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Blood lipid responses to plant stanol ester supplementation and aerobic exercise training

Sofiya Alhassan[a,b,*], Kristin A. Reese[a], Jack Mahurin[c], Eric P. Plaisance[a], Bethany D. Hilson[a], John C. Garner[a], Sang O. Wee[a], Peter W. Grandjean[a]

[a]*Department of Health & Human Performance, Auburn University, Auburn, AL 36849, USA*
[b]*Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA 94305, USA*
[c]*Baptist Family Medicine Residency Program, Montgomery, AL 36116, USA*

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Abstract

The purpose of this study was to determine the independent and combined effects of plant stanol ester (PSE) margarine and aerobic exercise on blood lipid concentrations and related intravascular enzymes in 26 healthy sedentary, middle-aged men and postmenopausal women (age, 53 ± 8 years; body mass index, 27 ± 1.0, % fat, 28.5 ± 2). In a stratified double-blind manner, participants were randomly assigned to either a PSE (n = 17) or a placebo (CON, n = 9) margarine group. Participants supplemented their daily diets with 42 g of margarine spread (PSE = 3 g; CON, PSE = 0 g, of approximately equal energy content) for 9 weeks. During the last 4 weeks of margarine supplementation (MS), participants expended 400 kcal on a treadmill 5 d/wk at 65% of $\dot{V}O_2$ reserve (2000 kcal/wk). Fasting blood samples were obtained before initiating and after 4 weeks of MS and after exercise training. All blood samples were analyzed for total cholesterol, low-density lipoprotein cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), hepatic lipase, lipoprotein lipase, and cholesterol ester transfer protein activities. Total cholesterol (−10%), low-density lipoprotein cholesterol (−13%), and triglyceride (−18%) concentrations decreased after 4 weeks of MS in the PSE group, but not in the CON group ($P < .05$ for all). Four weeks of aerobic exercise increased HDL-C by 21% in the CON group ($P < .05$) and by 4% in the PSE group ($P > .05$). Total cholesterol–HDL-C ratio decreased significantly ($P < .05$) in the PSE group, but not in the CON group. No other significant alterations were observed with either PSE or exercise. Our findings suggest that PSE is effective in reducing blood cholesterol concentrations and that exercise can increase HDL-C in middle-aged men and postmenopausal women. Our findings also suggest that PSE supplementation may attenuate the exercise-induced increase in HDL-C.

1. Introduction

The Adult Treatment Panel III of the National Cholesterol Education Program advocates the use of "therapeutic lifestyle changes" such as plant stanol esters (PSEs) and aerobic exercise to ameliorate dyslipidemia and, thereby, reduce coronary heart disease (CHD) risk [1]. Margarines and spreads enriched with 1.6 to 3.8 g of PSE consumed either once or in 3 equal portions per day have been reported to reduce total cholesterol (TC) by 8% to 11% and low-density lipoprotein cholesterol (LDL-C) by 8.5% to 14.6% with little or no effect on high-density lipoprotein cholesterol (HDL-C) or triglyceride (TG) concentrations in both normal and dyslipidemic individuals [2,3]. Plant stanol ester–induced changes in TC and LDL-C can occur within 2 to 3 weeks after initiating PSE consumption and dissipate approximately 2 weeks after terminating PSE consumption [4,5]. Aerobic exercise training resulting in 1200 to 2000 kcal/wk has been shown to significantly elevate HDL-C and reduce TG concentrations in both normal and dyslipidemic individuals [6,7]. Exercise-mediated changes in HDL-C and TG are transient and have been shown to occur in the hours and days after exercise [7].

Plant stanol ester is thought to reduce TC and LDL-C concentrations by decreasing intestinal cholesterol absorption, hepatic secretion of very low-density lipoprotein cholesterol, and indirectly influencing LDL receptor protein

* Corresponding author. Stanford Prevention Research Center, Stanford University School of Medicine, Palo Alto, CA 94305-5705, USA. Tel.: +1 650 723 1085; fax: +1 650 498 7623.
E-mail address: alhassan@stanford.edu (S. Alhassan).

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expression in peripheral tissues [2]. Exercise-induced alterations in HDL-C and TG are thought to result from postexercise changes in intravascular enzyme and transfer protein activities that regulate lipoprotein lipid composition [6,8,9]. Thus, PSE supplementation and exercise could potentially have a combined effect on blood lipid concentrations due to differences in their mechanisms of action (PSE on cholesterol absorption and exercise on circulating lipids).

Currently, there is only one published study that has examined the combined effects of PSE supplementation and aerobic exercise on blood lipid concentrations and, to the best of our knowledge, no published studies that include responses of related enzyme activities with these interventions. Knowledge of the short-term effects of these 2 therapeutic lifestyle interventions would be useful to health practitioners and clinicians as they consider the efficacy of nonpharmacologic strategies for reducing blood cholesterol levels. Therefore, the purpose of this study was to examine the independent and combined effects of PSE supplementation and aerobic exercise on blood lipids in healthy middle-aged men and postmenopausal women with varying baseline blood cholesterol concentrations. A secondary purpose was to characterize changes in the activities of lipoprotein lipase and cholesterol ester transfer protein because both are considered markers of reverse cholesterol transport and are integral to lipoprotein lipid modifications after exercise [6,8,9].

2. Materials and methods

2.1. Participants

Middle-aged male and postmenopausal female volunteers were recruited from Auburn-Opelika, Montgomery, and the surrounding areas in Alabama. Inclusion criteria for this study were (1) 40 to 65 years of age; (2) sedentary (not participating in any regular leisure-time physical activity or working in physically demanding jobs in the previous 6 months); (3) nonsmoking; (4) not currently using margarine containing PSE; (5) not taking any medications known to alter lipid metabolism (with the exception of hormone replacement); (6) not previously diagnosed with cardiovascular, metabolic, or pulmonary diseases; (7) not exhibiting any known contraindications to exercise; and (8) TC of 240 or less and TG of 200 mg/dL or less. Women were considered for the study if they were postmenopausal for at least 5 years. Forty-eight volunteers, who met study inclusion criteria, read and signed an institutionally approved consent document. Nine participants did not wish to participate in the study and were not randomized. Therefore, 39 participants were assigned through gender-stratified randomization to a PSE margarine or control (CON) group. To examine the effects of dietary PSE and aerobic exercise independent of changes in body weight and/or body fat, an a priori decision was made to exclude participants with meaningful changes in body weight (±2 kg) and/or body fat (±3%).

2.2. Overview of experimental procedures

After a general participant review meeting, all participants underwent physiologic assessments. This 9-week double-blind placebo-controlled parallel design study consisted of a margarine supplementation (MS) and an aerobic exercise training program. Participants were asked to ingest their margarine supplement for 4 weeks. They were then assessed over a 1-week period during which they maintained their margarine intake. Exercise training was then added to MS during the last 4 weeks of the experimental protocol. Participants' blood samples were obtained at baseline, after 4 weeks of MS, and again at the completion of the experimental protocol.

2.3. Dietary and physical activity assessment

Participants were asked to record their dietary intake over a 3-day period (2 weekdays and 1 weekend day). Each participant's total energy expenditure and percentages of carbohydrate, fat, and protein were estimated from their 3-day dietary record using a commercially available software package (Food Processor version 7.40, ESHA Research, Salem, OR). Participants were provided with individualized dietary guidelines based on information they provided in their 3-day dietary record. Dietary guidelines were used to help participants maintain a consistent nutrient intake and stable body weight (±2 kg) throughout the experimental protocol. Participants were asked to adhere to their individualized dietary regimen and refrain from strenuous physical activity for 7 days before each experimental blood sample. Participants completed daily physical activity records during each assessment week to assess physical activity habits, reduce the likelihood of large fluctuations of physical activity outside of what was prescribed experimentally, and to estimate daily energy expenditure [10].

2.4. Physiologic assessments

All participants underwent an examination by a physician and were measured for height and weight, waist and hip circumference, and body fat percentage [11]. Participants also completed a maximal graded-exercise test (GXT) on a motor-driven treadmill using the Bruce protocol to determine their peak oxygen consumption ($\dot{V}O_2$peak) [12]. During the GXT, respiratory gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) was measured using an automated breath-by-breath system (Medical Graphics Cardio 2 Integrated Metabolic system, MedGraphics, Minneapolis, MN). Physiologic measurements were repeated again midstudy (week 5) and at the completion of the experimental protocol (week 9). Data from the second GXT (week 5) were used to determine the intensity and duration of the exercise training program. Individual exercise intensity ($\dot{V}O_2$ reserve [$\dot{V}O_2R$]; $mL \cdot kg^{-1} \cdot min^{-1}$) was estimated as follows: $\dot{V}O_2R$ ($mL \cdot kg^{-1} \cdot min^{-1}$) = {[$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) − 3.5 mL ·

$kg^{-1} \cdot min^{-1}]0.65\}$ + 3.5 mL · kg^{-1} · min^{-1}. Exercise workload was then estimated using a modified American College of Sports Medicine's treadmill walking equation {workload (mL · kg^{-1} · min^{-1}) = 3.5 mL · kg^{-1} · min^{-1} + [2.68 mL · kg^{-1} · min^{-1} · mph^{-1} × speed (mph)] + [0.48 mL · kg^{-1} · min^{-1} · mph^{-1} · $\%grade^{-1}$ × speed (mph) × incline %grade]} [12].

2.5. Margarine supplementation

Participants in the PSE group were asked to consume 3 tablespoons (42 g) per day of a commercially available margarine spread containing 3 g of PSE (Benecol Light, McNeil Consumer & Specialty Pharmaceuticals, Ft Washington, PA). A serving size of Benecol Light (1 tablespoon) contains 1 g of PSE, 188.3 J, and 5 g of fat. Participants in the CON group were asked to consume the same amount of a placebo margarine spread (I Can't Believe It's Not Butter Light-soft, Unilever, London, UK). A serving size of the placebo margarine spread (1 tablespoon) contains 0 g of PSE, 209.2 J, and 5 g of fat. All participants were asked to consume their margarine spreads daily for 9 weeks and 3 days.

Margarine supplies were distributed twice per week, with each day's serving size packaged in individually coded containers. Participants were instructed to consume all the margarine in each container and to not empty any residual margarine. Participants were instructed not to use the margarine for cooking or frying. Participants were required to return the previous days' containers before they were given the subsequent days' supply of margarine. The amount of margarine consumed by each participant was quantified by weighing empty margarine containers before and after the addition of the margarine spread (preweight). All returned margarine containers were weighed (postweight), and the amount of margarine consumed was determined as the difference between the pre- and post-margarine container weight.

2.6. Exercise training program

The exercise training program was initiated after 4 weeks of MS. Each participant's first experimental exercise session was conducted in a laboratory setting to verify the energy expenditure estimates and prescribed exercise intensity. Participants were required to wear a Polar heart rate monitor (Polar A1, Polar Electro, Woodbury, NY) during all exercise sessions. After completing a brief warm-up, exercise time was initiated and treadmill speed and incline were adjusted to a workload estimated to elicit 65% of $\dot{V}O_2R$. Estimated exercise intensity and rate of energy expenditure were verified with respiratory gas exchange data. Treadmill speed and/or incline were adjusted whenever necessary to maintain the prescribed exercise intensity. The average heart rate obtained for this exercise session was used to prescribe a heart rate range (average exercise heart rate, ± 3 beats per minute) for all remaining exercise sessions, which were conducted at a fitness facility under the supervision of trained exercise technicians.

Participants were required to exercise 5 d/wk for 4 weeks. Each individual walked on a treadmill at a heart rate corresponding to 65% of $\dot{V}O_2R$ for a duration calculated to expend 400 kcal per exercise session for a total weekly energy expenditure of 2000 kcal. Heart rate and workload were used at each training session and verified at 10-minute intervals to assure that participants completed the prescribed exercise duration. To maintain the prescribed exercise intensity, we made workload adjustments by manipulating either the speed and/or incline component of the American College of Sports Medicine's treadmill walking equation [12].

2.7. Blood sampling

Blood samples were obtained at the same time of day after a 10- to 12-hour fast on 6 separate occasions: 24 hours before (baseline-A), immediately before (baseline-B), 4 weeks after (MS-A and MS-B), and 9 weeks after (MS + Ex-A and MS + Ex-B) initiation of the experimental protocol. For all participants, MS-B blood samples were obtained 24 hours after MS-A and before initiation of the exercise training. The last 2 blood draws were obtained 72 hours (MS + Ex-A) and 96 hours (MS + Ex-B) after the last exercise session to eliminate transient changes in blood lipid concentrations that occur after a single exercise bout [13].

All blood samples were obtained through the following steps. First, an intravenous catheter was inserted into an antecubital vein, and blood was drawn into 2 chilled 10-mL "green top" vacutainer tubes. Next, 75 IU/kg of heparin (1000 IU/mL) was injected into the catheter and allowed to circulate for 10 minutes. A 10-mL blood sample was then drawn into another chilled green top vacutainer tube [14].

2.8. Biochemical analysis

Small samples of preheparin blood were used to determine hematocrit and hemoglobin concentrations for calculation of plasma volume changes [15]. Plasma samples were then isolated by centrifugation at 1500*g* for 20 minutes, aliquoted, and stored at −70°C for analysis after all samples were collected. Before freezing, aliquots of plasma samples were used to separate the HDL and HDL_3 subfractions [16,17]. Plasma samples from A and B at each time point (baseline, MS, and MS + Ex) were compared to determine daily variation in blood lipid concentrations in 10 randomly selected participants. As no significant differences were found ($P > .05$), equal volumes of plasma from A and B at each sampling time were pooled for all remaining biochemical analysis for all participants.

Plasma samples were analyzed enzymatically for TC, HDL-C, HDL_3-C, and TG using commercially available diagnostic kits [18,19]. HDL_2-C was calculated as the difference between HDL-C and HDL_3-C. Low-density lipoprotein cholesterol was calculated according to the equation of Friedewald et al [20]. Total lipase activity (TLa) and hepatic TG lipase activity (HLa) were determined by the methods described by Krauss et al [21] with

modification by Thompson et al [14]. Lipoprotein lipase activity (LPLa) was calculated as the difference between TLa and HLa. Cholesterol ester transfer protein activity (CETPa) was determined by fluorescence using a commercially available kit containing donor and acceptor particles (Roar Biomedical, New York, NY) [22]. Participants' samples were analyzed in random order for all biochemical analysis. The intra-assay coefficients of variation determined from control plasma measured multiple times within each assay were TC = 0.4%, TG = 0.7%, HDL-C = 0.3%, HDL_3-C = 0.3%, TLa = 4.1%, HLa = 5.3%, and CETPa = 2.3%. The interassay coefficients of variation determined from control plasma measured with each assay were TC = 2.8%, HDL-C = 1.9%, HDL_3-C = 1.9%, TG = 10.9%, TLa = 12.5%, HLa = 11.4%, LPLa = 15.9%, and CETPa = 14.4%.

2.9. Statistical analysis

Two (group) × three (time) analysis of variance repeated for the second factor was used to analyze changes in blood lipid concentrations and intravascular enzyme activities. Simple main effects and Duncan new multiple-range post hoc tests were used to further explore significant differences. Pearson product moment correlations were used to determine the relationship between changes in dependent variables of interest and baseline measurements. The comparison-wise error rate was set at the $P < .05$ level. Paired t tests were used to compare daily variations in TC, TG, HDL-C, and HDL_3-C in 10 randomly selected participants. A Bonferroni adjustment α level of .0042 (.05/12) was used to correct for the multiple comparisons.

3. Results

3.1. Study compliance and physiologic characteristics

Four participants dropped out of the study because of medical problems (n = 1) and time conflicts (n = 3). As a result, 35 participants met the criteria and completed the study (Fig. 1). Of these 35 participants, 9 participants were

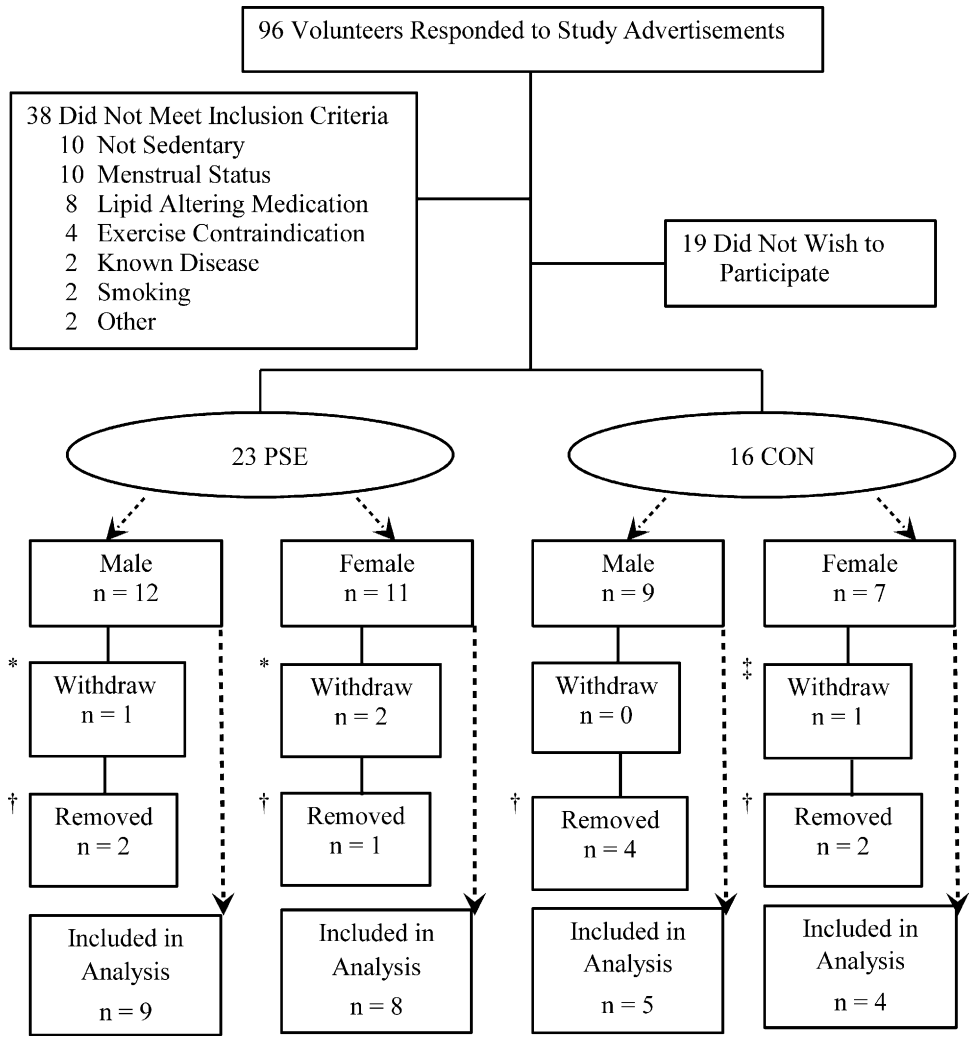

Fig. 1. Participants' selection and randomized group assignment. *Withdraw because of time conflict. ‡Withdraw because of medical problems. †Changes in body weight ±2 kg or body fat ±3%.

Table 1
Physiologic characteristics

Variable	Baseline	MS	MS + Ex
Age (y)			
PSE	53 ± 7		
CON	52 ± 9		
Weight (kg)			
PSE	74.9 ± 3.3	75.7 ± 3.4	75.0 ± 3.3
CON	81.9 ± 5.3	82.2 ± 5.0	82.7 ± 5.2
BMI (kg/m^2)			
PSE	26.5 ± 1.0	26.4 ± 0.9	26.3 ± 1.0
CON	26.9 ± 1.0	27.2 ± 1.1	27.5 ± 1.1
Waist girth			
PSE	35.9 ± 5.7	36.2 ± 4.8	35.3 ± 4.7
CON	36.8 ± 5.6	37.1 ± 5.2	36.7 ± 5.0
% FAT			
PSE	28 ± 2	29 ± 2	29 ± 2
CON	29 ± 2	30 ± 2	29 ± 2
$\dot{V}O_2$peak ($mL \cdot kg^{-1} \cdot min^{-1}$)[a]			
PSE	25.7 ± 1.8	25.4 ± 1.6	27.1 ± 1.8
CON	27.4 ± 1.7	26.2 ± 1.6	27.7 ± 1.5

Values are means ± SE. PSE indicates stanol ester margarine group (n = 17); CON, control margarine group (n = 9); MS, 4 weeks of MS; MS + Ex, 9 weeks of MS plus 4 weeks of exercise training; BMI, body mass index; % FAT, body fat expressed as a percentage of body weight.

[a] $\dot{V}O_2$peak, significantly increased in the combined participant population from baseline (26.3 ± 1.3) to MS + Ex (27.3 ± 1.3).

removed from statistical analysis because changes in their body weight exceeded the a priori cutoff (±2 kg). The remaining 26 participants (PSE, n = 17; CON, n = 9) were included in the final statistical analysis.

We observed a high degree of compliance with the MS and exercise training program. Daily margarine intake averaged 41.4 and 41.3 g for the PSE and CON group, respectively ($P \geq .05$). The average daily margarine intake for both groups ranged from 40.6 ± 4.5 to 41.7 ± 0.4 g and was not different between groups nor did it change over the course of the study ($P \geq .05$ for all). Participants in both groups completed 98% of exercise sessions. Four participants in both groups missed 1 exercise session each, and in the PSE group, 2 additional participants missed 2 exercise sessions each. Daily exercise energy expenditure for the PSE and the CON groups were 415 ± 0.9 and 415 ± 2.5 kcal, respectively, and did not vary over the exercise period ($P > .05$). Average exercise heart rate was similar for both groups and ranged from 130 to 134 beats per minute for all 4 weeks. The rate of oxygen consumption, averaged over each exercise session and estimated from treadmill workouts, increased significantly ($P = .008$), and exercise session duration decreased ($P < .0001$) in all participants over the 4-week training program.

Baseline physiologic characteristics and blood lipid concentrations were not different between groups. Body weight and body fat were not different between groups and did not change over time ($P > .05$). Participants VO_2peak did not change over the first 4 weeks of MS, but increased approximately 4% in all participants with 4 weeks of exercise training ($P = .002$) (Table 1).

3.2. Blood lipid, lipoprotein, and enzyme changes

An average relative plasma volume reduction of 2% was measured across groups after 4 weeks of MS. However, plasma volume then increased by 1% after 4 weeks of exercise ($P > .05$). All blood lipid and intravascular enzyme variables were adjusted for these plasma volume changes.

Total cholesterol changes observed over the experimental protocol were different in the PSE vs the CON group ($P = .019$; Table 2). Although TC was not different between groups at any of the blood sampling time points, TC decreased significantly by 20 mg/dL in the PSE group after the first 4 weeks of MS and remained lower than baseline after 4 weeks of exercise. However, in the CON group, TC increased progressively throughout the 9-week protocol. In the PSE group, LDL-C decreased significantly by 17 mg/dL with margarine alone and by an additional 3 mg/dL with exercise ($P = .042$; Table 2). Although not

Table 2
Changes in plasma lipid and lipoprotein concentrations

Variable	Baseline	MS	MS + Ex
TC (mg/dL)			
PSE	202 ± 8[a]	182 ± 7[b]	184 ± 7[b]
CON	187 ± 10[a]	192 ± 9[a,b]	204 ± 9[b]
LDL-C (mg/dL)			
PSE	128 ± 7[a]	111 ± 7[b]	108 ± 7[b]
CON	113 ± 10[a]	121 ± 9[a]	121 ± 8[a]
HDL-C (mg/dL)			
PSE	49 ± 3[a]	50 ± 3[a]	52 ± 4[a]
CON	51 ± 4[a]	47 ± 4[a]	57 ± 4[b]
HDL_2-C (mg/dL)			
PSE	11 ± 3[a]	13 ± 2[a]	13 ± 2[a]
CON	14 ± 3[a]	10 ± 2[a]	17 ± 3[a]
HDL_3-C (mg/dL)			
PSE	38 ± 2[a]	37 ± 2[a]	39 ± 2[a]
CON	38 ± 2[a]	37 ± 2[a]	41 ± 3[a]
TG (mg/dL)			
PSE	123 ± 14[a]	101 ± 9[b]	118 ± 8[a]
CON	113 ± 21[a]	128 ± 38[a]	110 ± 21[a]
TC/HDL ratio			
PSE	4.3 ± 1.4[a]	3.8 ± 1.0[b]	3.8 ± 1.0[b]
CON	3.7 ± 0.7[a]	4.3 ± 1.3[b]	3.8 ± 0.9[a]

Values are means ± SE. TC, LDL-C, HDL-C, and TG were not significantly different between groups at any time point. Means within group with different lower case letter are significantly different from each other ($P < .05$). $HDL_{2,3}$-C indicates HDL-C subfractions.

Table 3
Changes in intravascular lipoprotein enzyme activities

Variable	Baseline	MS	MS + Ex
LPLa (μmol FFA $\cdot$ $mL^{-1} \cdot h^{-1}$)			
PSE	13.4 ± 1.2	15.7 ± 1.3	14.0 ± 1.5
CON	12.0 ± 0.7	11.2 ± 0.6	10.3 ± 2.2
HLa (μmol FFA $\cdot$ $mL^{-1} \cdot h^{-1}$)			
PSE	19.7 ± 1.5	18.7 ± 1.6	18.0 ± 1.6
CON	17.8 ± 2.3	18.5 ± 3.4	17.4 ± 2.3
CETPa (pmol $\cdot$ $\mu L^{-1} \cdot h^{-1}$)			
PSE	25.1 ± 2.7	28.1 ± 3.1	28.9 ± 3.8
CON	19.7 ± 2.5	24.9 ± 3.3	22.5 ± 2.4

Values are combined group means ± SE. CETPa values are expressed as a percentage of cholesterol ester transferred from a donor to acceptor particle. FFA indicates free fatty acid.

significant, LDL-C rose by 8 mg/dL in the CON group over the 9-week experimental protocol.

The HDL-C response to MS and aerobic exercise training differed significantly between groups ($P = .046$; Table 2). The CON group experienced a nonsignificant 4 mg/dL decrease in HDL-C with margarine alone; with exercise, however, HDL-C significantly increased by 10 mg/dL. High-density lipoprotein cholesterol concentrations remained unchanged in the PSE group with MS; however, it increased slightly with exercise training (2 mg/dL, $P > .05$). Neither margarine nor exercise had any significant effect on $HDL_{2,3}$-C. The TC/HDL-C ratio was significantly different between groups ($P = .003$, Table 2). In the PSE group, TC/HDL-C ratio decreased with 4 weeks of MS and remained unchanged with the addition of exercise training, whereas in the CON group, TC/HDL-C ratio increased with margarine alone and then decreased after exercise training.

Triglyceride concentrations significantly decreased in the PSE group, whereas in the CON group, TG concentrations were slightly elevated over the first 4 weeks of MS (Table 2). Triglyceride concentrations returned toward baseline values in both groups with exercise training. The different TG response between groups was found to be statistically significant ($P = .042$). Neither margarine nor exercise had any significant effect on any intravascular enzyme activities (Table 3).

3.3. Diet and physical activity records

No differences were found between groups for any dietary variables, and with the exception of dietary fat intake, none of the dietary variables changed over the course of the study ($P > .05$ for all). Average dietary fat intake was found to be greater midstudy vs baseline or posttraining values ($P = .007$) (Table 4).

Average daily energy expenditure, as estimated from physical activity records, did not differ between groups; however, reported daily energy expenditures outside the exercise intervention were statistically lower after 4 weeks of MS (799 ± 80) then at baseline (877 ± 85) or at postexercise (945 ± 65) ($P = .009$).

Table 4
Average daily energy expenditure and nutrient composition

Variable	Baseline	MS	MS + Ex
EI (kJ/d)	7778 ± 418	8238 ± 523	7883 ± 414
Protein (g/d)	93 ± 6	86 ± 7	77 ± 6
Fat (g/d)	73 ± 4	85 ± 5*	75 ± 4
Carbohydrate (g/d)	216 ± 16	221 ± 15	224 ± 14
Protein (%)	20 ± 1	17 ± 1	17 ± 1
Fat (%)	36 ± 1	39 ± 1	36 ± 1
Carbohydrate (%)	46 ± 2	44 ± 2	47 ± 1
P/S ratio	0.8 ± 0.4	1.0 ± 0.1	1.0 ± 0.1

Values are combined group means ± SE. All dietary variable values are daily values averaged over 7 days before blood sampling. Nutrients expressed as percentages are percentages of the average daily energy expenditure (kcal). EI indicates energy intake; P/S ratio, polyunsaturated to saturated fat ratio.

* $P < .05$, MS > all other time points.

Baseline physiologic characteristics, dietary energy, and nutrient intake did not correlate with any of the observed changes in blood lipids. Similarly, changes in participants' physiologic characteristics were not related to observed blood lipid changes ($P > .05$ for all).

4. Discussion

This double-blind, randomized placebo-control study examined the independent and combined effects of short-term PSE supplementation and aerobic exercise training on blood lipid concentrations and related intravascular enzyme activities in middle-aged men and postmenopausal women with varying baseline blood cholesterol concentrations. The present data indicate that exercise independently increased HDL-C and PSE supplementation independently decreased TC, LDL-C, and TG and appeared to attenuate elevations in HDL-C that occurred with aerobic exercise.

Our results support previous findings that the consumption of 3 g/d of PSE margarine can effectively reduce TC and LDL-C concentrations between 10% and 15% [23,24]. For example, using a similar PSE margarine dosage and duration as in the present study, both Plat and Mensink [24] and Gylling and Miettinen [23] were able to demonstrate reductions in TC and LDL-C in non-hypercholesterolemic individuals and patients with non–insulin-dependent diabetes, respectively. Results on the effect of aerobic exercise training on TC and LDL-C concentrations have not been consistent. In most cases when TC and/or LDL-C were reduced with exercise, it was usually accompanied by an exercise-induced reduction in body weight and/or body fat [7]. However, in the absence of body weight or body fat changes, aerobic exercise training does not seem to have any significant effect on TC or LDL-C concentration [25-27]. These findings are supported by the present results, in that a moderate-intensity aerobic exercise program expending 2000 kcal/wk in the absence of body weight or body fat changes did not have any effect on TC or LDL-C.

The addition of 4 weeks of exercise training to PSE supplementation did not result in any further alteration in TC or LDL-C beyond what was observed with PSE supplementation alone. It is possible that with a longer exercise training period and/or changes in participants' body weight and/or body fat, we might have observed a further reduction in TC or LDL-C. In hypercholesterolemic individuals, Varady et al [28] were able to demonstrate that exercise training and PSE supplementation reduced TC by 5% using a longer 8-week exercise training period. Participants in that study also exhibited a significant reduction in their body fat, which likely enhanced the TC changes observed with the combined PSE plus exercise intervention.

Interestingly, in the present investigation, a significant elevation in TC was observed in the CON group over the 4-week exercise training period. The observed 12 mg/dL increase in TC may be largely attributed to the exercise-induced increase in HDL-C because LDL-C did not

change and HDL-C increased by 10 mg/dL over the same time frame.

High-density lipoprotein cholesterol increased in both groups with the current exercise stimulus; however, the increase was only significant in the CON group. The 21% increase in HDL-C observed in the CON group is substantial, but comparable to what has been reported in the literature (4%-22%) after short-term exercise programs [6,7]. Although the observed HDL-C increase in the PSE group (2 mg/dL) was not significant in the present study, other studies have found the same magnitude of increase in HDL-C to be significant [29-31]. Furthermore, Gordon et al [32] examined the relationship between HDL-C and CHD in 4 large prospective observational studies and reported that a 1 mg/dL increase in HDL-C was associated with a 2% to 3% reduction in CHD risk for both men and women. Therefore, the modest HDL-C increase in the PSE group may still have some physiologic significance.

Although HDL-C concentration increased by 10 mg/dL with exercise training in the CON group, the simultaneous change in TC and HDL-C over the 4-week exercise period was reflected in the nonsignificant change in the TC/HDL-C ratio. On the other hand, the reduced TC/HDL-C ratio observed over the first 4 weeks of MS in the PSE group was maintained with exercise training, although HDL-C concentrations did not significantly increase. Therefore, the combined effects of PSE and exercise are reflected in the fact that TC/HDL ratio was reduced in the PSE group, but not in the CON group.

One can argue that the effect of exercise was actually suppressed by PSE MS because HDL-C increased significantly with exercise in the CON, but not in PSE group. The mechanisms responsible for this change are currently not known, and nothing was measured in the present investigation that would address these biologic mechanisms. Certainly, PSE margarine reduced blood cholesterol and LDL-C; however, exercise did not significantly alter lipoprotein lipase or cholesterol ester transfer protein activities. At least part of the reason why no significant changes were observed in any of these enzyme activities in the present investigation could be because of the short duration of the training intervention and the fact that the blood samples were obtained at least 3 days after the last bout of exercise when most of the short-term effects of the last exercise session had dissipated [33]. Similar to the current investigation, other researchers have also not been able to observe an exercise-induced alteration in the activities of LPL [25,27], HL [25,34,35], or CETP [36].

We observed an 18% reduction in TG concentration with PSE supplementation. Although the PSE-induced reduction in TG concentration was significant, TG concentrations returned toward baseline values so that by the end of the study they were not different from initial TG concentration, indicating that there was no physiologically meaningful shift in TG concentration with the combined interventions. The inclusion of both men and postmenopausal women in the present investigation may have accounted for much of the variability in the TG responses observed with exercise. Significant reductions in TG concentration in men [7], but not in postmenopausal women [37,38], have been reported after exercise training regimens of similar weekly energy expenditure. Moreover, the nonsignificant changes in TG observed in the present investigation are similar to what King et al [39] and Fonong et al [40] reported in a mixed-sex population.

The prescribed exercise stimulus resulted in a significant 4% increase in participants' $\dot{V}O_2$peak, which is comparable to what has been reported for short-term exercise training in middle-aged men and women exercising at a similar intensity [26]. $\dot{V}O_2$ peak improvements however have been greater for those whose body weight and body fat decreased at a similar exercise intensity and frequency [41,42]. In the present investigation, changes observed in $\dot{V}O_2$ or any other physiologic variables were not associated with any of the reported blood lipid changes.

In both groups, participants' daily energy and nutrient intake did not change over the course of the study with the exception of their dietary fat intake, which was highest midway through the study compared with values reported at the beginning or end of the study. Because an increase in dietary fat intake has been associated with an increase in TC [43], it is possible that the added dietary fat from the MS could have contributed, in part, to the increased TC observed in the CON group. It is also feasible that the reason TC did not increase in the PSE group can be attributed to the inhibitory role PSE has on intestinal cholesterol absorption [3]. It is also possible that the change in dietary fat was not responsible for the change in TC because there was only a 10 g/d change in dietary fat, and also because of the fact that the study was of short duration and dietary fat-induced changes in blood cholesterols are usually delayed [43].

It is well recognized that variations in daily physical activity levels can influence blood lipid concentrations and thereby affect blood lipid changes reported with exercise training. Participants' self-reported physical activity assessed at the end of the study varied significantly from the midpoint, but not from initial assessment. On average, only a 68-kcal increase in daily energy expenditure was observed between baseline and the end of the study. Therefore, it is reasonable to conclude that the experimental exercise intervention and not the participants' habitual physical activity were responsible for the exercise-induced changes observed in blood lipids.

In conclusion, changes in blood lipids with PSE plus exercise demonstrated a modest effect above what is reported for PSE or exercise alone. Our results demonstrated that independent of body weight and/or body fat changes, short-term PSE MS and aerobic exercise training can result in changes in blood lipids that improve important markers of physical health and CVD risk in both men and women with varying baseline cholesterol status. Our findings are of

practical significance for clinicians and other health care professionals who are attempting to control blood lipids or ameliorate mild dyslipidemia by nonpharmacologic means. Further research is needed to determine if PSE supplementation consistently attenuates exercise-induced increases in HDL-C and, if so, the mechanisms responsible for such an effect.

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