from R&D Systems. C-reactive protein (CRP) kits were purchased from ALPCO Diagnostics. 8-Isoprostane isolation columns and kits were purchased from Cayman Chemicals. Urinary creatinine concentrations were determined using kits from Wako Diagnostics. The LGP was provided by the California Table Grape Commission.

Study population. Women (24 premenopausal and 20 postmenopausal) were recruited through the use of local newspapers and brochure distribution within the university community. Menopause was defined as not having menses for at least 1 y. Exclusion criteria included use of lipid-lowering drugs or aspirin, cardiovascular disease, diabetes, and hormone replacement therapy for $<$5 y. Subjects gave their informed consent to participate in the intervention, and all protocols were approved by the University of Connecticut Institutional Review Board.

Study design. The study utilized a single-blind, crossover design in which subjects consumed the LGP or placebo for 4 wk followed by a 3-wk washout period after which subjects consumed the alternate treatment for an additional 4 wk. At baseline, participants were randomly assigned to the LGP or placebo group.

The California Table Grape Commission provided LGP. The chemical composition of the grape preparation was as follows: protein, 40 g/kg; sugar, 920 g/kg; fat, 6 g/kg; and fiber, 34 g/kg. Selected phytochemical components were determined, and had the following values: total phenols, 5.8 g/kg; flavans, 4.1 g/kg; anthocyanins, 0.77 g/kg; quercetin, 102 μmol/kg; myricetin, 8 μmol/kg; kaempferol, 11 μmol/kg; and resveratrol, 7 μmol/kg. Total phenols of the grape powder were analyzed with Folin & Ciocalteu's phenol reagent; flavans were analyzed by reaction with vanillin; anthocyanins were analyzed spectrophotometrically; flavonols and resveratrol were analyzed by HPLC after acid hydrolysis (13). The placebo was prepared from an equal ratio of fructose and dextrose (1:1). It was similar in appearance and energy content to the grape preparation.

Supplements were distributed weekly. Subjects were asked to mix their supplement with water before intake. The subjects consumed 36 g/d of LGP. Because 1 kg of fresh grapes yields 182 g of powder, this is equivalent to 200 g/d (1.5 cups/d) of grapes. The energy intake provided by the 2 supplements was 554 kJ/d. Compliance was monitored by asking subjects to document supplement consumption.

Diet. Throughout the intervention, subjects were asked to consume their regular diet while avoiding foods rich in polyphenols. These foods included tea, grape products, fruit juice, citrus, berries, onions, apples, and broccoli. In both 4-wk interventions, subjects were asked to complete two 7-d dietary records to monitor compliance. Diets were analyzed by the Nutrition Data System (Nutrition Coordinating System, University of Minnesota).

Anthropometrics. Subjects' height, weight, blood pressure, and waist circumference (WC) were recorded at baseline. After a 5-min rest period, subjects' blood pressure was measured with a Welch Allyn, Tycos cuff. The mean of 2 blood pressure readings per week was used to ensure accuracy. The WC was measured at the point midway between the lowest rib and the iliac crest to the nearest 0.1 cm (14). Baseline physical activity was measured using the International Physical Activity Questionnaire short form (15), which has 3 categories of physical activity: Category 1, insufficiently active; Category 2, sufficiently active; and Category 3, highly active.

Urine collection. Nonfasting subjects were asked to provide two 24-h urine collections at the end of each treatment. Subjects voided the first urine for the day and then collected for 24 h. Subjects recorded the time of first void and the time of first collection. Total urine volume was recorded, and samples were centrifuged for 10 min at 5000 $\times$ g to remove any sediment. Aliquots were stored at -80°C until needed for creatinine and F_2 isoprostane measurements.

Plasma lipids. The mean of 2 blood draws obtained in the same week from fasting (12 h) subjects was used to determine plasma lipids at baseline and at the end of each supplement period. Whole blood was collected in EDTA tubes. After plasma samples were isolated, preservation cocktail was added to the samples (5 mL/L aprotinin, 1 mL/L PMSF, and 1 mL/L sodium azide). Plasma TC and HDL cholesterol (HDL-C) concentrations were determined using enzymatic kits from Boehringer Mannheim (16). Plasma TG concentrations were determined by blanking free glycerol (17). HDL-C was analyzed after precipitation of apo B–containing lipoproteins with dextran sulfate (18). LDL-C concentrations were then determined using the Friedewald equation (19).

Our laboratory has been part of the Lipid Standardization Program of the Centers for Disease Control—National Heart, Lung, and Blood Institute since 1989. The CVs assessed by the standardization program during the last human study were 0.76–1.42% for TC, 1.71–2.72% for HDL-C, and 1.64–2.47% for TG (20).

Plasma lecithin cholesteryl acyltransferase and cholesterol ester transfer protein determinations. Lecithin cholesteryl acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) activities were determined according to Ogawa and Fielding (21). Physiological CETP activity was determined without inhibition of LCAT by measuring the mass transfer of cholesterol ester between HDL- and apo B–containing lipoproteins. Samples were incubated at 37°C for 6 h in a shaking water bath, and TC and free plasma cholesterol were measured at both time points (22). LCAT activity was determined by mass analysis of the decrease in plasma free cholesterol between 0 and 6 h at 37°C. Assays were carried out concurrently with measurements of CETP (23).

Plasma apolipoproteins and glucose. Apo B concentrations were determined using an immunoturbidimetric method (Wako Diagnostics) (24). Apo C-III and apo E were measured on a Hitachi Autoanalyzer 740 with Wako kits used according to the manufacturer's instructions. Apo C-1 concentrations were determined using an ELISA (25).

LDL size and LDL oxidation. The Lipoprint LDL system (Quantimetrix) was used to identify the size of LDL using a nongradient high-resolution PAGE (polyacrylamide gel electrophoresis) system (26). LDL oxidation was determined according to Abbey et al. (27). LDL was isolated by ultracentrifugation in a L8-M ultracentrifuge (Beckman Instruments). LDL was isolated at a density of 1.09 g/L at 65,000 $\times$ g for 45 min. Samples were dialyzed overnight in 1.5 g/kg NaCl and 0.1 g/kg Na_2HPO_4, pH 7.4.

After dialysis, protein was assayed in the LDL samples (28). Samples were diluted to 102 μg LDL/0.0012 L using dialysis buffer

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(10 mmol/L Na_2HPO_4, 0.15 mol/L NaCl, pH 7.4). After the dilution, samples were transferred to a DU-640 UV spectrophotometer (Beckman Coulter), and 25 μL of 0.25 mmol/L $CuSO_4$ was added to initiate oxidation. Kinetics of samples proceeded at 37°C for 180 min, and absorbance was plotted every 120 s. The rate of oxidation was determined from the slope of the propagation phase. Lag time was determined from the intercept of the lag and propagation phases.

Plasma glucose. Glucose concentrations were determined using an enzymatic method (Wako Diagnostics) and read with the DU-640 spectrophotometer.

Plasma TNF-α, IL-6, and CRP determination. TNF-α, IL-6, and CRP concentrations were determined at the end of each treatment period in samples from fasting subjects. High-sensitivity ELISA kits were used to determine both TNF-α and IL-6 concentrations. The sensitivity for the TNF-α assay ranged from 0.06 to 0.32 ng/L, whereas the range of sensitivity for the IL-6 assay was from 0.016 to 0.110 ng/L. The CRP calibrators were standardized against WHO Certified Reference Material 470. The sensitivity for the CRP ELISA was 0.124 ng/L.

Urinary isoprostane isolation and determination. An 8-isoprostane affinity column was used to isolate isoprostanes from 24-h urine collections at the end of each treatment period. Briefly, 1 mL of urine was passed through the column. The column was then washed with ultrapure water and column buffer (0.1 mol/L phosphate buffer). After the wash, a 95% ethanol solution was passed through the column and collected for analysis. Samples were dried under nitrogen and reconstituted with 500 μL of buffer. Samples were then applied directly to the competitive enzyme immunoassay plate.

Urinary creatinine quantification. Creatinine was assayed to serve as a control for the 24-h urine collection and isoprostane determination. Creatinine was assayed using the Jaffé reaction method (Beckman Coulter).

Statistical analysis. An independent *t* test was used to compare baseline characteristics between pre- and postmenopausal women. Repeated-measures ANOVA was used to determine the effects of LGP on plasma lipids, lipoprotein metabolism, inflammation, and oxidative stress. Each individual's response to diet (LGP or placebo) was considered as the repeated measure and the menopausal status (pre- vs. post-) as the between-subject factor. Differences with $P < 0.05$ were considered significant. Data are presented as means ± SD for the number of subjects in each group. Statistical analysis was conducted using SPSS version 12 for Windows.

RESULTS

Baseline measurements. Pre- and postmenopausal women did not differ in baseline smoking status, physical activity level, BMI, or blood pressure (**Table 1**). Similarly, TC, HDL-C, and LDL-C concentrations did not differ between the groups. In contrast, TG concentrations in postmenopausal women were ~33% higher at baseline compared with those of premenopausal women ($P < 0.05$).

Dietary analysis and anthropometrics. Energy intake and macronutrient intake, including carbohydrate, fat, and protein, did not differ between the periods (**Table 2**). In addition, intakes of Vitamin A, E, and C (natural antioxidants) did not differ between periods.

Furthermore, throughout the intervention there were no changes in weight, WC, or blood pressure for either pre- or postmenopausal women (data not shown).

Plasma lipids and apolipoproteins. Intake of LGP had no effect on TC or HDL-C concentrations in either pre- or postmenopausal women (**Table 3**). However, grape treatment decreased TG concentrations by 15 and 6% in pre- and postmenopausal women, respectively ($P < 0.002$). Similarly, LDL-C concentrations were decreased due to LGP treatment ($P < 0.05$).

As expected, postmenopausal women had higher TC and TG concentrations than premenopausal women during both treatment periods ($P < 0.05$). Following the trend in lipid metabolism alterations, apo B and apo E concentrations (**Table 4**) were decreased due to LGP treatment in both pre- and postmenopausal women ($P < 0.05$). In addition, there was a significant menopausal effect on apo B, apo E, and apo C-III concentrations. In contrast, plasma apo C-I concentrations were not affected by diet or menopausal status (Table 4).

TABLE 1

Baseline characteristics of the women[1]

Variable	Premenopausal	Postmenopausal
n	24	20
Age, *y*	39.7 ± 8.5	58.5 ± 7.5
Smokers, *n*	2	2
Physical activity category[2]	2	2
BMI, *kg/m²*	31.06 ± 4.4	31.13 ± 4.6
Systolic blood pressure, *mm Hg*	121.5 ± 9.5	123.6 ± 9.5
Diastolic blood pressure, *mm Hg*	77.6 ± 7.8	78.9 ± 6.1
WC, *cm*	88.1 ± 8.9	88.2 ± 11.1
Plasma, *mmol/L*		
TC	5.0 ± 1.03	5.6 ± 0.98
LDL-C	2.9 ± 0.80	2.9 ± 0.87
HDL-C	1.7 ± 0.23	1.8 ± 0.35
TG	1.0 ± 0.55	1.5 ± 1.2*

[1] Values are means ± SD. * Different from premenopausal, $P < 0.05$.
[2] Category 2: sufficiently active.

Although alterations due to LGP treatment occurred in TG, apo B, and apo E concentrations, there were no significant effects on LDL size (data not shown). Similarly, there were no significant effects on LDL lag time or conjugated diene formation by either treatment or menopausal status (data not shown). However, postmenopausal women (0.25 ± 0.28 mmol/L) had higher concentrations ($P < 0.05$) of the smaller LDL subfractions compared with premenopausal women (0.11 ± 0.19 mmol/L).

Plasma LCAT and CETP activities. LGP treatment reduced CETP activity by 9 and 29% in pre- and postmenopausal women, respectively ($P < 0.05$) (**Table 5**). LCAT activity did not differ during the LGP and placebo periods.

Plasma glucose concentrations. Glucose concentrations were not affected by LGP treatment or menopausal status. For premenopausal women, glucose concentrations were 4.9 ± 0.78 and 5.0 ± 0.48 mmol/L for the LGP and placebo periods, respectively. For postmenopausal women, glucose concentrations were 5.1 ± 0.77 and 5.1 ± 0.31 mmol/L for the LGP and placebo periods, respectively.

Plasma cytokine concentrations. IL-6 and CRP concentrations did not differ after LGP treatment in either pre- or postmenopausal women (**Table 6**). However, LGP treatment significantly decreased TNF-α concentrations in both groups ($P < 0.05$). Menopausal status had no effect on IL-6, CRP, or TNF-α concentrations.

Urinary isoprostane concentrations. Unlike LDL oxidation parameters, in vivo lipid peroxidation quantified by isoprostane concentrations was decreased by LGP treatment in both pre- and postmenopausal women ($P < 0.05$) (**Fig. 1**). Menopausal status had no effect on isoprostane concentrations.

DISCUSSION

In this study, we demonstrated that LGP had significant cardioprotective effects in both pre- and postmenopausal women by lowering plasma TG, LDL-C, apo B, apo E, and

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TABLE 2

Dietary analysis of pre- and postmenopausal women during the placebo and LGP periods[1]

	Placebo		LGP	
Component	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
n	24	20	24	20
Energy, *kJ/d*	7447 ± 1588	7657 ± 1184	7451 ± 1470	7560 ± 2516
Carbohydrate, *% of energy*	52.4 ± 4.9	51.0 ± 5.8	53.0 ± 6.4	52.2 ± 7.9
Protein, *% of energy*	15.4 ± 3.1	16.8 ± 2.8	15.7 ± 3.4	16.1 ± 3.5
Fat, *% of energy*	33.8 ± 4.0	33.7 ± 4.3	33.1 ± 4.7	33.6 ± 5.2
Saturated fat, *% of energy*	11.4 ± 2.1	11.5 ± 1.4	11.0 ± 1.8	11.2 ± 2.0
Monounsaturated fat, *% of energy*	13.0 ± 1.8	12.9 ± 2.1	12.7 ± 2.4	13.1 ± 2.4
Polyunsaturated fat, *% of energy*	6.9 ± 1.9	6.5 ± 2.1	6.9 ± 1.5	6.5 ± 1.8
Dietary cholesterol, *mg/d*	228.1 ± 101.1	274.0 ± 103.0	223.3 ± 91.6	279.7 ± 127.0
Vitamin A, *μg/d*	954.1 ± 640.6	933.3 ± 457.4	827.6 ± 507.6	977.1 ± 498.2
Vitamin C, *mg/d*	145.3 ± 356.0	105.9 ± 59.9	169.5 ± 439.0	112.3 ± 67.5
Vitamin E, *mg/d*	14.45 ± 13.0	16.0 ± 15.4	12.3 ± 12.8	17.0 ± 18.4

[1] Values are means ± SD. Groups and dietary periods did not differ.

oxidative stress, as determined by urinary isoprostane and plasma TNF-α concentrations.

During both the placebo and LGP periods, postmenopausal women had elevated plasma TC, TG, apo B, apo C-III, and apo E concentrations compared with premenopausal women. The higher plasma TG in postmenopausal women could be associated with reductions in lipoprotein lipase (LPL) activity as well as increases in the overall secretion of VLDL from the liver. Furthermore, low doses of 17β-estradiol were shown to increase LPL activity in abdominal adipocytes isolated from women (29). In our population, postmenopausal women had elevated apo C-III concentrations, indicating a higher inhibition of LPL activity compared with premenopausal women. Due to the inhibition of LPL, elevated concentrations of VLDL particles could yield smaller, denser, TG-laden LDL particles (30), as occurred in this study. These smaller particles are believed to be more susceptible to oxidation. However, there were no significant increases in LDL oxidation associated with menopausal status in the women in the current study. Together with previous data (2,3,30), the current findings further confirm that menopausal status has an effect on CHD risk factors and overall lipoprotein metabolism.

In previous studies, grape juice and other treatments were shown to significantly increase plasma TG concentrations (5,31). In contrast, in our study, LGP treatment significantly decreased TG concentrations compared with placebo. It is important to note that LGP did not raise TG concentrations from baseline. However, there was a significant increase in TG concentrations from baseline to the end of the placebo period in all subjects ($P < 0.0001$).

Grape polyphenols were shown to alter lipoprotein metabolism by decreasing plasma TG and apo B concentrations. Studies using Hep G2 cells showed that naringenin, a grapefruit flavonoid, decreased apo B secretion, thereby reducing the concentration of TG secreted into the medium (10,11). In addition, MTP was inhibited by naringenin, thereby interrupting VLDL packaging. Similarly, red wine treatment in Hep G2 cells was shown to reduce apo B-100 secretion (12). In a previous study in our laboratory, the same LGP reduced VLDL-C and TG by 50 and 39%, respectively, in ovariectomized guinea pigs compared with controls (7). LGP also decreased hepatic acyl-CoA cholesterol acyltransferase activity, an important enzyme involved in the packaging of VLDL. It is evident that grape polyphenols modify the assembly of VLDL through alteration in MTP activity and apo B secretion. These modifications appear to decrease the overall secretion of the

TABLE 3

Plasma TC, LDL-C, HDL-C, and TG of pre- and postmenopausal women during the placebo and LGP periods[1]

Group	*n*	TC	LDL-C	HDL-C	TG
			mmol/L		
Premenopausal	24				
Placebo		5.2 ± 1.0	3.0 ± 1.0	1.8 ± 0.22	1.3 ± 0.44
LGP		5.1 ± 1.1	2.7 ± 0.76	1.8 ± 0.21	1.1 ± 0.45
Postmenopausal	20				
Placebo		5.8 ± 1.1	2.8 ± 0.82	1.9 ± .37	1.7 ± 1.0
LGP		5.7 ± 1.1	2.7 ± 0.92	1.9 ± .34	1.6 ± 1.1
Repeated-measures ANOVA					
Diet effect		NS[2]	$P < 0.05$	NS	$P < 0.002$
Menopause status		$P < 0.05$	NS	NS	$P < 0.05$
Interaction		NS	NS	NS	NS

[1] Values are means ± SD.
[2] NS, nonsignificant, $P \geq 0.05$.

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TABLE 4

Plasma apo B, apo C-I, apo C-III, and apo E of pre- and postmenopausal women during the placebo and LGP periods[1]

Group	*n*	Apo B	ApoC-I	Apo C-III	Apo E
			mg/L		
Premenopausal	24				
Placebo		689 ± 20.3	100 ± 23	99 ± 32	34.7 ± 6.1
LGP		632 ± 166	94 ± 28	98 ± 39	33.0 ± 5.5
Postmenopausal	20				
Placebo		779 ± 185	105 ± 30	141 ± 59	42.7 ± 10.7
LGP		695 ± 2.51	100 ± 15	139 ± 57	41.0 ± 10.0
Repeated-measures ANOVA					
Diet effect		$P < 0.05$	NS[2]	NS	$P < 0.05$
Menopause status		$P < 0.05$	NS	NS	$P < 0.05$
Interaction		NS	NS	NS	NS

[1] Values are means ± SD.
[2] NS, nonsignificant, $P \geq 0.05$.

VLDL particles, thereby resulting in a reduction in plasma TG concentrations.

Perhaps due to decreases in TG concentrations, LGP treatment may have affected overall lipoprotein metabolism. Decreased concentrations of plasma TG could alter substrate availability in the delipidation cascade, leading to the observed decrease in LDL-C concentrations. CETP activity was also significantly decreased due to LGP treatment in both pre- and postmenopausal women. CETP is a key enzyme in the reverse cholesterol transport system. It regulates the mass transfer of TG from TG-rich lipoproteins to HDL in exchange for cholesteryl ester. Overall substrate availability mediates CETP activity. Within normal VLDL concentrations, CETP preferentially exchanges CE with LDL particles; however, when VLDL concentrations are elevated, CETP preferentially transfers CE to VLDL. The former transfer promotes the synthesis of cholesterol-enriched VLDL particles, which are known to be proatherogenic (32). Due to the significant decrease in plasma TG observed in the current study, we believe that substrate availability to promote the activity of CETP may have been downregulated. Furthermore, the decrease in TG concentrations in relation to the decrease in CETP activity can be considered to have an antiatherogenic effect.

After a decrease in TG, it is not surprising to find a significant decrease in apo E concentrations. Apo E is a crucial apolipoprotein involved in VLDL lipolysis and hepatic uptake. In general, apo E displaces apo C-II from the VLDL particle, thereby inhibiting LPL activity and overall lipolysis. Furthermore, Huang et al. (33) showed that by adding apo C-II to transgenic mice, apo E3–enriched VLDL increased LPL activity in a dose-dependent manner. In the current study, LGP treatment significantly decreased apo E and TG concentrations, which suggests less displacement of apo C-II by apo E, thereby promoting LPL activity and further reducing plasma TG concentrations.

Although grape treatment significantly reduced plasma TG, LDL-C, apo B, and apo E concentrations as well as CETP activity, the LDL particle was not affected. Similarly, there were no changes in LDL oxidation throughout the intervention. As previously stated, several animal and human studies showed that grape polyphenols decrease LDL oxidation (5,6).

TABLE 5

Plasma LCAT and CETP activities of pre- and postmenopausal women during the placebo and LGP periods[1]

Group	*n*	LCAT	CETP
		μmol/(L · h)	
Premenopausal	24		
Placebo		18.5 ± 5.6	20.5 ± 14.0
LGP		19.6 ± 7.9	18.8 ± 15.0
Postmenopausal	20		
Placebo		21.3 ± 9.5	29.9 ± 13.1
LGP		22.9 ± 6.4	21.1 ± 14.1
Repeated-measures ANOVA			
Diet effect		NS[2]	$P < 0.05$
Menopause status		NS	NS
Interaction		NS	NS

[1] Values are means ± SD.
[2] NS, nonsignificant, $P \geq 0.05$.

TABLE 6

Plasma TNF-α, IL-6, and CRP concentrations of pre- and postmenopausal women during the placebo and LGP periods[1]

Group	*n*	TNF-α	IL-6	CRP
		ng/L	*pg/L*	*mg/L*
Premenopausal	24			
Placebo		1.53 ± 1.03	3.14 ± 4.30	3.37 ± 5.64
LGP		1.36 ± 0.89	2.71 ± 4.10	2.45 ± 3.44
Postmenopausal	20			
Placebo		2.45 ± 1.78	2.25 ± 1.17	4.88 ± 7.74
LGP		2.20 ± 1.97	2.23 ± 1.27	4.55 ± 8.91
Repeated-measures ANOVA				
Diet effect		$P < 0.05$	NS[2]	NS
Menopause status		NS	NS	NS
Interaction		NS	NS	NS

[1] Values are means ± SD.
[2] NS, nonsignificant, $P \geq 0.05$.

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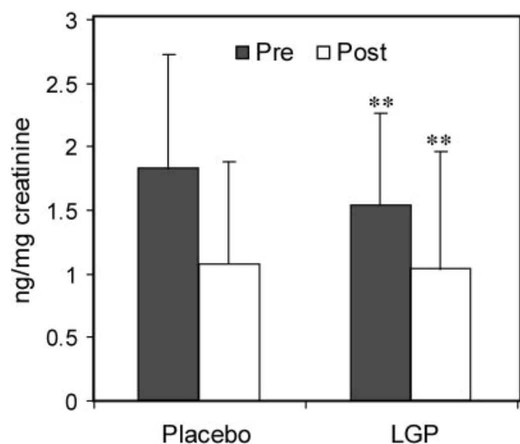

FIGURE 1 Urinary isoprostane concentrations of pre- and postmenopausal women during the placebo and LGP periods. Values are expressed as means ± SD, $n = 24$ premenopausal and $n = 20$ postmenopausal women. **LGP period differs from the placebo period, $P < 0.05$ (repeated-measures ANOVA).

However, in the current study and others, polyphenols did not have a measurable effect on LDL oxidation (34). This may be due in part to the partition of polyphenols in plasma, in which only 10–15% appear to be associated with the lipoproteins (35). Therefore, the direct effect of polyphenols on protecting circulating LDL from oxidation is minimal.

Although there were no changes in LDL oxidation, there was a significant decrease in isoprostane concentrations due to LGP treatment in both pre- and postmenopausal women. Isoprostanes are formed from the free radical–mediated peroxidation of arachidonic acid. Quantification of isoprostanes has been referred to as the gold standard of in vivo lipid peroxidation and oxidative stress (31). Many studies have reported an increase in F_2 isoprostane concentrations in smokers and in patients with hypercholesterolemia, obesity, and diabetes mellitus (36). Caccetta et al. (37) showed that red wine polyphenols significantly decreased F_2 isoprostane concentrations in smokers. These results together with data from the present study indicate that polyphenols may act as free radical scavengers and inhibit peroxidation in vivo.

LGP treatment also decreased TNF-α concentrations in both pre- and postmenopausal women. The decrease in TNF-α concentrations may be due to the decrease in lipid peroxidation. As previously stated, TNF-α, IL-6, and other cytokines are stimulated by reactive oxygen species (4,9). However, CRP release from the liver is stimulated by IL-6 (4). Although TNF-α concentrations were decreased due to LGP, IL-6 concentrations did not differ between the LGP and placebo groups. This may explain in part the nonsignificant change in CRP due to LGP. Although CRP tended ($P = 0.10$) to decrease during the LGP periods in both pre- and postmenopausal women, there may not have been a sufficient decrease in stimulatory cytokines to lower CRP release.

In the present study, LGP affected mainly plasma lipids and apolipoproteins and lowered CETP activity in all subjects. These data further support the current knowledge that grape polyphenols may disrupt VLDL assembly and secretion, thereby altering overall lipoprotein metabolism. Moreover, LGP treatment effectively reduced the major CHD risk factors that are elevated after menopause. Although the majority of polyphenol research is focused on alterations in LDL oxidation, the current study, along with our previous research, demonstrates that LGP has a distinct role in altering intravascular processing of lipoproteins, in vivo lipid peroxidation, and inflammation.

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