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Weight loss associated with reduced intake of carbohydrate reduces the atherogenicity of LDL in premenopausal women☆

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Abstract

The effect of a 3-tier intervention including dietary modifications (ie, moderate energy restriction, decreased carbohydrate, increased protein), increased physical activity, and the use of carnitine as a dietary supplement was evaluated on plasma lipids and the atherogenicity of low-density lipoprotein (LDL) particles in a population of overweight and obese premenopausal (aged 20-45 years) women. Carnitine or a placebo (cellulose) was randomly assigned to the participants using a double-blind design. Carnitine supplementation was postulated to enhance fat oxidation resulting in lower concentrations of plasma triglycerides. Seventy women completed the 10-week protocol, which followed a reduction in their energy intake by 15% and a macronutrient energy distribution of 30% protein, 30% fat, and 40% carbohydrate. In addition, subjects increased the number of steps taken per day by 4500. As no differences were observed between the carnitine and placebo groups in all the measured parameters, all subjects were pooled together for statistical analysis. Participants decreased ($P < .01$) their caloric intake (between 4132.8 and 7770 kJ) and followed prescribed dietary modifications as assessed by dietary records. The average number of steps increased from 8950 ± 3432 to 12 764 ± 4642 ($P < .001$). Body weight, plasma total cholesterol, LDL cholesterol, and triglyceride were decreased by 4.5%, 8.0%, 12.3%, and 19.2% ($P < .0001$), respectively, after the intervention. Likewise, apolipoproteins B and E decreased by 4.5% and 15% ($P < .05$) after 10 weeks. The LDL mean particle size was increased from 26.74 to 26.86 nm ($P < .01$), and the percent of the smaller LDL subfraction ($P < .05$) was decreased by 26.5% ($P < .05$) after 10 weeks. In addition, LDL lag time increased by 9.3% ($P < .01$), and LDL conjugated diene formation decreased by 23% ($P < .01$), indicating that the susceptibility of LDL to oxidation was decreased after the intervention. This study suggests that moderate weight loss (<5% of body weight) associated with reduced caloric intake, lower dietary carbohydrate, and increased physical activity impacts the atherogenicity of LDL.

1. Introduction

Coronary heart disease (CHD) is the leading cause of death in the United States. In 1999, CHD claimed over 950 000 lives and resulted in $112 billion expense in direct costs [1]. Elevated concentrations of plasma total cholesterol (TC) (≥240 mg/dL) and LDL cholesterol (LDL-C) (≥160 mg/dL) classify individuals at risk for CHD [2,3]. Since the first recommendations for treatment were made, the number of known CHD risk factors has increased, and treatment has become more aggressive [2-6]. Recently, evidence has demonstrated that measures of LDL atherogenicity, LDL particle size, and oxidation potential, in addition to LDL-C, are associated with CHD incidence and progression [6-11].

Very low density lipoproteins (VLDL) are the initial substrates in the delipidation cascade, which produces primarily 2 LDL phenotypes, pattern A and pattern B. The LDL phenotype is dependent on the amount of hepatic VLDL secreted and which apolipoproteins (apos) are present [10]. Apolipoproteins transport hydrophobic lipids, activate and inhibit lipases and other lipid modifying enzymes, and act as ligands for the receptors responsible for lipoprotein removal from circulation by the liver and extrahepatic tissue [12]. These factors interact to produce

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heterogeneous LDL particles in relation to size, density [9], and apo content [13]. Generally, larger, more buoyant LDL particles characterize pattern A [9]. In contrast, pattern B is typified by smaller, denser, more triglyceride (TG)-rich particles, which are more atherogenic [9] and is associated with a 3-fold increase in CHD risk [14]. In addition, pattern B particles have decreased affinity for hepatic LDL receptors, extended residence time in circulation, increased migration into endothelial cells, increased propensity for oxidation [8,9], and enhanced coagulant activity [15]. Studies have shown that converting from pattern B to pattern A decreases CHD risk [8].

We previously reported that this population of premenopausal overweight/obese women under study were at higher risk for both diabetes mellitus type 2 and CHD [16]. For example, insulin resistance, determined by the homeostasis model assessment [17,18], was present in 37.5% of participants, and 12.5% of these women had the metabolic syndrome [16].

Because lifestyle modifications have been shown to influence expression of pattern A and pattern B LDL [8,10], we decided to explore the influence of dietary modifications, increased exercise, and the use of carnitine as a supplement on plasma lipids and LDL atherogenicity. Carnitine, a necessary component of the system responsible for transporting long-chain fatty acids across the mitochondrial membrane for β-oxidation [19,20], could also increase the use of lipids when provided in supplemental form [21-24]. Our hypothesis was that carnitine supplementation would improve the plasma lipid profile when given in combination with dietary modifications and increased physical activity.

Our second hypothesis was that lifestyle modifications leading to moderate weight loss would alter LDL metabolism resulting in a less atherogenic LDL particle. Consequently, the purpose of this study was to evaluate the effect of a dietary modification (energy restriction and modified macronutrient composition), increased physical activity (measured as increased number of steps per day), and carnitine supplementation on lipoprotein metabolism and LDL atherogenicity.

2. Materials and methods

2.1. Materials

Enzymatic cholesterol and TG kits were obtained from Roche-Diagnostics (Indianapolis, Ind). Acetyl coenzyme A, carnitine acyltransferase, EDTA, aprotinin, sodium azide, 5,5′ -dithiobis(2-nitrobenzoic acid), and phenyl methyl sulfonyl fluoride were obtained from Sigma (St Louis, Mo). Malonaldehyde bis(diethyl acetal) was obtained from Aldrich (Arlington Heights, Ill). Apolipoprotein E and CIII immunoturbidometric kits were obtained from Wako (Osaka, Japan). Carnitine and placebo supplements were provided by Lonza (Lonza Inc, Fair Lawn, NJ).

2.2. Study population

Eighty-five overweight and obese, premenopausal women were recruited from the University of Connecticut and surrounding communities to participate, and 70 completed the 10-week protocol. Reasons for attrition were personal reasons unrelated to the study (n = 5), time constraints because of the study (n = 5), and difficulty with adhering to dietary modifications (n = 5). The participants who dropped out of the study were not significantly different from those who remained in the study in regard to baseline diet and plasma profile. The 70 participants (74% white) who completed the protocol were between the age of 20 and 45 years, and body mass indexes (BMIs) ranged from 25 to 37 kg/m^2. Exclusionary criteria were pregnancy, lactation, and history of CHD, kidney or liver disease, and diabetes. Eight of the women were identified as current smokers, and 52 reported some alcohol consumption. Eight participants were taking a multivitamin, 5 were taking calcium, and 1 participant was taking calcium, vitamins A and E, and folic acid. Twenty-five participants were taking oral contraceptives, 8 were on thyroid medication (stable for at least 2 years), and 1 participant had been taking a cholesterol-lowering medication for over 5 years. Three participants reported anemia, but did not report taking any prescription or nonprescription drugs for the condition. Using the International Physical Activity Questionnaire [25], the majority of participants considered themselves to be sedentary to moderately active.

2.3. Experimental design

The 10-week study protocol was approved by the University of Connecticut Institutional Review Board, and informed consents were obtained from all subjects. All participants were placed on a energy-restricted diet (85% of energy needs based on the Harris-Benedict formula and a small activity factor). The distribution of energy in the diets was 30% protein, 30% fat, and 40% carbohydrate. The carbohydrate content of the diet (ie, 40% of total energy) was lower than current governmental recommendations and was chosen to enhance the likelihood of carnitine supplementation having a positive effect. Carnitine palmitoyltransferase I, the first of the carnitine-dependent long-chain fatty acid transport proteins, is inhibited when carbohydrate consumption is high [19,20]. A moderate (40% of total energy) rather than a more severe carbohydrate restriction was also chosen to improve dietary compliance. Participants received the food and the menus to follow based on their energy level and macronutrient composition. Based on their calculated energy expenditure, we assigned our participants into 5 different groups based on kilojoule intake: 5880, 6300, 6720, 7140, and 7560 kJ/day. An example of a week menu for the 5880-kJ group is presented in Table 1. Each participant also received an Omron HJ-104 pedometer (Omron Healthcare, Inc, Vernon Hills, Ill), which was set

Table 1
An example of a typical menu for the 5880-kJ group provided to the participants*

Breakfast	Omelet:
	½ Cup egg substitute, ¼ cup chopped onion
	¼ Cup chopped green pepper, ¼ cup chopped tomato
	1 Teaspoon olive oil
	Sauté onion, pepper, and tomato in oil. Add egg and let cook
	Optional: salt and pepper to taste
Snack	1 Medium slice wheat bread
Lunch	Chef salad:
	2 Cup chopped lettuce, ¼ cup chopped tomato
	¼ Cup chopped green pepper, ¼ cup chopped onion
	2 Teaspoon olive oil, 2 teaspoon vinegar
	2 Oz deli turkey, 1 oz deli ham
	Dessert:
	¼ Cup raisins
Snack	1 Oz Provolone cheese, 1 medium apple
Dinner	Entrée:
	Sauté chicken in oil mixed with BBQ sauce
	6 Oz chicken breast, 1 teaspoon olive oil,
	2 teaspoon BBQ sauce
	1 Cup spinach (sauté or steam)
	1 Cup asparagus (sauté or steam)
Snack	1 Oz tortilla chips, 1 tablespoon salsa,
	¼ Cup mozzarella cheese

* Ninety percent of the food was provided to the participants except for the condiments and nonfat milk.

to their individual stride length. A double-blind randomized design was used for the assignment of carnitine (active) or placebo.

On day 0, subjects were assigned their corresponding energy level, received daily menus, 90% of the groceries for a week, a pedometer, and the supplement for that week either the carnitine (3 g of carnitine) or placebo (3 g of cellulose). They were instructed to follow the menus and to record any modifications made and to consume 3 pills of active or placebo with both breakfast and lunch. For that first week, participants were also instructed to maintain normal activity so that baseline number of steps could be determined. Log sheets were provided to record the number of steps taken as well as supplement intake on a daily basis. Subjects continued collecting their food and supplements each week during the 10 weeks of the study.

Baseline number of steps was determined by averaging the number of steps taken each day during the first week. At the beginning of week 2, participants were instructed to walk 1500 more steps than those calculated at baseline. The steps-per-day goal was increased 2 more times at the beginning of weeks 4 and 6 for a total increase of 4500 steps per day.

Two separate fasting (12 hours) blood samples were collected in weeks 1 and 10. Blood was collected into tubes containing 0.15 g/100 g EDTA. Plasma was separated by centrifugation at 1500 × *g* for 20 minutes at 4°C and placed into vials containing phenyl methyl sulfonyl fluoride (0.05 g/100 g), sodium azide (0.01 g/100 g), and aprotinin (0.01 g/100 g). Samples were then placed into −80°C freezer until analysis was performed. Plasma samples were used to determine plasma lipids, LDL size, and apo concentrations. Twenty-four–hour urine samples were collected by participants at baseline and 10 weeks to determine compliance with supplement consumption.

2.4. Dietary assessment

Baseline dietary data were collected using a 120-item food-frequency questionnaire (FFQ) developed by the Fred Hutchinson Cancer Research Center (Seattle, Wash). One participant did not have a complete FFQ. For each food, participants selected their serving size in comparison to the medium size listed. Pictures of small, medium, and large food items were provided to increase reporting accuracy. Participants recorded how many times, on average, they had consumed each food listed in the FFQ for the past 3 months. During the intervention, 3 nonconsecutive weeks (15 weekdays and 6 weekend days) of the participants' menus with modifications were entered into the Nutrient Database Systems for Research, version 4.05_33 (Nutrition Coordinating Center, University of Minnesota) for nutrient analysis.

2.5. Physical activity assessment

The International Physical Activity Questionnaire was completed by participants on day 0 to determine normal activity. Baseline number of steps was determined during the first week using the pedometer provided to each participant. The pedometer counted the number of steps taken over a 24-hour period, after which, it reset to 0. Log sheets were provided for recording the number of steps taken at the end of each day and were collected at the beginning of the following week. As an additional feature, the pedometer was equipped with a 7-day memory function. Subjects were selected at random to verify the accuracy of the reported number of steps taken per day compared to what the pedometer had recorded.

2.6. Anthropometric measurements

Weight was measured to the closest 0.5 lb, and height was measured to the closest 0.5 kg in on a portable stadiometer scale [26]. Weight and height were converted into metric measures to calculate BMI (kg/m^2). Waist circumference (WC) was measured midway between the lowest rib and iliac crest to the nearest 0.1 cm [26,27]. Hip circumference was measured at the widest point on the hip. Blood pressure was measured on the right arm using a Welch Allyn Tycos blood pressure cuff (Welch Allyn, Arden, NC) with the participant seated, after a 5-minute rest.

2.7. Plasma lipids and apos

Our laboratory is a participant in the Centers for Disease Control–National Heart, Lung and Blood Institute Lipid Standardization Program since 1989 for quality control and standardization for plasma TC, HDL-C, and TG assays. Coefficients of variance assessed by the

program during the study period were 0.76 to 1.42 for TC, 1.71 to 2.72 for HDL-C, and 1.64 to 2.47 for TG. Total cholesterol was determined enzymatically [28]. High-density lipoprotein cholesterol was measured in the supernatant after precipitation of apo B–containing lipoproteins [29], and LDL-C was determined using the Friedewald equation [30]. Triglycerides were determined adjusting for free glycerol [31]. Apolipoprotein B concentrations were measured by an immunoturbidimetric method, and turbidity was determined at 340 nm [32]. Apolipoprotein CIII [33] and apo E [34] were measured with a Hitachi Autoanalyzer 740 using Wako kits. Plasma apo CI was measured by ELISA [35].

2.8. Low-density lipoprotein size determination

The Lipoprint LDL system (Quantimetrix, Redondo Beach, Calif) was used to determine LDL particle size. Briefly, 25 μL of plasma was added to precast polyacrylamide gel tubes and overlayered with 200 μL of loading gel. Tubes were then photopolymerized for 30 minutes and placed into the electrophoresis chamber. Electrophoresis buffer (Tris-hydroxymethyl aminomethane 66.1 g/100 g, boric acid 33.9 g/100 g, pH 8.2-8.6) was added to the top and bottom portion of the chamber. The gel was run until the HDL fraction was approximately 1 cm from the end of the gel tube, approximately 90 minutes at 72 mV. Gels were allowed to sit for 30 minutes and then scanned with a densitometer. The Lipoprint system quantifies 6 different LDL subclasses based on size. The majority of subjects did not have LDL-4, LDL-5 and LDL-6 in an amount that could be detected; therefore, only 3 fractions are reported here. However, for those subjects who carried a detectable amount of cholesterol in the smaller LDL fractions, these concentrations were added into LDL-3 fraction.

2.9. Susceptibility of LDL to oxidation

Low-density lipoprotein oxidation was determined according to Abbey et al [36]. Low-density lipoprotein was isolated by ultracentrifugation in an L8-M ultracentrifuge (Beckman Instruments, Fullerton, Calif) at a density of 1.006 g/mL at 60 000 × *g* for 45 minutes in a VT5 65.5 rotor. Samples were dialyzed overnight in 10 mmol/L Na_2HPO_4, 0.15 mmol/L NaCl, pH 7.4, and protein was determined after dialysis [37]. Low-density lipoprotein samples were diluted to 102 μg LDL/1.2 mL of the dialysis buffer used. Samples were then transferred to cuvettes, which were placed in a DU-640 UV spectrophotometer (Beckman Coulter, Fullerton, Calif) to be read. $CuSO_4$ was added to initiate oxidation. Kinetics of the samples proceeded at 37°C, for 180 minutes, and absorbance was plotted every 120 seconds. Conjugated diene formation was determined from the differences between the intercept of the propagation and termination phases and absorbance at time 0. Conjugated diene concentrations were determined by using the extinction coefficient for conjugated dienes at 234 nm (29 500 L/[mol cm]). Lag time was determined from the intercept of the lag and propagation phases.

2.10. Urinary carnitine

Urinary carnitine was determined by a spectrophotometric method. Briefly, 1 mL of perchloric acid was added to 1 mL of sample and mixed. After centrifugation at 5000 × *g* for 10 minutes at 4°C, 200 μL of 1.75 mmol/L of potassium phosphate was added to the supernatant, mixed, and centrifuged at 5000 × *g* for 5 minutes at 4°C. Five hundred microliters of the supernatant was then mixed with 2.5 μL of 5 mmol/L potassium hydroxide and incubated in a water bath for 15 minutes at 60°C. After incubation, 120 μL of perchloric acid was added, mixed, and centrifuged at 5000 × *g* for 5 minutes at 4°C. From this mixture, 100 μL was mixed with 1 mL of 0.1 mmol/L Tris buffer and 100 μL of 1 mM 5,5′ -dithiobis(2-nitrobenzoic acid). In a timed sequence, 5 μL of carnitine acetyltransferase and 30 μL of acetyl coenzyme A were added to this final mixture every 30 seconds. Samples were incubated at room temperature for 15 minutes and read on the Spectrophotometer (Biomate 3, Thermo Spectronic, Rochester, NY) at 412 nm. Calculations were performed to determine the concentrations of total acid soluble carnitine (TASC) in μmol/L.

2.11. Statistical analysis

Values are reported as mean ± SD. Repeated measures analysis of variance was used to compare changes in plasma lipids, apos, LDL size, and parameters of oxidation between baseline and posttreatment in the 2 groups, the carnitine and the placebo-treated. A P value of $<.05$ was considered significant. As no statistical significance was found in any of the measured parameters due to carnitine supplementation, data for all 70 subjects were pooled to evaluate the effects of the weight-loss program (dietary modifications and increased physical activity) on lipoprotein metabolism and LDL particle atherogenicity. A stepwise linear regression was conducted to evaluate the major determinants of weight loss (increased physical activity, caloric reduction, increased intake of protein, or decreased intake of carbohydrate). Similarly, changes in lipid parameters were evaluated by a stepwise linear regression to determine the major determinants of the observed reductions in LDL-C, TG, and apos, and in the parameters of LDL oxidation and LDL size. Significant correlations between changes in the intervention and the resulting variables were also evaluated. All data were analyzed using SPSS version 11.5 (SSPS, Chicago, III).

3. Results

The significant increase observed in urinary TASC in those participants consuming carnitine strongly suggests compliance with the protocol. Urinary TASC for those taking carnitine significantly increased from 128.9 ± 145.3 μmol/L at baseline to 583.4 ± 295.2 μmol/L ($P < .01$) at

Table 2
Changes in BMI, WC, number of steps per day, and percent of energy from macronutrients of premenopausal women at baseline and after 10 weeks of intervention*

	Baseline	10 wk
Weight (kg)	79.4 ± 11.1[a]	75.8 ± 11.4[b]
BMI (kg/m^2)	29.6 ± 3.2[a]	28.3 ± 3.4[b]
WC (cm)	90.1 ± 8.0[a]	84.3 ± 7.8[b]
Number of steps per day	8950 ± 3432[a]	12 764 ± 4642[b]
Carbohydrates (% energy)	50.9 ± 10.1[a]	42.1 ± 1.2[b]
Total fat (% energy)	32.7 ± 7.7[a]	31.8 ± 1.1[a]
Saturated fat (% energy)	11.0 ± 0.4[a]	9.7 ± 0.5[b]
Monosaturated fat (% energy)	12.3 ± 3.2[a]	13.8 ± 0.7[b]
Polysaturated fat (% energy)	6.8 ± 1.9[a]	5.6 ± 0.4[b]
Protein (% energy)	16.8 ± 3.3[a]	28.1 ± 1.0[b]

* Values are presented as mean ± SD for n = 70 participants. Values in the same row with different superscripts are significantly different as determined by paired t test ($P < .001$).

week 10. Participants consuming the placebo had comparable baseline TASC value of 113.6 ± 77.6 μmol/L, but a significantly lower concentration at 10 weeks, 160.1 ± 145.0 μmol/L ($P < .001$).

Participants' mean age was 29.4 ± 8.8 years. From baseline to the end of the protocol (10 weeks), there was a significant decrease in both BMI and WC. Mean BMI changed from 29.6 ± 3.2 to 28.3 ± 3.4 kg/m^2 ($P < .0001$), and mean WC changed from 90.1 ± 8.0 to 84.3 ± 7.8 cm ($P < .0001$) (Table 2). The mean number of steps taken each day increased significantly from 8950 ± 3434 to 12 764 ± 4642 ($P < .0001$) (Table 2). Energy intake decreased from 8588.1 ± 3148.9 to 6301.1 ± 502.5 J/day. Macronutrient intake closely reflected expected composition of 30% protein, 30% fat, and 40% carbohydrate (Table 2). Mean contribution of carbohydrate intake to total energy decreased from 50.9% ± 10.1% to 42.1% ± 1.2% ($P < .0001$), and mean protein intake increased from 16.8% ± 3.3% to 28.1% ± 1.0% ($P < .0001$) (Table 2). Fat was the only macronutrient that did not change significantly over the intervention period. Fat contribution to total energy intake was 32.7% ± 7.7% compared to 31.8% ± 1.1% ($P = .293$), baseline and 10 weeks, respectively (Table 2). However, the type of fat was significantly modified by the intervention and reflected the diet provided to the subjects. Intake of both saturated and polyunsaturated fat was lower ($P < .05$) during the intervention, whereas monounsaturated fat intake was higher ($P < .05$) (Table 2). As discussed later, these modifications had no effect on plasma lipid levels.

Table 3
Plasma TC, LDL-C, HDL-C, TG, and apo B, CI, CIII, and E concentrations of premenopausal women at baseline and after 10 weeks of intervention

	Baseline	10 wk	P
TC (mg/dL)	182.8 ± 37.6	168.2 ± 25.8	<.0001
LDL-C (mg/dL)	99.8 ± 25.1	87.5 ± 24.0	<.0001
HDL-C (mg/dL)	61.4 ± 10.8	61.3 ± 12.4	NS
TG (mg/dL)	120.1 ± 64.0	97.1 ± 43.0	<.0001
Apo B (mg/dL)	74.9 ± 15.1	71.8 ± 13.5	<.05
Apo CI (mg/dL)	5.2 ± 2.3	4.5 ± 1.6	<.05
Apo CIII (mg/dL)	18.8 ± 5.1	17.2 ± 4.4	NS
Apo E (mg/dL)	3.5 ± 1.5	3.0 ± 1.1	<.05

Values are expressed as mean ± SD for n = 70 subjects. Baseline and 10-wk values were compared by using paired t test. NS indicates nonsignificance.

Table 4
Distribution of cholesterol in LDL subfractions, LDL peak size, and presence of apo B phenotype in premenopausal women at baseline and after 10 weeks of intervention[a]

	Baseline	10 wk	P
LDL peak size (nm)	26.74 ± 3.70	26.86 ± 3.60	<.0001
Pattern B (%)	44	31	.06
LDL 1 (mg/dL)	68.0 ± 12.7	62.5 ± 18.7	<.005
LDL 2 (mg/dL)	24.5 ± 8.6	21.0 ± 8.0	<.0001
LDL 3 (mg/dL)	5.3 ± 6.0	3.2 ± 4.7	<.01
LDL 1 (%)	70.5 ± 8.8	72.1 ± 10.4	NS
LDL 2 (%)	24.6 ± 5.7	24.3 ± 7.2	NS
LDL 3 (%)	4.9 ± 4.8	3.6 ± 4.9	<.05

Baseline and 10-week values were compared by using paired t test.
[a] Values are expressed as mean ± SD for n = 70 subjects.

Plasma lipid profile improved after the intervention. Total cholesterol significantly decreased 8.0% ($P < .0001$) due primarily to the 12.3% decrease in LDL-C ($P < .001$), with no significant change in HDL (61.4 mg/dL at baseline and 61.3 mg/dL at 10 weeks) (Table 3). Apolipoprotein concentrations significantly decreased after the intervention. Apolipoprotein B decreased by 4.1% ($P < .05$), apo CI by 13.5% ($P < .05$), and apo E by 14.3% ($P < .05$) (Table 3).

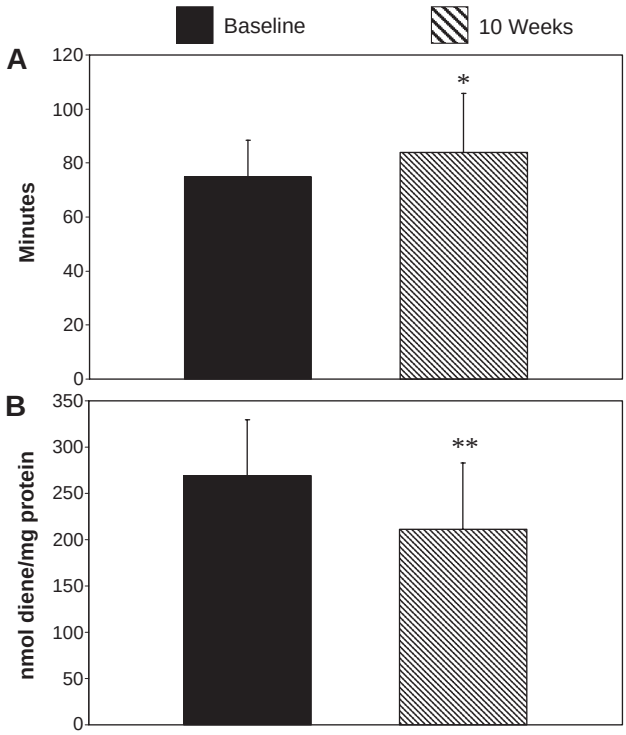

Fig. 1. Parameters of LDL oxidation. Lag time (panel A) in LDL isolated at baseline (solid bar) and after 10 weeks (hatched bar), and conjugated dienes formation (panel B) in LDL isolated at baseline (solid bar) and after 10 weeks (hatched bar). *Significantly different at $P < .01$; **significantly different at $P < .0001$.

Table 5
Presentation of models which determined the major changes (CH) in weight (WT), TG, LDL-C, and in the parameters of LDL oxidation (lag time and conjugated dienes)

Change	Model	R^2	β	P
CH WT	CH carbohydrate	0.072	.268	.033
CH TG	1. CH energies	0.114	.338	.007
	2. CH energies and CHWC	0.194	.348	.003
CH LDL-C	CH carbohydrates	0.057	.270	.033
CH lag time	CH LDL-C	0.142	−0.377	.005
CH conjugated dienes	CH LDL-C	0.157	.396	.003

In contrast, apo CIII concentrations did not change after the intervention (Table 3).

Low-density lipoprotein peak size significantly increased at week 10 ($P < .0001$), and there was a trend of fewer participants expressing the pattern B phenotype, 44% at baseline vs 31% at 10 weeks ($P = .06$) (Table 4). All LDL subfractions, LDL 1 through LDL 3, significantly decreased in absolute amount (Table 4). The percent change was only significant for subfraction LDL 3, a 26.5% decrease ($P < .05$). Measures of LDL oxidation also significantly changed. The lag time increased by 11.9% ($P < .01$) and conjugated dienes decreased by 21.5% ($P < .001$) (Fig. 1).

As indicated in Table 5, the major determinant of the weight loss was the reduction in dietary carbohydrate. Carbohydrate intake explained 7.2% of the changes in weight loss. No other parameter including number of steps or reduction in energy explained the changes in weight. For the changes in TG, there were 2 significant models. The first model was the reduction in carbohydrates, which explained 11.4% of the changes, and the second one included the change in WC. Thus, the combination of reduced carbohydrate intake and reductions in WC explains 19.4% of the changes seen in plasma TG (Table 5). For LDL-C, the reduction in dietary carbohydrate again was the major determinant of decreases in LDL-C. Although it was significant, it explained only 5.7% of the changes. The reduction in LDL-C was the major determinant of the LDL oxidation parameters, lag time, and conjugated dienes, explaining 14.2% and 15.7% of the changes, respectively. Changes in apo concentrations were not explained by one single factor or those presented in Table 5. Interestingly, increased physical activity as measured by the increased number of steps did not explain any of the observed changes in the plasma lipids or the parameters for LDL oxidation. Similarly, the decreases in saturated fat or the increases in monounsaturated fatty acids did not explain the observed reductions in LDL-C after the intervention.

4. Discussion

This study was conducted to determine the combined effects of carnitine supplementation, caloric restriction, and increased physical activity on weight loss. The rationale was that under the conditions of the experiment, carnitine effect would be more pronounced as the metabolic conditions would favor the oxidation of body fat and produce a decrease in plasma TG. Although carnitine had no effect on any parameters measured, the weight loss intervention produced significant changes in plasma lipids and anthropometrics.

In the present study, we found that in this population of overweight, obese premenopausal women, the intervention resulted in significant decreases in weight (4.5%), BMI (4.4%), and a mean decrease of 5.8 cm in WC. The decrease in BMI from 29.6 to 28.3 kg/m^2 removed the group from the cusp of obesity (BMI $\geq$30 kg/m^2). The average WC (84.3 cm) after the intervention placed the subjects at reduced risk for the metabolic syndrome, type 2 diabetes, and CHD based on the high-risk guidelines of WC $\geq$88 cm [16,26]. Although there were both a significant decrease in energy intake (26.6%) and a significant increase (42.6%) in the number of steps taken per day, stepwise linear regression indicated that the carbohydrate reduction was the best predictor of weight loss. Macronutrient intake suggests that participants were compliant with the expected changes of 30%, 30%, and 40% energy from protein, fat, and carbohydrate, respectively. It is interesting to note that although there was a decrease in saturated fat intake from 11% to 9.7% of total energy and an increase in monounsaturated fat intake from 12.3% to 13.8% of total energy after 10 weeks, the changes in LDL-C were associated only with the reductions in carbohydrate. In addition, the decreases in LDL-C were significantly associated with the decreases in the atherogenicity of LDL, which include the formation of the phenotype, pattern A, and the decreases in the susceptibility of LDL to oxidation.

Modest weight loss has been shown to decrease the incidence of cardiac events and to decrease cardiac mortality and total mortality [38], which is generally attributed to the beneficial effects of weight loss on TG , LDL-C, and HDL-C [38]. The effects of modest weight loss on other significant factors of CHD such as LDL size ant its oxidative potential are less known [6,11]. Studies have shown that modest weight losses improve plasma lipid profiles by decreasing TG, TC, and LDL-C [38-40]. In addition, other characteristics, particularly the size and oxidative potential of LDL, are important risk factors of CHD [6,11]. In one of the few studies exploring the effect of weight on LDL characteristics in addition to LDL-C concentrations, Vasankari et al [41] reported decreased concentrations of oxidized LDL in obese premenopausal women after weight loss.

4.1. Effects of diet modifications and increased activity on plasma lipids and apos

In this population of overweight, obese premenopausal women, there was a significant decrease in plasma apo B concentrations after the 10-week weight loss intervention.

Apolipoprotein B concentration is a proxy measurement for VLDL and LDL concentrations because one apo B is present per particle [8]. Evidence has shown in some female populations that it is the best parameter to predict CHD [8] because the apo B–containing lipoproteins retained in endothelial tissue are the initiators of the atherogenic plaque formation [42]. A possible explanation for the reduced plasma apo B concentrations could be decreased VLDL secretion and therefore a decreased conversion into LDL particles. Another possibility is increased hepatic clearance of VLDL. The decrease in caloric intake could be one of the primary reasons for the decrease in apo B concentrations. The decreased caloric intake decreases the TG concentration in the liver available for VLDL synthesis. Therefore, the critical mass of lipids needed for VLDL synthesis is not reached, and there is an increased degradation of apo B100 and a decrease in VLDL secretion. The increase seen in the number of steps taken per day would enhance the energy deficit. Lipids are the primary source of energy during low- and moderate-intensity exercise [43,44] such as walking. Low to moderate exercise has been recommended for overweight and obese individuals because this type of exercise can increase fat oxidation [45]. Therefore, the significant increase in steps taken per day may have increased lipid oxidation, decreased free fatty acids in circulation, and further lowered hepatic lipid concentrations.

The intervention also favorably affected lipoprotein metabolism. While in circulation, VLDL binds with lipoprotein lipase (LPL) and hepatic lipase (HL), and TGs are hydrolyzed from the particles and transported into hepatic and extrahepatic tissue [10]. Once LDL is formed, it accepts TG in exchange for cholesterol esters via the cholesterol ester transport protein from the VLDL [8]. Triglyceride in LDL is a prime substrate for HL. Hepatic lipase hydrolyzes TG from the LDL, which further develops into small, dense pattern B LDL [8] The levels of the small apos may modify this process. Apolipoprotein CII activates the action of LPL, whereas both apo CI and apo CIII have an inhibitory effect [46]. In transgenic mice studies wherein apo CI was overexpressed, decreased hepatic VLDL clearance was observed due to either interference by apo CI with apo E–mediated binding to the LDL receptor or by a direct related effect [46]. These changes result in increased residence time and promote the formation of LDL [46]. Apolipoprotein CI has also been shown to block apo E from associating with β-VLDL thereby preventing hepatic clearance by the LDL receptor–related protein [47]. In this population of premenopausal women, there was a significant decrease in apo CI (13.5%), but there was no significant decrease in apo CIII. However, evidence suggests that apo CI may also inhibit LPL as well as HL [48]. Therefore, the decrease in apo CI may have partially decreased LPL inhibition as well as increased the affinity for the LDL receptor and the LDL receptor related protein. Together, these would result in an increased clearance of VLDL by hepatic, muscle, and adipose tissue, which in turn would decrease TG concentrations and the number of VLDL converted to LDL.

Apolipoprotein E is needed for hepatic clearance of VLDL and its remnants. However, different isoforms of apo E are associated with elevated LDL-C and TG levels [49]. Data from a study using a mouse model reported that VLDL may actually be able to cross the endothelial lining and be taken up by macrophages via apo E receptors [50]. It has also been shown in human plasma that VLDL, via apo E, can be taken up by macrophages [51]. Hypertriglyceridemia in humans has been shown to be associated with elevated levels of apo E [49]. Huang et al [49] studied the effect of apo E3 on VLDL synthesis and lipolysis in transgenic rabbits. The overexpression of apo E3 in rabbits resulted in increased VLDL production, decreased lipolysis, and the hindrance of hepatic LDL clearance [49]. Although in our study apo E isoform status was not explored, the improvement in the lipoprotein profile and decrease in LDL atherogenicity may be partially explained by the observed decreases in apo E concentrations.

4.2. Effects of diet modifications and increased activity on LDL atherogenicity

Expanding the characterization of heterogeneous LDL particles has revealed additional components of CHD risk assessment including LDL size, LDL oxidation potential, and the apos that moderate LDL metabolism. Furthermore, dietary intake has been shown to affect these parameters. For example, high carbohydrate intake has been shown to increase TG and decrease LDL particle size [52]. In a comparison between the Framingham cohort and a population from Costa Rica, Campos et al [53] reported that Costa Ricans who consumed more carbohydrates also had higher plasma TG and apo B concentrations, and decreased LDL particle size than the population from Framingham [53]. Krauss and Dreon [52] reported similar results in 105 healthy men who consumed both high- and low-fat diets using a crossover design. During the low-fat diet period, 36 subjects converted from pattern A to pattern B, whereas on the high-fat diet, there was a high prevalence of large, buoyant LDL particles [52]. In general, high-carbohydrate diets have resulted in conversion of the pattern A phenotype to pattern B [54], whereas low-carbohydrate diets have resulted in a shift from pattern B to pattern A [55,56]. In the present study, we found similar results with 13% of the subjects shifting from pattern B to pattern A after they followed a low-carbohydrate diet for 10 weeks.

In addition, the pattern B LDL particles are more susceptible to oxidation in part because of their increased residence time in circulation [8,9,54]. Oxidized LDL is thought to be one of the initiators of atherosclerotic plaque formation [50]. The oxidized LDL, partly because of its small size, crosses the endothelial lining easily [57] and causes a further increase in endothelial permeability [50,58]. This subsequently cascades further, with inflammatory and

coagulant responses, proliferation of smooth muscle cells to "remodel" the artery, and eventual occlusion [50,58]. Obesity is associated with an increase in serum lipids as well as an increase in oxidized LDL [59], and weight loss has been shown to decrease concentrations of oxidized LDL [41]. As has been found in earlier research [58], weight loss increased mean LDL diameter in our population. There was also a significant increase in LDL oxidation lag time and a significant decrease in conjugated dienes formed after the 10-week intervention, consistent with previous data [41].

The current study was conducted with overweight and obese premenopausal women, and the results may not be applicable to the general public. Also, the intervention period was 10 weeks, and whether these positive responses would persist over the long-term is unknown. We suspect these favorable responses would be maintained as long as significant weight is not regained; however, additional studies are required to support this premise.

In conclusion, after the short-term weigh-loss intervention, plasma lipoprotein profile improved with significant decreases in TC, LDL-C, and TG, and the maintenance of HDL levels. Concentrations of apos active in the metabolism of LDL also improved after the intervention, with a significant decrease of apo B, apo CI, and apo E. There was also a decrease in LDL atherogenicity as evidenced by the significant increase in LDL peak size; significant decrease in percentage of small, dense LDL particles; and decreased LDL oxidation. There were also a significant trend ($P < .06$) of subjects converting from pattern B to pattern A. These data provide further evidence in support of weight-loss programs that incorporate lifestyle modifications as part of a regimen aimed at improvement of the lipoprotein profile and the potential atherogenicity of LDL. The fact that these favorable changes in lipoprotein metabolism were achieved with modest weight loss (<5%) is important because this can provide motivation for overweight/obese individuals to strive for small weight loss while achieving a relatively large reduction in cardiovascular risk.

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