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Beneficial effects of weight loss on plasma apolipoproteins in postmenopausal women

Maria Luz Fernandez[a,*], Shiva Metghalchi[b], Sonia Vega-López[a], Karin Conde-Knape[a], Timothy G. Lohman[c], Zaida R. Cordero-MacIntyre[b]

[a]*Department of Nutritional Sciences, University of Connecticut, 3624 Horsebarn Road Ext., Storrs, CT 06268, USA*
[b]*School of Public Health, Loma Linda University, Loma Linda, CA 92350, USA*
[c]*Department of Physiology, University of Arizona, Tucson, AZ 85721, USA*

Abstract

A total of 39 postmenopausal women 40–70 years of age and undergoing hormone replacement therapy participated in a 6-month weight reduction program, which consisted of a low calorie diet (5040 KJ/day) and phentermine hydrochloride therapy. Subjects had an average body mass index of 35.95 ± 5.32 kg/m^2 and 42.20 ± 11.0 kg of total fat. Body mass index, plasma lipids, total and trunk fat, and plasma apoproteins were measured at baseline and after 3 and 6 months of the weight reduction program. Subjects experienced an overall 10% weight loss during the treatment period ($P<0.001$). Plasma LDL cholesterol and triglycerides were reduced by 18% and 15% ($P<0.01$) respectively, whereas HDL cholesterol was increased by 9% ($P<0.01$) over the 6-month period. Plasma apoproteins were significantly affected by weight loss. Plasma apolipoprotein (apo) B concentrations were reduced 6.5% ($P<0.01$), and apo C-III and apo E were reduced by 9% over 6 months ($P<0.01$). The observed decreases in plasma apo B were significantly correlated with the observed changes in plasma cholesterol ($r=0.356$, $P<0.01$) over 3 months. In addition, changes in plasma triglycerides were correlated with changes in both apo C-III ($r=0.436$) and apo E ($r=0.354$) over 6 months. These results suggest that weight loss may have multifactorial effects on lipoprotein metabolism, resulting in better plasma lipid and apoprotein profiles.

Keywords: Weight loss; Apolipoprotein B; Apolipoprotein C-III; Apolipoprotein E

1. Introduction

Coronary heart disease (CHD) is the major cause of death for men and women in the United States [1]. Numerous factors are associated with increased CHD risk including obesity, hypertension, elevated plasma cholesterol and triglyceride concentrations, and low concentrations of HDL cholesterol (HDL-C) [2,3]. Due to higher levels of plasma triglycerides, and LDL cholesterol (LDL-C) as well as increases in body fat, postmenopausal women are at greater risk for heart disease than are their younger counterparts [4].

In women, the risk of fatal and nonfatal myocardial infarction is increased by 42% with body mass index (BMI) >25 [5]. Results from the Minnesota Heart Survey demonstrated that BMI values have increased significantly over the last decade. In 1982, the mean BMI for women was 24.8 kg/m^2, increasing to 26.9 kg/m^2 by 1997 [6]. By 2000, the third National Health and Nutrition Examination Survey (1988–1994) estimated that 64.5% of US adults were overweight or obese, with the prevalence of obesity escalating to 30.5% [7]. Abdominal obesity, which increases after menopause, is commonly associated with lipid abnormalities, insulin resistance, and increased risk for CHD. These data indicate that overweight and obesity is a significant problem in women, specifically in postmenopausal women.

Plasma apolipoproteins (apo) play a major role in the synthesis, processing, and removal of plasma lipoproteins. Apo B-100 is a large protein involved in the transport of cholesterol and triglycerides from the liver to the peripheral tissues. It is required for secretion of very–low–density lipoprotein (VLDL) and does not exchange with other lipoproteins in plasma [8]. In contrast, apo C-III and apo E are synthesized in the liver, secreted as part of the nascent HDL, and exchanged with triglyceride-rich lipoproteins in plasma [9]. High levels of apo B [10], apo C-III [11], and apo

* Corresponding author. Tel.: +1 860 486 5547; fax: +1 860 486 3674.
E-mail address: maria-luz.fernandez@uconn.edu (M.L. Fernandez).

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E [12] have been correlated with an increased risk for coronary heart disease.

In our previous report, postmenopausal women participating in a weight reduction program had significant modifications in body composition parameters and beneficial changes in their plasma lipid profiles [13]. The purpose of this study was to investigate whether weight loss affected the concentrations of plasma apoproteins, which are related to increased risk for CHD. We hypothesized that plasma apo B, apo C-III, and apo E concentrations would decrease after weight loss, and that this decrease would be correlated with the observed beneficial effects in body weight and plasma cholesterol and triglycerides [13].

2. Methods and materials

2.1. Subjects

A total of 49 postmenopausal women of white ethnicity who were 40–70 years of age and on hormone replacement therapy (HRT) were recruited to participate in a 6-month prospective clinical trial to examine the effects of weight loss on body composition, plasma lipids, and apoproteins. Of these women, 39 completed the study. A BMI of ≥30 kg/m2 or a BMI of 27 kg/m2 plus a cardiovascular risk factor such as hypertension, diabetes mellitus, hyperlipidemia, or degenerative joint disease were required for participation in the study. Exclusion criteria were participation in any weight control program during the previous 3 months, use of serotonin reuptake inhibitors, untreated hypertension, hyperparathyroidism, or use of monoamine oxidase inhibitors. All subjects gave written informed consent to participate. The study protocol was approved by the Loma Linda University Institutional Review Board.

2.2. Experimental design

Phentermine hydrochloride (Fastin, GlaxoSmithKline Pharmaceuticals, Philadelphia, PA) at a dose of 15 mg/day was initially prescribed for study subjects. If a subject did not experience depressed appetite in response to this dose, the Fastin dose was increased to 15 mg b.i.d. (8 AM and 5 PM). In addition to the drug therapy, a low-calorie diet (5040 KJ/day) was prescribed, and attendance at monthly support sessions was required. Participants were followed over a 6-month period.

Food frequency dietary records were collected from all participants at baseline, and at 3 and 6 months to ascertain compliance with the low–energy diet and to assess dietary intakes of total and saturated fat, cholesterol, and fiber. The Nutrient Profile Plus Program (Well Force Inc., Clackamas, OR) was used to calculate nutrient consumption. Results from dietary compliance evaluations are reported elsewhere [13].

2.3. Plasma lipids and apoproteins

Two blood samples were obtained to determine plasma lipids (total, HDL, and LDL cholesterol, and triglyceride) concentrations at baseline and at 3 and 6 months for all subjects. Standardization and quality control for plasma total cholesterol and triglyceride assays have been maintained by participation in the Centers for Disease Control National Heart, Lung and Blood Institute (CDC-NLBI) Lipid Standardization Program since 1989. An enzymatic method [14] was used to determine plasma total cholesterol against cholesterol standards (Boehringer Mannheim Corp., Indianapolis, IN). Plasma HDL cholesterol was measured in the supernatant after precipitation of apo B–containing lipoproteins [15] and LDL-C was calculated as described by Friedewald et al. [16]. Plasma triglycerides were determined after adjusting for free glycerol [17]. Apo B concentrations were measured by an immunoturbidimetric method in a microplate spectrophotomer at 340 nm [18]. Apo C-III [19] and apo E [20] were measured with a Hitachi Autoanalyzer 740 using kits from Wako (Richmond, VA).

2.4. Body composition

Total and regional body composition was measured by DXA using the Hologic QDR–4500A instrument and body composition analysis software, version 8.21 (Hologic Inc., Waltham, MA). Scans were obtained with the subject in the supine position, wearing only a hospital gown and undergarment, and with metal and jewelry removed. Whole body scans were taken and regions of interest were isolated. Scan time was approximately 3 minutes for each assessment, with a radiation exposure of 1.5 mrem. DXA scans were obtained at baseline and at 3 and 6 months for all subjects.

2.5. Statistical analysis

Statistical analyses were calculated using SPSS for Windows, version 10.05 (SPSS Inc, Chicago, IL) with significance defined as $P<0.05$ Data are presented as mean ± SD. Repeated-measures ANOVA was performed to detect differences over time within subjects in the measures of plasma lipids and apoproteins. Means and standard deviation were used to summarize the outcomes at each time point. Pearson product–moment correlations were performed to relate trends in plasma lipid profile and body composition changes with changes in apoprotein concentrations in plasma.

3. Results

3.1. Characteristics at baseline

Characteristics of the subjects at baseline have been reported elsewhere [13]. Subjects were postmenopausal with an average age of 58.4 ± 4.7 years. Participants had a mean weight of 91.5 ± 17.6 kg, and a BMI of 35.95 ± 5.32 kg/m^2. Women experienced significant changes in these parameters after 3 and 6 months of the weight loss program [13]. Weight was reduced to 88.5 ± 16.0 and 85.4 ± 15.4 kg over 3 and 6 months respectively (Table 1), whereas mean

Table 1
Plasma lipids and apoprotein concentrations in postmenopausal women during the 6-month weight loss treatment

Parameter	Baseline	3 Months	6 Months
Weight (kg)	91.5 ± 17.6	88.5 ± 16.0	85.4 ± 15.4
Cholesterol (mmol/L)	5.38 ± 0.63[a]	4.84 ± 0.95[b]	4.76 ± 0.83[b]
Triglycerides (mmol/L)	1.64 ± 0.86[a]	1.45 ± 0.85[b]	1.40 ± 0.63[b]
HDL-C (mmol/L)	1.28 ± 0.26[b]	1.23 ± 0.24[b]	1.40 ± 0.26[a]
LDL-C (mmol/L)	3.32 ± 0.91[a]	2.94 ± 0.80[b]	2.72 ± 0.77[c]
Apo B (mg/L)	540 ± 136[a]	493 ± 137[b]	505 ± 121[b]
Apo C-III (mg/L)	152 ± 46[a]	136 ± 44[b]	137 ± 39[b]
Apo E (mg/L)	36.8 ± 11.0[a]	34.6 ± 10.5[ab]	33.3 ± 8.2[b]

Values represent mean ± SD for $N=39$ subjects. Values with in the same row with different superscript letters are significantly different as determined by repeated-measures analysis of variance and Tukey post hoc test ($P<0.01$).
Apo = apolipoprotein; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

BMI values were 32.89 ± 5.27 over 3 months and were 32.53 ± 5.33 kg/m^2 at the end of the study.

3.2. Plasma lipids and apoproteins

There was a significant decrease in total cholesterol, triglycerides, and LDL-C over 3 and 6 months (Table 1). Participants experienced a 12% reduction in total and an 8% reduction in LDL-C, and a 15% reduction in triglycerides after 6 months of the weight loss program. In contrast, plasma HDL cholesterol concentrations were 9% higher ($P<0.01$) over 6 months.

Plasma apo B concentrations were reduced by 8.7% and 6.5% over 3 and 6 months respectively ($P<0.01$). In addition, concentrations of plasma apo C-III and apo E were 10 and 9% lower over 6 months ($P<0.01$).

Significant correlations were found between changes in plasma apoprotein concentrations and changes in body weight, body composition, and total and abdominal fat (Table 2). Values for total and trunk fat at baseline and over 3 and 6 months have been previously reported [13]. Total and trunk fat mass were 42.2 ± 11.0 and 20.3 ± 5.7 kg respectively at baseline, 38.6 ± 10.0 and 18.2 ± 5.2 over 3 months, and 33.7 ± 9.1 and 16.8 ± 4.7 over 6 months. The strongest correlations were found over 3 months when the most pronounced changes in body composition and weight occurred. Changes in plasma apo B concentrations were significantly correlated with changes in total cholesterol ($r=0.413$), triglycerides ($r=0.527$), total fat ($r=0.531$), trunk fat ($r=0.413$), weight ($r=0.420$), and BMI ($r=0.427$). Correlations between changes in apo B concentrations and these parameters reached significance only with weight ($r=0.496$) and with BMI ($r=0.453$) over 6 months.

Changes in apo C-III and apo E concentrations with changes in plasma triglycerides were quite consistent over the whole treatment period (Table 2). There were significant correlations between apo C-III and triglycerides at 3 months ($r=0.634$) and 6 months ($r=0.436$). Similarly, changes in apo E were correlated with plasma triglycerides at 3 ($r=0.354$) and 6 ($r=0.352$) months.

4. Discussion

In this study, we have demonstrated that weight loss not only results in an improved lipid profile but it also reduces the concentrations of apo B, apo C-III, and apo E, which are associated with atherosclerosis and increased risk for coronary heart disease.

Apo B is the predominant apolipoprotein in LDL and is required for the secretion of VLDL. Because both of these lipoproteins have been associated with increased risk for heart disease, plasma apo B concentrations constitute an independent risk factor for CHD [21]. Interestingly, changes

Table 2
Correlation between changes in total cholesterol (TC), triglycerides (TG), total fat, trunk fat, weight, body mass index (BMI), and plasma apoprotein changes over 3 and 6 months in study subjects ($N=39$)

	ΔTC	ΔTG	ΔTotal fat	ΔTrunk fat	ΔWeight	ΔBMI
Apo B						
Baseline–3 months	0.413*	0.527†	0.531†	0.413*	0.420*	0.427*
Baseline–6 months					0.496†	0.453†
Apo C–III						
Baseline–3 months		0.634†				
Baseline–6 months		0.436†				
Apo E						
Baseline–3 months	0.356*	0.354*				
Baseline–6 months		0.352*			0.465†	

Apo = apolipoprotein.
* $P<0.05$.
† $P<0.01$.

in apo B concentrations were significantly correlated with changes in both body composition and body weight at 3 months, when the most significant decreases in body fat and weight loss occurred. Studies have shown that abdominal obesity can be related to increased secretion of VLDL apo B. Pont et al. [22] compared apo B kinetics in lean women with those in women with abdominal obesity who were not hypertriglyceredimic or had type II diabetes (similar to the subjects in our study). These investigators demonstrated that obese subjects had increased production of VLDL, Intermediate density lipoprotein (IDL), and LDL apo B. The reduction in apo B concentrations observed in obese postmenopausal women in the present study could be related to decreased production of apo B as weight loss progresses.

Apo C-III is the inhibitor for lipoprotein lipase thus playing a major role in decreasing lipolysis and increasing the concentrations of plasma triglycerides [23]. In addition, apo C-III may be a marker for lipoproteins, which have an increased ability to bind to proteoglycans [11], thus having a major role in the atherosclerotic process. Proteoglycans are vascular matrix molecules that contribute to the retention of lipoproteins in the arterial wall [24]. In this study we observed a significant reduction of apo C-III over the 6-month period of weight loss, which was correlated with the observed decreases in plasma triglycerides.

Apo E is an apoprotein that is secreted by many tissues and has a variety of physiological roles [25] in addition to its specific functions related to lipoprotein metabolism. Although apo E is the major ligand for the removal of chylomicron remnants returning to the liver, it may have a role in altering plasma triglycerides. It has been demonstrated that apo E displaces apo C-II in triglyceride-rich lipoproteins, decreasing the interaction of lipoprotein lipase with its activator (C-II) and contributing to elevations in plasma triglycerides [26]. In the present study, plasma apo E and triglyceride concentrations were decreased in postmenopausal women over the 6 months of weight loss, suggesting that the lower apo E levels had less of an effect in displacing apo C-II. In addition, decreases in apo B were significantly correlated with decreases in plasma triglycerides at 3 and 6 months, suggesting that apo E may have displaced apo C-II in VLDL and therefore affected lipoprotein lipase activity, as has been observed in other studies.

We conclude from these studies that weight loss may have multifactorial effects on lipoprotein metabolism. The observed decreases in potentially atherogenic apolipoproteins suggest that decreases in body fat, specifically trunk fat, may be associated with decreased production of these apolipoproteins.

References

[1] Minino AM, Arias E, Kochanek KD, Murphy SL, Smith BL. Deaths: final data for 2002. Natl Vital Stat Rep 2000;50:1–119.

[2] Stamler J, Wentforth D, Neaton JD. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356, 222 primary screens of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 1986;256: 2823–8.

[3] Castelli WP. Cholesterol and lipids in the risk of coronary heart disease. The Framingham Heart Study. Can J Cardiol 1988;4:5A–10A.

[4] Bonithon-Kopp C, Scarabin P-Y, Darne B, Malmejac A, Guize L. Menopause-related changes in lipoproteins and some other cardiovascular risk factors. Int J Epidemiol 1990;19:42–8.

[5] Willett WC, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE, et al. Weight, weight change and coronary heart disease in women. Risk within the "normal" weight range. JAMA 1995;273: 461–5.

[6] Arnett DK, McGovern PG, Jacobs Jr DR, Shahar E, Duval S, Blackburn H, et al. Fifteen-year trends in cardiovascular risk factors (1980–1982 through 1995–1997). The Minnesota Heart Survey. Am J Epidemiol 2002;156:929–35.

[7] Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity amoung US adults, 1999–2000. JAMA 1998;288: 1723–7.

[8] Dixon JL, Ginsberg HN. Hepatic synthesis of lipoproteins and apolipoproteins. Semin Liver Dis 1992;12:364–72.

[9] Jong MC, Hofker MH, Havekes LM. Role of apoCs in lipoprotein metabolism. Functional differences between apoC1, apoC2 and apoC3. Arterioscler Thromb Vasc Biol l999;19:472–84.

[10] Hodis HM, Mack WJ. Triglyceride-rich lipoproteins and the progression of coronary artery disease. Curr Opin Lipidiol 1995;6: 209–14.

[11] Olin-Lewis K, Krauss RM, La Belle M, Blanche PJ, Barrett PHR, Wight TN, et al. Apo C-III content of apoB-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan. J Lipid Res 2002;43:1969–77.

[12] Wang-Iverson P, Ginsberg HN, Peteanu LA, Le NA, Brown WV. Apo E-mediated uptake and degradation of very low density lipoproteins by human monocyte macrophages: a saturable pathway distinct from the LDL receptor. Biochem Biophys Res Commun 1985;126:578–86.

[13] Cordero-MacIntyre ZR, Lohman TG, Rosen J, Peters W, Espana RC, Dickinson B, et al. Weight loss is correlated with an improved lipoprotein profile in obese postmenopausal women. J Am Coll Nutr 2000;19:275–84.

[14] Allain CC, Poon LC, Chan CS, Richard W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–5.

[15] Warnick GR, Benderson J, Albers JJ. Dextran-sulphate-Mg^{+2} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982;28:1379–88.

[16] Friedewald WT, Levy RI, Fredirckson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1978;18: 499–502.

[17] Carr T, Anderssen CJ, Rudel LL. Enzymatic determination of triglycerides, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 1993;26:39–42.

[18] Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins A-I, A-II and B in serum. Clin Chem 1986;32:957–60.

[19] Frendenrich A, Giroux LM, Tremblay M, Krimbou L, Davingnon J, Cohn JS. Plasma lipoprotein distribution of apo C-III in normolipidemic and triglyceridemic subjects: comparison of the apo C-III to apo E ratio in different lipoprotein fractions. J Lipid Res 1997;38: 1421–32.

[20] Cohn JS, Tremblay M, Amiot M, Bouthillier D, Roy M, Genest Jr J, et al. Plasma concentration of apolipoprotein E in intermediate-size remnant-like lipoproteins in normolipidemic and hyperlipidemic subjects. Arterioscler Thromb Vasc Biol 1996;16:149–59.

[21] Vega GL, Denke MA, Grundy SM. Metabolic basis of primary hypercholesterolemia. Circulation 1991;84:118–28.

[22] Pont F, Duvillard L, Florentin E, Gambert P, Verges B. Early kinetic abnormalities of apo B-containing lipoproteins in insulin-resistant women with abdominal obesity. Arterioscler Thromb Vasc Biol 2002;22:1726–32.

[23] Shachter NS. Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. Curr Opin Lipidol 2001;12:297–304.
[24] Camejo G. The interaction of lipids and lipoproteins with the intercellular matrix of arterial tissue: its possible role in atherogenesis. Adv Lipid Res 1982;19:1–53.
[25] Mahley RW. Apolipoprotein E: cholesterol transport protein with expending role in cell biology. Science 1988;240:622–30.
[26] Huang Y, Liu XQ, Rall SC, Taylor JM, von Eckardstein A, Assman G, et al. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. J Biol Chem 1998;273:26388–93.