The Lowering of Plasma Lipids following a Weight Reduction Program Is Related to Increased Expression of the LDL Receptor and Lipoprotein Lipase

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ABSTRACT To determine whether changes in plasma lipids following a weight loss program were related to modifications in gene expression of the LDL receptor (LDL-R), lipoprotein lipase (LPL), and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, overweight/obese premenopausal women were recruited. The 10-wk, randomized, double-blind intervention consisted of a hypoenergetic diet, high in protein (30% energy) and low in carbohydrate (40% energy), increased physical activity (number of steps taken per day), and intake of a supplement (carnitine or placebo). Our initial hypothesis was that carnitine would enhance the beneficial effects of weight loss on plasma lipids and anthropometrics. Because the carnitine and placebo groups did not differ in any of the measured variables, data for all subjects were pooled and comparisons were made between baseline and postintervention. Mean weight loss was 4.4 kg (P < 0.001), and plasma triglycerides (TG), total, and LDL cholesterol (LDL-C) were reduced by 31.8, 9.9, and 11.9%, respectively (P < 0.001). The expression of the genes of interest was measured in RNA extracted from mononuclear cells at baseline and postintervention using a semiquantitative RT-PCR method. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. After 10 wk, there was a 25.7% increase in the abundance of LPL mRNA (P < 0.01) and a 27.7% increase in that of LDL-R mRNA (P < 0.01). The expression of HMG-CoA reductase was not altered by weight loss. The results suggest that the increased expression of the LDL-R and LPL after the intervention might have contributed to the lower plasma LDL-C and TG observed in these women. J. Nutr. 135: 735–739, 2005.

KEY WORDS: • weight reduction • plasma lipids • lipoprotein lipase • LDL receptor • premenopausal women

Cardiovascular disease (CVD) is the leading cause of death in the United States; ~24% of men and 42% of women die in y 1 after a myocardial infarction. Dyslipidemias and excess weight are identified as some of the major risk factors for CVD (1,2). The importance of weight control and improved serum lipid profile has been widely emphasized (3,4). Weight reduction in overweight and obese people has a more appreciable lipid-lowering effect than in normal subjects (5). The duration of weight loss programs also has varying influence on plasma lipid concentrations. A meta-analysis of 70 studies on the effect of weight reduction on blood lipids confirmed that weight reduction lowers total (TC) and LDL cholesterol (LDL-C) and triglyceride (TG), whereas an increase in HDL cholesterol (HDL-C) depends on whether weight is stabilized (4). The association between cholesterol synthesis and increased activity on plasma lipid profiles and the expression of genes involved in lipid metabolism in a population of overweight/obese but otherwise healthy premenopausal women who lost substantial amounts of weight (7), suggesting that reductions in cholesterol synthesis may contribute to the lowering of plasma cholesterol. The main function of lipoprotein lipase (LPL) is to regulate plasma TG by facilitating the uptake of fatty acids by tissues. Interventions involving increased physical activity (8), which will increase fatty acid utilization in peripheral tissues, will have a major effect on this regulatory enzyme of plasma TG homeostasis. On the basis of these observations, we speculated that the reductions in plasma lipids after a weight loss intervention could be the result of decreased cholesterol synthesis and increased lipolysis in the plasma compartment. In addition and because the LDL receptor (LDL-R) is a major regulator of plasma LDL-C concentrations (9), it was of interest to evaluate whether the removal of LDL from the circulation contributed to the lowering of plasma cholesterol after a weight reduction program. Therefore the purpose of this study was to determine the effect of weight reduction through energy restriction and increased activity on plasma lipid profiles and the expression of genes involved in lipid metabolism in a population of overweight/obese but otherwise healthy premenopausal women. Our initial hypothesis was that using carnitine as a supplement would enhance the beneficial effects of weight loss on plasma lipids.
lips and anthropometries. We also postulated that the expected changes in lipid profile and BMI would be related to alterations in the expression of LPL, the LDL-R, and 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, major regulators of plasma cholesterol and TG. Because it is not feasible to obtain human liver samples, we assessed the effects of weight loss on monocytes, the peripheral mononuclear cells readily accessible from the blood, which appear to resemble hepatocytes in the regulation of lipoprotein uptake (10). We reported previously that dietary fiber selectively modifies LPL and HMG-CoA reductase mRNA abundance in men and postmenopausal women using RNA extracted from mononuclear cells (11).

SUBJECTS AND METHODS

Materials. Enzymatic TC and TG kits were obtained from Roche-Diagnostics. Apolipoprotein (apo) C-III and apo E kits were obtained from Wako Pure Chemical and human insulin RIA kits from Linco Research. Apo B kits, EDTA, HBSS, and Histopaque 1077 were purchased from Sigma Chemical. Trizol reagent, 1Kb plus DNA ladder, and agarose were obtained from Invitrogen. A One-Step RT-PCR kit was purchased from Qiagen.

Study protocol. Overweight/obese premenopausal women (n = 70; 74% Caucasian, 15% Hispanic, 6% African American, and 5% other) with a BMI between 25 and 37 kg/m², aged 20 to 45 y, free of type 2 diabetes, liver disease, or cardiovascular diseases participated in a weight loss intervention study. This was a randomized, double-blind intervention that had 3 different components, i.e., dietary restriction with a high-protein diet, increased physical activity, and the use of a dietary supplement, either L-carnitine or a placebo. Written informed consent was obtained from the subjects and the study protocol was approved by the Committee on the Use of Human Subjects in Research of the University of Connecticut.

The subject’s energy intake was restricted to 85% of each subject's energy expenditure as determined by the Harris-Benedict equation3 plus an activity factor of 1.2 because all subjects were classified as sedentary after evaluation of an exercise questionnaire (12). Diets were formulated to meet the energy requirements of the population studied.

Participants were given 90% of the food to be consumed every week and detailed menus for food preparation. All foods were individually prepared to meet the 85% energy restriction for each subject. The only foods not provided were milk (subjects were instructed to drink skim milk), condiments (they were written into the menus), butter, or margarine. On day 0, subjects were assigned their corresponding energy level, received daily menus, the necessary groceries, and the supplement (1 g of carnitine or placebo) for that week. They were instructed to follow the menus, to record any modifications made, and to consume 3 pills of active or placebo with both breakfast and lunch. Log sheets were provided to record supplement intake.

Each participant also received an Omron HJ-104 pedometer (Omron Healthcare), which was set to their individual stride length. During wk 1, participants were instructed to maintain normal activity so that a baseline number of steps could be determined. Each subject was asked to increase their baseline number of steps gradually by increments of 1500 throughout the intervention for a maximum of 4500 additional steps compared with baseline. Log sheets were also provided to record the number of steps taken each day. This report represents data from a representative subsample of 30 individuals from both the L-carnitine (n = 15) and the placebo (n = 15) groups.

Dietary assessment. Baseline dietary data were collected utilizing a 120-item FFQ developed by the Fred Hutchinson Cancer Research Center (Seattle, Washington). 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, in the past 3 mo they had consumed each food listed in the FFQ. In addition, subjects provided three 7-d dietary records during the wk 1, 5, and 10 of the study to record any modifications in diet. These data were entered into the Nutrient Database Systems for Research, version 4.05.33 (Nutrition Coordinating Center, University of Minnesota) for nutrient analysis.

Plasma lipids and apolipoproteins. Two (12-h) blood samples were collected from each fasting subject on different days in the same week into tubes containing 0.15 g/100 g EDTA to determine plasma lipids, insulin, and apolipoprotein levels. Plasma was separated by centrifugation at 1500 × g for 20 min at 4°C and placed into vials containing phenylenedimethyl sulfonate fluoride (0.05 g/100 g), sodium azide (0.01 g/100 g), and aprotinin (0.01 g/100 g).

Our laboratory has participated in the Centers for Disease Control National Heart, Lung and Blood Institute (CDC-NHLBI) Lipid Standardization Program since 1989. Under this program, the CVs of the study were 0.76–1.42% for TC, 1.71–2.72% for LDL-C, and 1.64–2.47% for TG.

Anthropometrics. Measurement of waist circumference (WC) and calculation of BMI were done according to standard techniques and equipment (20). WC was measured by the same research assistant (12) for all women at all times at the midpoint between the lowest rib and the iliac crest to the nearest 0.1 cm (20). Weight was measured in pounds to the closest 0.5 lb and height was measured in inches to the closest 0.5-inch on a portable stadiometer. The weight and height were converted into metric measures to calculate the BMI (kg/m²).

Mononuclear cell isolation. Mononuclear cells were isolated from whole blood through centrifugation on a Ficoll gradient according to the method of Boyum (21). Briefly, 20 mL of whole blood was diluted with 10 mL of HBSS without Ca²⁺ and Mg²⁺, layered over 10 mL of Histopaque 1077, and centrifuged at 500 × g for 30 min. The mononuclear cell interface was removed, washed with HBSS, and centrifuged twice at 600 × g for 10 min. The cell pellet was resuspended in 0.2 mL of Tris buffer (10 mmol of Tris, 150 mmol of NaCl and 1 mmol CaCl₂/L; pH: 7.4) and kept at −80°C until RNA was extracted.

RNA extraction. The extraction of total RNA from the mononuclear cells was based on the method of Chomczynski and Sacchi (22). Trizol reagent was used according to the manufacturer's instructions; the method was slightly modified by using isopropanol alcohol for RNA precipitation (11). Estimation of RNA concentration was done by UV spectrophotometer, measured at 260 nm.

RNA quantification. A semiquantitative RT-PCR method adapted from that of Powell and Kroon (11,23) was used to determine the expression of LPL, the LDL-R, and HMG-CoA reductase mRNA abundance. Mononuclear cell mRNA abundance in men and postmenopausal women represents data from a representative subsample of 30 individuals from both the L-carnitine (n = 15) and the placebo (n = 15) groups.

Dietary assessment. Baseline dietary data were collected utilizing a 120-item FFQ developed by the Fred Hutchinson Cancer Research Center (Seattle, Washington). For each food, participants selected their serving size in comparison to the medium size listed.

3 Harris-Benedict equation: Women = 655 + (9.6 × weight) + (1.8 × height) – (4.7 × age) where weight is in kilograms, height is in centimeters, and age is in years.
control in all reactions. A Gene Amp. PCR System 9700 (Applied Bio-systems) thermal cycler was used to carry out RT-PCR using the one-step Qiagen RT-PCR kit. The reaction mixture contained 1 μL of total cellular RNA at a concentration of 1 μg/μL. RNase inhibitor and Q-solution were not used during the procedure. For the sake of accuracy and precision, quantitative data were collected at a point at which every sample was in the exponential phase of amplification. Amplification was carried out at an annealing temperature of 56°C for 34 cycles for LPL, 60°C for 30 cycles for LDL-R, 56°C for 30 cycles for HMG-CoA reductase, and 56 and 60°C for 26 cycles for GAPDH. Electrophoresis through a 2% agarose gel (20 g of agarose/L in 1X Tris-borate:EDTA buffer) was used to size fractionate each of the reaction mixtures (10 μL). The bands were visualized by staining with ethidium bromide. The products were quantified by measuring the relative band intensity using a NucleoVision documentation system with Gel Expert 3.0 software (NucleoTech). Band intensities were corrected on the basis of the GAPDH signal, coamplified in the same reaction. Comparison among the gels was achieved by running a pooled RNA sample in each gel.

**Urinary carnitine.** Urinary carnitine was determined by a spectrophotometric method as described by de Souza et al. (29). Calculations were performed to determine the concentrations of total acid-soluble carnitine in mmol/L.

**Statistical analysis.** Paired Student t tests were used to detect differences in plasma lipids and apoproteins, insulin levels, anthropometrics, and mRNA abundance between baseline and postintervention. Repeated-measures ANOVA was used to test differences between the carnitine and placebo groups. Because the carnitine and placebo groups did not differ, data were pooled from all subjects to compare baseline with postintervention. The data are presented as means ± SD for the number of subjects in each group. Differences with P < 0.05 were considered significant. Statistical analysis was conducted using SPSS for windows 11.0.

## RESULTS

Because the L-carnitine (n = 15) and the placebo groups (n = 15) did not differ in lipid variables or weight loss (data not shown), we pooled the results from all subjects (n = 30) for the different variables measured in this study to compare baseline and postintervention. The significant increase observed in urinary carnitine in those participants supplemented with L-carnitine strongly suggested compliance with the protocol. Urinary carnitine for those subjects taking L-carnitine increased from 128.9 ± 145.3 mmol/L at baseline to 583.4 ± 295.2 mmol/L (P < 0.01) at 10 wk. Participants consuming the placebo had comparable urinary carnitine values at baseline (113.6 ± 77.6 mmol/L) and 10 wk (160.1 ± 145.0 mmol/L) (P > 0.05), which differed from those of subjects taking carnitine at 10 wk (P < 0.01).

Subjects had a significant increase in the number of steps taken per day (Table 1) as well as significant changes in macronutrient intake in agreement with the prescribed diets. There was a significant decrease in energy, with no changes in energy derived from fat. In contrast, the contribution of protein to total energy was increased (P < 0.0001), whereas carbohydrate intake was decreased (Table 1). In addition, dietary cholesterol and energy from saturated fat and polyunsaturated fat were lower postintervention, while monounsaturated fat was higher (Table 1).

The energy-restricted diet and increased physical activity markedly reduced weight, BMI, and WC after the 10-wk weight loss intervention (P < 0.001; Table 2). Plasma insulin was reduced by 17% after the intervention (P < 0.001; Table 2). In addition, there were significant reductions in plasma concentrations of total cholesterol (9.9%), TG (31.8%), LDL-C (12%), and apo C-III (7.5%). In contrast, plasma HDL-C concentrations, apo B, and apo E did not change for this subset of subjects after 10 wk of the intervention (Table 2).

**DISCUSSION.** This study was conducted to determine the combined effects of carnitine supplementation, energy restriction, and increased physical activity on weight loss. The rationale was that under the conditions of the experiment, the effect of carnitine would be more pronounced because the metabolic conditions would favor the oxidation of body fat and produce a decrease in plasma TG. Although carnitine had no effect on any variables measured, the weight loss alone caused significant changes in plasma lipids and anthropometrics. Weight loss subsequent to this 10-wk intervention program had a favorable effect on BMI, WC, and plasma lipid variables. These results confirm previous findings that weight loss is associated with significant improvement in serum lipids and lipoproteins (4). Several studies also showed that plasma HDL-C concentrations decrease with active weight loss early during the intervention, but it begins to increase at a later stage when body weight stabilizes or during a weight maintenance diet (4,30). The final portion of the current study can be viewed as the weight-stabilizing stage in which the HDL-C concentrations return to baseline values and begin to rise. Moreover, the average concentration of HDL-C in our study at baseline was high [61 ± 9 mg/dL (1.59 ± 0.23 mmol/L)] (31); changes in HDL-C concentration have also been related to the sex of the study subjects, with men tending to have increases and women no change or decreases in HDL-C during weight reduction (32,33).

In this study, serum fasting insulin concentrations were significantly reduced with weight loss as shown in previous studies (34). The changes tended to parallel those of plasma TG and are correlated with measures of adiposity (35). Insulin is known for its antilipolytic activity by inhibiting hormone-sensitive lipase within the adipocytes. With decreases in in-

### TABLE 1

<table>
<thead>
<tr>
<th>Weight, BMI, WC, number of steps per day, total energy, and percentage of energy from macronutrients in overweight/obese premenopausal women at baseline and after 10 wk of weight loss intervention†</th>
<th>Baseline</th>
<th>10 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>78.4 ± 13.0</td>
<td>74.0 ± 12.8*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.3 ± 3.0</td>
<td>27.6 ± 3.0*</td>
</tr>
<tr>
<td>WC, cm</td>
<td>90.5 ± 8.4</td>
<td>83.2 ± 7.8*</td>
</tr>
<tr>
<td>Steps/d, n</td>
<td>9480.5 ± 3230</td>
<td>13,569.9 ± 5615*</td>
</tr>
<tr>
<td>Energy, kJ/d</td>
<td>8608.4 ± 3408.5</td>
<td>6133.8 ± 619.7*</td>
</tr>
<tr>
<td>Carbohydrates, % energy</td>
<td>50.9 ± 10.1</td>
<td>42.1 ± 12*</td>
</tr>
<tr>
<td>Total fat, % energy</td>
<td>32.7 ± 7.7</td>
<td>31.8 ± 1.1</td>
</tr>
<tr>
<td>Protein, % energy</td>
<td>16.8 ± 3.3</td>
<td>28.1 ± 1.0*</td>
</tr>
<tr>
<td>Cholesterol, mg/d</td>
<td>287.5 ± 180.1</td>
<td>205.6 ± 18.5*</td>
</tr>
<tr>
<td>Dietary fiber, g/d</td>
<td>20.8 ± 8.4</td>
<td>19.4 ± 2.5</td>
</tr>
</tbody>
</table>

† Values are means ± SD, n = 30. * Different from baseline, P < 0.001.
Plasma insulin, animals. L-Carnitine supplementation during energy restriction influences lipid metabolism and body composition in Apo C-III, mg/L, a measurement of abdominal adiposity, which may be associated with the observed decreases in WC, lipase and an increased release of fat from the adipocytes, insulin levels, there may be less inhibition of hormone-sensitive triglycerides due to increased mitochondrial supplementation encourages a preferential energetic utilization of rats (36). Martin-Privat (37) hypothesized that carnitine supplementation caused weight loss among obese dogs and cats but not in monkeys after 10 wk could very well mimic the expression of hepatic LDL-R postintervention. The receptor-mediated uptake of LDL by the liver is responsible for 75% of LDL removal from plasma (9); thus an increase in LDL-R expression could partly explain the lowering of LDL-C in these women. Although HMG-CoA reductase and the LDL-R are regulated in coordination at the level of gene expression (43), these 2 genes are regulated differently at the translational and post-translational levels (44). This independent regulation was also shown by other investigators (45). In agreement with these reports, in our study, these 2 genes were not regulated in coordination because we found a significant increase in LDL-R expression, whereas HMG-CoA reductase mRNA abundance was unaffected.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Postintervention</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>5.05 ± 0.70</td>
<td>4.52 ± 0.48</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.77 ± 0.55</td>
<td>2.42 ± 0.51</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.57 ± 0.23</td>
<td>1.59 ± 0.28</td>
<td>NS</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.86 ± 0.88</td>
<td>1.24 ± 0.54</td>
<td>0.001</td>
</tr>
<tr>
<td>Apo B, mg/L</td>
<td>785.4 ± 132.0</td>
<td>733.3 ± 123.0</td>
<td>NS</td>
</tr>
<tr>
<td>Apo E, mg/L</td>
<td>35.5 ± 14.0</td>
<td>33.1 ± 11.0</td>
<td>NS</td>
</tr>
<tr>
<td>Apo C-III, mg/L</td>
<td>208.8 ± 50.0</td>
<td>194.5 ± 46.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma insulin, pmol/L</td>
<td>136 ± 77</td>
<td>113 ± 68</td>
<td>0.01</td>
</tr>
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</table>

1 Values are means ± SD, n = 30. NS, not significant.

**Effects of weight loss on LPL mRNA.** The regulation of LPL is complex and often opposite in adipose and muscle tissue in response to the same physiologic stimulus. LPL also performs a noncatalytic bridging function that allows it to bind simultaneously with beta lipoproteins and LDL-Rs, LDL-R related proteins, VLDL receptors, and apo E receptors (38,39). These interactions increase the cellular uptake and clearance of lipoproteins from the plasma. In addition, much regulation of LPL occurs post-transcriptionally. Ranganathan et al. (40) demonstrated that human adipose tissue and muscle express different mRNA forms with different lengths of the 3′-untranslated region. This may be of functional importance because the longer mRNA, which is expressed preferentially in muscle, is more efficiently translated. These data illustrate an important tissue-specific difference in LPL gene expression and an interesting mechanism for translational regulation. Also, muscle LPL levels were shown to increase with exercise (41). Hypoenergetic weight loss increases adipocyte-related LPL activity, which would facilitate lipid hydrolysis. In the current study, we demonstrated that there was a significant increase in LPL mRNA level in monocytes after the weight reduction intervention. The receptor-mediated uptake of LDL by the liver is responsible for 75% of LDL removal from plasma (9); thus an increase in LDL-R expression could partly explain the lowering of LDL-C in these women. Although HMG-CoA reductase and the LDL-R are regulated in coordination at the level of gene expression (43), these 2 genes are regulated differently at the translational and post-translational levels (44). This independent regulation was also shown by other investigators (45). In agreement with these reports, in our study, these 2 genes were not regulated in coordination because we found a significant increase in LDL-R expression, whereas HMG-CoA reductase mRNA abundance was unaffected.
Acute weight reduction was shown to decrease cholesterol synthesis in obese type 2 diabetic subjects as measured by indicators of plasma cholesterol markers (7); therefore, a weight loss would be predicted to downregulate the activity of HMG-CoA reductase, the regulatory enzyme of cholesterol synthesis. In our current study, there were no changes in HMG-CoA reductase gene expression after the intervention, suggesting that either the amount of weight loss achieved by the women in the study was not of sufficient magnitude to suppress synthesis or that HMG-CoA reductase activity would have to be measured directly to determine its contribution to plasma cholesterol homeostasis.

From this study, we conclude that weight loss via a hypoen- ergetic diet and increased physical activity led to a favorable lipid profile in overweight/obese women. The decrease in plasma TG, TC, and LDL-C can be explained in part by the increased expression of LDL and LDL-R. However, the synthesis of cholesterol, as measured by HMG-CoA reductase gene expression in monocytes did not contribute to the lowering of plasma cholesterol in this group of women.

**LITERATURE CITED**