Effects of Oral Combined Hormone Replacement Therapy on Plasma Lipids and Lipoproteins

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Hormone replacement therapy has been shown to decrease the risk of coronary heart disease (CHD) in menopausal women. In this cross-sectional study, we addressed the following question: What effects would combined oral hormone replacement therapy have on plasma lipid and lipoprotein profiles independent of the other known CHD risk factors? We analyzed the plasma lipoproteins of two groups of menopausal women who were randomly selected from a large database of individuals. One group (n = 10) was not taking any hormone replacement therapy (NO HRT), while the second group (n = 8) was taking a daily dose of 0.625 mg conjugated estrogen and 2.5 mg medroxyprogesterone orally (PremPro, Wyeth-Ayerst, Philadelphia, PA) for at least 6 months (HRT). The two groups were not different in age, body weight, percent body fat, body mass index (BMI), waist to hip ratio, blood pressure, or insulin and glucose levels. High-density lipoprotein (HDL)-cholesterol was significantly higher ($P < .05$) in the HRT group. The total cholesterol (TC) to HDL-cholesterol ratio was significantly lower for HRT versus NO HRT ($P < .05$). Apolipoprotein (apo) A-1, the apo A-1/B ratio, and lecithin:cholesterol acyltransferase (LCAT) activity were significantly higher in HRT ($P < .05$). Lipoprotein subclass profiles measured by nuclear magnetic resonance (NMR) spectroscopy showed an increase in larger HDL subpopulations (H3 and H4) in HRT ($P < .05$), which are considered antiatherogenic. No differences were seen in the cholesterol concentration or size of low-density lipoprotein (LDL) subpopulations in HRT compared with NO HRT. These results indicate that the combined estrogen and progesterone treatment leads to beneficial effects on plasma lipoproteins. The beneficial effects include (1) increases in HDL-cholesterol and predominance of HDL_2, (2) no adverse effects on LDL subpopulation distribution, and (3) increases in apo A-1 levels and LCAT activity, which indicate an improvement in reverse cholesterol transport.

THE RISK FOR CORONARY heart disease (CHD) has been associated with several factors, including elevations in plasma cholesterol and low-density lipoprotein (LDL)-cholesterol, decreases in high-density lipoprotein (HDL)-cholesterol, age, diabetes and insulin resistance, body fat distribution, smoking, blood pressure, and gender. Males have a higher risk for CHD than females mainly because of higher plasma cholesterol and LDL-cholesterol and lower HDL-cholesterol levels. However, with menopause, plasma cholesterol and LDL-cholesterol increase and HDL-cholesterol decreases in females, and as a result, there is a concomitant increase in CHD risk in menopausal women.[1,2]

In addition to the association of CHD risk with variations in plasma lipid levels, studies have shown that alterations in the structure and/or composition of plasma lipoproteins have a role in CHD. Chemical and physical characterization of the lipoproteins have shown that the structure and composition among the subfractions in a given class of lipoprotein particles may differ among individuals according to the risk for CHD.[3] It has been shown, for example, that in patients with documented heart disease, small and dense LDLs are more prevalent than the large, more buoyant LDLs.[4-8] Small and dense LDLs have been shown to be more prone to oxidation,[9-10] more readily penetrate the intima,[11] have an altered reactivity to antibodies that recognize specific epitopes of apolipoprotein (apo) B that bind to the LDL receptor,[12-13] and have a longer residence time in circulation.[14] Similarly, the larger cholesterol-rich subfraction of HDL, namely HDL_2, which is more responsive to external interventions than HDL_3, has a more protective effect on CHD than the smaller, less buoyant HDL_3 subfraction. Thus, characterization of the subpopulation distribution of the lipoproteins is helpful in evaluating CHD risk.

Shifts in the subpopulation distribution of plasma lipoproteins, which may be more predisposing to CHD risk, have been demonstrated in menopausal women.[1,15-16] Li et al[15] reported that menopausal status was associated with lower levels of HDL-cholesterol and smaller HDL particle size, and Campos et al[1] reported higher LDL-cholesterol levels and smaller LDL particle size. These changes in the nature and concentration of plasma lipoproteins may contribute to the increased risk of CHD associated with menopause. Hormone replacement therapy has been reported to decrease the risk for CHD by decreasing plasma cholesterol and LDL-cholesterol levels and increasing HDL-cholesterol levels.[17] However, estrogen replacement therapy has been shown to have adverse effects on LDL subfraction distribution. Using polyacrylamide gel electrophoresis to analyze the subpopulation distribution of plasma LDL, Rajman et al[18] reported an increase in the concentration of smaller LDL after 6 weeks of estrogen-only hormone replacement therapy. Thus, in addition to the beneficial effect with regard to lipids, estrogen replacement therapy also appears to affect LDL in a detrimental way.

The combined estrogen-progesterone hormone replacement therapy is more commonly used with postmenopausal women with intact uteri. However, reports show that this form of hormone replacement therapy does not produce the same positive effects on plasma lipid levels as estrogen-alone replacement therapy. In a recent study, evidence was presented

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showing that the combined hormone replacement therapy resulted in a reduction in large, buoyant LDL, and an increase in the small, dense LDL subfraction without a change in the plasma LDL concentration.[19] To date, information on the influence of combined hormone replacement therapy on the subpopulation distribution of HDL is lacking.

The purpose of this cross-sectional study was to examine the effects of combined estrogen-progesterone replacement therapy on plasma lipid and apoprotein levels, the subpopulation distribution of plasma LDL and HDL, and the activity of cholesterol ester transfer protein (CETP) and lecithin:cholesterol acyl transferase (LCAT). One group of menopausal women who have been on this combined replacement therapy for at least 6 months were compared with a control group not on replacement therapy. To minimize the confounding effects of factors that influence plasma lipid concentrations and the subpopulations of the lipoproteins, the two groups of subjects did not differ in age, body weight, fat mass, lean mass, body mass index (BMI), waist to hip ratio, treadmill time to exhaustion (a measure of physical fitness), blood pressure, or fasting plasma glucose and insulin concentrations.

SUBJECTS AND METHODS

Subjects

Twenty-eight middle-aged white women who had not engaged in exercise in the previous 2 years were enrolled in this study. Subjects were taken from a database of participants in the Cardiovascular Disease Risk Factor Identification Program in the Human Performance Laboratory at East Carolina University. From this subject pool, all individuals who met the inclusion criteria, which included nonsmokers, no cardiovascular disease or diabetes, and no current use of medications that interfere with carbohydrate or lipid metabolism, were considered candidates for study. Complete data were obtained from 18 subjects. One group (n = 10) was not taking any hormone replacement therapy (NO HRT), while the second group (n = 8) was taking a daily dose of 0.625 mg conjugated estrogen and 2.5 mg medroxyprogesterone orally (PremPro) for at least 6 months (HRT). Alcohol consumption was limited to social drinking (not more than 4 drinks/wk). The protocol for this study was approved by the Institutional Review Board for Human Subject Research of this university before initiating data collection.

Anthropometric Tests

Body density was determined by hydrostatic weighing following expiration to residual volume as determined by oxygen dilution.[20] The percentage of body fat and fat-free mass was calculated from body density using the Siri equation.[21] Dry body mass was recorded to the nearest 0.1 kg and height to the nearest 0.1 cm. The BMI was calculated as mass in kilograms divided by height in meters squared. Umbilicus, minimal waist, and maximal hip girths were obtained as described earlier.[22] All circumferences were obtained with a spring-tension, stretchless Gulick tape (Lafayette Instruments, Lafayette, IN) to the nearest 1 mm.

Cardiorespiratory Fitness

Time to exhaustion was determined during a standard (Bruce protocol) incremental treadmill test.

Chemical and Biochemical Analyses

Fasting blood samples were collected and treated with the addition of sodium azide (0.1 mg/mL) and aprotinin (10 KIU/mL). Plasma was prepared by centrifugation at 2,800 rpm for 30 minutes at 4°C. Plasma samples were aliquotted and stored at −80°C until analyzed. The estradiol concentration was determined by radioimmunoassay (Diagnostic Systems, Webster, TX). Plasma insulin was determined by microparticle enzyme immunoassay (IMX; Abbott, Abbott Park, IL), and glucose was determined spectrophotometrically (16-UV; Sigma, St Louis, MO). Plasma lipid concentrations were determined by microplate assays similar to those described by Shireman and Durieux.[23] Microassays for total cholesterol (TC), HDL cholesterol, and total triglycerides (TG) were modified for use with Sigma Diagnostics Cholesterol and Triglyceride Reagents. All lipid determinations by microplate were made in quadruplicate with standards and controls recommended by the assay procedure.

LDL and HDL profiles were determined by nuclear magnetic resonance (NMR) profiling.[24] NMR lipoprofiling allows deconvolution and calculation of the cholesterol concentration of three LDL (L3 to L1) and five HDL (H5 to H1) subclasses. The average particle diameter and the number of particles per unit volume are derived from the NMR analysis. With this method, larger, less dense particles are denoted by larger numbers (ie, L3 and H5). Diameter ranges (mean ± SD) for the two lipoprotein classes and their subfractions are as follows (in nanometers): LDL average diameter = 21.1 ± 0.5 and subclasses L3 (large LDL) = 22 ± 0.7, L2 (intermediate LDL) = 20.5 ± 0.7, and L1 (small LDL) = 19 ± 0.7; HDL average diameter = 9.4 ± 0.4 and subclasses H5 (HDL_{2b}) = 11.5 ± 1.5, H4 (HDL_{2a}) = 9.4 ± 0.6, H3 (HDL_{3a}) = 8.5 ± 0.3, H2 (HDL_{3b}) = 8.0 ± 0.2, and H1 (HDL_{3c}) = 7.5 ± 0.2.

In addition to the analysis of LDL by NMR, we determined plasma LDL particle diameter by gel electrophoresis as described by Rajman et al[18] using the Lipoprint LDL System (Quantimetrix, Hawthorne, CA). The Lipoprint LDL System profiles LDL by size from 25 μL plasma by polyacrylamide gel electrophoresis. Sample loading and determination of the relative concentrations of LDL subclasses were performed according to the recommendations of the supplier. Profiles obtained with this procedure provide seven "lipoprint" subclasses (*lp* 1 to *lp* 7), with the larger, less dense particles denoted by smaller numbers. LDL scores were calculated from these profiles as described by Rajman et al,[18] which is a modification of the scoring system used by Campos et al.[5] This scoring system allows a comparison of intermediate LDL patterns, not clearly identified as pattern A or pattern B. Larger scores indicate a smaller average diameter determined by gel electrophoresis. We also isolated LDL from the plasma of each patient and determined the concentration of each of its components. LDL was isolated by ultracentrifugation in a Beckman Optima TL Ultracentrifuge as previously described.[25] The isolated LDL was analyzed for free cholesterol, TC, protein, phospholipids, and TG as described earlier. The cholesterol ester concentration was calculated from TC and free cholesterol measurements. Particle diameter was determined using 2% to 16% polyacrylamide gradient gel electrophoresis (PAGGE) as described previously.[26,27]

Cholesterol esterification rates (a measure of LCAT activity) were estimated by the disappearance of free cholesterol from the plasma over a 4-hour incubation at 37°C.[28] CETP activity was measured by determining the rate of transfer of radiolabeled cholesteryl esters from exogenously prepared donor to acceptor lipoproteins during an incubation at 37°C.[29] Both donor and acceptor lipoproteins were prepared from pooled, blood-bank plasma. Donor preparations were labeled with ^{3}H-CE by endogenous LCAT during an incubation at 37°C in the presence of ^{3}H-cholesterol (Dupont NEN, Boston, MA). Apo A1 and apo B were determined by turbidometric immunoassays using commercial kits (Incstar, Stillwater, MN).

Statistical Analyses

Results are presented as the mean ± SD for the number of patients indicated. The results were analyzed by ANOVA (one-way ANOVA combined with the Fisher protected least-significant difference test for

determining differences between groups) using Super ANOVA software (Abacus Concepts, Berkeley, CA). P values less than .05 were accepted as significant.

RESULTS

Table 1 shows the physical and biochemical characteristics of the subjects. There were no statistically significant differences between the two groups of subjects in age, body weight, percent body fat, BMI, waist to hip ratio, or blood pressure. In addition, no difference in treadmill time to exhaustion, a measure of physical fitness, was observed between the two groups. Fasting plasma insulin and glucose concentrations were similar in the groups and were in the normal range, indicating the absence of diabetes. Estradiol levels were significantly higher in the HRT versus NO HRT group.

Table 2 shows the concentrations of plasma lipids and apoproteins and the activities of CETP and LCAT in the two groups. No statistical differences in plasma TC, TG, or LDL-cholesterol levels were observed between the two groups. However, HDL-cholesterol was significantly higher ($P < .05$) in the HRT group. The TC/HDL-cholesterol ratio was significantly lower in the HRT versus NO HRT group. Apo A-1 and the apo A-1/B ratio were significantly higher in the HRT group ($P < .05$). Accompanying these changes in HDL, LCAT activity was significantly higher ($P < .05$) in the HRT group, but CETP activity was not different in the two groups.

Table 3 shows the concentration of cholesterol in plasma LDL and in each LDL subfraction, LDL average size determined by NMR and by PAGGE, LDL score derived from the lipophore system, LDL particle concentration, and chemical composition of plasma LDL expressed as a percent of weight. No differences between the two groups were observed in any of the parameters measured. Furthermore, there were no differences in the relative concentration of cholesterol in each LDL subclass when expressed as a percent of cholesterol in the individual subfractions relative to LDL-cholesterol (data not shown).

Table 4 shows that HDL-cholesterol was 37% ($P < .05$) higher in the HRT group versus the nontreated group. No difference in HDL average particle diameter between the two groups was observed. The cholesterol content in H4 and H3 in the HRT group was higher than in the NO HRT group, but no statistical differences were found in the other classes of HDL. The five HDL subclasses measured by NMR can be related to the traditional subfraction designation of HDL_2 (the sum of H4 and H5) and HDL_3 (the sum of H1, H2, and H3). The data are presented in this traditional classification in Fig 1, which shows that HDL_2 was 78% ($P < .05$) higher and HDL_3 was 12% higher in the HRT group versus the NO HRT group.

Table 1. Physical and Biochemical Characteristics of the HRT and NO HRT Groups

Characteristic	NO HRT	HRT
No. of subjects	10	8
Age (yr)	56.3 ± 3.2	54.4 ± 3.39
Weight (kg)	66.8 ± 14	63.0 ± 5.09
Fat (%)	29.9 ± 6.32	26.7 ± 3.68
Fat mass (kg)	20.9 ± 9.5	17.1 ± 3.39
Lean mass (kg)	46.5 ± 6.3	45.9 ± 2.83
BMI (kg/m^2)	24.5 ± 3.8	23.6 ± 1.98
Treadmill time (s)	804 ± 133	888 ± 175
Waist to hip ratio	0.89 ± 0.09	0.86 ± 0.06
Blood pressure (diastolic/systolic)		
Sitting	71/111 ± 7.3/11	73/117 ± 5.1/12
Standing	68/109 ± 7.3/8.5	69/115 ± 6.2/12
Glucose (mmol/L)	5.72 ± 0.41	5.48 ± 0.62
Insulin (pmol/L)	63 ± 29	63.6 ± 17
Estradiol (pmol/L)	34.7 ± 24	151 ± 110*

NOTE. Values are the mean ± SD.
*Significantly different v NO HRT ($P < .05$).

Table 2. Enzyme Activity and Lipid and Apoprotein Concentrations in Plasma of the HRT and NO HRT Groups

Parameter	NO HRT (n = 10)	HRT (n = 8)
TC (mmol/L)	5.22 ± 1.14	5.40 ± 1.1
HDL-cholesterol (mmol/L)	1.14 ± 0.16	1.53 ± 0.31*
TG (mg/dL)	133 ± 63	122 ± 40
LDL-cholesterol (mmol/L)	3.65 ± 0.98	3.46 ± 0.82
TC/HDL	4.58 ± 1.45	3.53 ± 0.62*
Apo A-1 (mg/dL)	136 ± 35	166 ± 28*
Apo B (mg/dL)	102 ± 32	91 ± 23
Apo A-1/B ratio	1.33 ± 0.21	1.82 ± 0.59*
CETP (nmol/mL/h)	68 ± 10	65 ± 7.10
LCAT (nmol/mL/h)	12.9 ± 3.79	21.9 ± 4.67*

NOTE. Values are the mean ± SD.
*Significantly different v NO HRT ($P < .05$).

DISCUSSION

In addition to the known CHD risk factors such as age, diabetes, body fat distribution, smoking, high blood pressure, and gender, menopause increases CHD risk by adversely affecting plasma lipid concentrations. The question this study aimed to address was as follows: What effects would combined hormone replacement therapy have on plasma lipid and lipoprotein profiles independent of the other CHD risk factors? This was accomplished by comparing the effects of this regimen of

Table 3. Physical Characteristics, Chemical Composition, and Subpopulation Distribution of LDL in NO HRT and HRT Groups

Parameter	NO HRT (n = 10)	HRT (n = 8)
Physical characteristics		
LDL-cholesterol (mmol/L)	3.65 ± 0.98	3.46 ± 0.82
LDL size (nm, by NMR)	21.1 ± 0.32	21.3 ± 0.28
LDL particle concentration (nmol/L)	1,486 ± 525	1,330 ± 291
LDL score (Lipophore)	3.42 ± 0.32	3.24 ± 0.20
LDL diameter (PAGGE)	25.8 ± 0.73	25.7 ± 0.85
Chemical composition (%)		
Protein	23.4 ± 2.21	24.7 ± 6.51
Phospholipids	23.7 ± 4.11	23.3 ± 4.81
TG	7.7 ± 2.53	8.1 ± 2.83
Cholesterol esters	31.7 ± 8.54	32.3 ± 7.92
Free cholesterol	13.6 ± 6.32	11.5 ± 4.53
Cholesterol concentration in subfractions (mmol/L)		
IDL	0.18 ± 0.16	0.15 ± 0.14
L3	2.03 ± 0.92	2.29 ± 0.79
L2	0.88 ± 0.88	0.78 ± 0.23
L1	0.55 ± 0.54	0.24 ± 0.23

NOTE. Values are the mean ± SD.

Table 4. Physical and Chemical Characteristics of HDL of the HRT and NO HRT Groups

Parameter	NO HRT (n = 10)	HRT (n = 8)
HDL-cholesterol (mmol/L)	1.14 ± 0.16	1.53 ± 0.31*
HDL size (nm)	9.1 ± 0.32	9.3 ± 0.28
Subfraction distribution (mmol/L)		
H5	0.27 ± 0.13	0.34 ± 0.14
H4	0.11 ± 0.06	0.33 ± 0.17*
H3	0.25 ± 0.09	0.32 ± 0.14*
H2	0.32 ± 0.13	0.42 ± 0.23
H1	0.20 ± 0.09	0.11 ± 0.14

NOTE. Values are the mean ± SD.
*Significantly different *v* NO HRT ($P < .05$).

replacement therapy in two groups of subjects in which the contribution of other CHD risk factors is minimal. Since the two groups (Table 1) were not obese (BMI < 27) or diabetic (normal glucose and insulin values), had normal blood pressure, had a similar pattern of body fat distribution (similar waist to hip ratio), and were of comparable physical fitness (similar treadmill time to exhaustion), the changes in plasma lipids and lipoproteins observed in the present study most likely resulted from this regimen of hormone replacement therapy. Nevertheless, undetected differences between the two study groups may have magnified or diminished some of the differences observed in the present study.

The effects of estrogen-alone replacement therapy on plasma lipids are fairly well documented and include a decrease in plasma cholesterol and LDL-cholesterol and increases in HDL-cholesterol.[30,31] In addition to these positive influences, reports show a preponderance of small LDL particles and an increase in TG levels in patients receiving this form of therapy. Thus, estrogen replacement therapy leads to positive effects with regard to plasma cholesterol and HDL-cholesterol, but may also be associated with a detrimental effect with regard to the subpopulation distribution of plasma LDL.

Although the effects of the combined hormone replacement therapy on plasma lipid concentrations were reported to be similar to those observed with estrogen replacement, the magnitude of the changes was attenuated. However, the effects

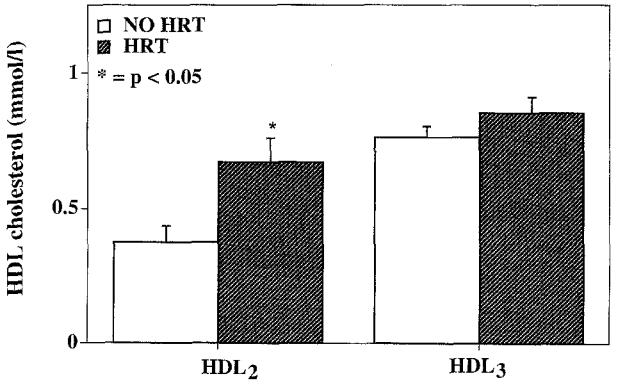

Fig 1. HDL subfractions in HRT (n = 8) and NO HRT (n = 10) groups. HDL_2, calculated as the sum of H4 and H5 of NMR profiling; HDL_3, calculated as the sum of H1, H2, and H3 of NMR profiling. Values are the mean ±SEM.

of this regimen of therapy on the subpopulation distribution of plasma lipoproteins are not clearly established. In patients treated with estrogen-progesterone for 12 months, van der Mooren et al[32] reported an increase in the small LDL subfraction when lipoprotein profiles were analyzed by gradient gel electrophoresis. In a prospective study, Tilly-Kiesi et al[19] treated patients with a regimen consisting of 28-day cycles with patches delivering 50 μg 17β-estradiol combined with oral cyclic medroxyprogesterone acetate 10 mg/d on days 17 to 28 (E2/MPA).[19] In E2/MPA-treated patients, no decrease in LDL-cholesterol was observed, although there was a 20% decrease in the cholesterol content of large LDL. Our results are consistent with their finding in that the LDL-cholesterol concentration did not change in our patients. However, we did not observe shifts in the subpopulation distribution of LDL even when LDL subpopulation distribution was analyzed by three different methods. The disparity between the two studies may be due to the following: (1) our study is a cross-sectional study in which both groups of patients were matched for various risk factors, whereas the Tilly-Kiesi study was prospective over a 12-month period; (2) there are differences in the treatment regimen and mode of administration of drugs between the two studies; and (3) there are differences in the methodology used for determination of LDL subpopulations.

The established effects of estrogen on blood lipids are an elevation in plasma HDL-cholesterol and a decrease in both TC and LDL-cholesterol levels. Similarly, the results of this study showed that the combined hormone replacement therapy was associated with an increase in plasma HDL-cholesterol greater than 34% ($P < .05$; Table 2). This is consistent with other reports wherein the increase in HDL was reported to be 5% to 26% with combined hormone replacement therapy. However, in one of the prospective studies, Omu and Al-Qattan[33] did not find any significant changes in HDL levels after 12 months of combined HRT. But when the results were analyzed in relation to BMI, subjects who had a BMI less than 26 had a modest (15%) increase in HDL levels after HRT. In the present study, the BMI of all subjects was less than 26 (Table 1). In addition to the increase in HDL-cholesterol concentration, we also observed a decrease in the TC/HDL-cholesterol ratio ($P < .05$), which resulted from a significant increase in HDL-cholesterol in treated patients. Accompanying these changes in HDL-cholesterol, there was a significant increase in apo A-1 and a concomitant increase in the apo A-1/apo B ratio (the atherogenic index). Similarly, there was a significant increase in LCAT activity that was indicated by a higher esterification rate of free cholesterol on HDL particles. This increase in the esterification rate was manifested by a concomitant increase in the more buoyant HDL_2 subfraction (Fig 1). This finding is in agreement with the prior observation by Walsh et al[34] of an increase in HDL_2 levels of 36% due to an increase in production rather than changes in catabolism after 6 weeks of an oral estradiol regimen. Thus, the increase in HDL-cholesterol concentration in HRT subjects along with the increase in the more antiatherogenic subfraction of HDL, namely HDL_2, may provide added protection against CHD in postmenopausal women on this regimen of therapy.

The results from this and other studies demonstrate that estrogen-progesterone replacement therapy in menopausal

women has favorable effects on lipid and lipoprotein profiles with respect to cardiovascular disease risk. Even though the beneficial effects of increasing HDL-cholesterol while decreasing TC and LDL-cholesterol are not as large as those observed with estrogen-alone therapy, the combined therapy does not adversely affect LDL subpopulation distribution in this small group of subjects. Furthermore, this is the first report that a combined hormone replacement therapy increased LCAT activity along with apo A-1 and HDL_2, indicating that the beneficial process of reverse cholesterol transport may be increased.

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REFERENCES

1. Campos H, McNamara JR, Wilson PW, et al: Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women. J Clin Endocrinol Metab 67:30-35, 1988

2. Jensen J, Nilas L, Christiansen C: Influence of menopause on serum lipids and lipoproteins. Maturitas 12:321-331, 1990

3. Rudel LL, Parks JS, Johnson FL, et al: Low density lipoproteins in atherosclerosis. J Lipid Res 27:465-474, 1986

4. Austin MA, Breslow JL, Hennekens CH, et al: Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 260:1917-1921, 1988

5. Campos H, Genest JJ Jr, Blijlevens E, et al: Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 12:187-195, 1992

6. Griffin BA, Freeman DJ, Taait GW, et al: Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: Relative contribution of small, dense LDL to coronary heart disease risk. Atherosclerosis 106:241-253, 1994

7. Stampfer MJ, Krauss RM, Ma J, et al: A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 276:882-888, 1996

8. Gardner CD, Fortmann SP, Krauss RM: Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 276:875-881, 1996

9. de Graaf J, Hak-Lemmers HLM, Hectors MPC, et al: Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler Thromb 11:298-306, 1991

10. Tribble DL, van den Berg JJ, Motchnik PA, et al: Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content. Proc Natl Acad Sci USA 91:1183-1187, 1994

11. Nordestgaard BG, Nielsen LB: Atherosclerosis and arterial influx of lipoproteins. Curr Opin Lipidol 4:252-257, 1994

12. Kinoshita M, Krul ES, Schonfeld G: Modification of the core lipids of low density lipoproteins produces selective alterations in the expression of apoB-100 epitopes. J Lipid Res 31:701-708, 1990

13. Kleinman Y, Schonfeld G, Gavish D, et al: Hypolipidemic therapy modulates expression of apolipoprotein B epitopes on low density lipoproteins. Studies in mild to moderate hypertriglyceridemic patients. J Lipid Res 28:540-548, 1987

14. Feingold KR, Grunfeld C, Pang M, et al: LDL subclass phenotypes and triglyceride metabolism in non–insulin-dependent diabetes. Arterioscler Thromb 12:1496-1502, 1992

15. Li Z, McNamara JR, Fruchart J-C, et al: Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. J Lipid Res 37:1886-1896, 1996

16. Barrett-Connor E, Miller V: Estrogens, lipids, and heart disease. Clin Geriatr Med 9:57-67, 1993

17. Walsh BW, Schiff I, Rosner B, et al: Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 325:1196-1204, 1991

18. Rajman I, Lip GY, Cramb R, et al: Adverse changes in low-density lipoprotein subfractions profile with oestrogen-only hormone replacement therapy. Q J Med 89:771-778, 1996

19. Tilly-Kiesi M, Puolakka LJ, Luotola H, et al: Different effects of continuous oestrogen-progestin and transdermal oestrogen with cyclic progestin regimens on low-density lipoprotein subclasses. Eur J Clin Invest 26:1125-1133, 1996

20. Wilmore JA, Vodak P, Parr R, et al: Further simplification of a method for determination of residual lung volume. Med Sci Sports Exerc 12:216-218, 1980

21. Siri WE: Body composition from fluid spaces, in Brozek J, Henschel A (eds): Techniques for Measuring Body Composition. Washington, DC, National Academy of Science, 1961, pp 223-244

22. Houmard JA, Wheeler WS, McCammon MR, et al: An evaluation of waist to hip ratio measurement methods in relation to lipid and carbohydrate metabolism in men. Int J Obes 15:181-188, 1990

23. Shireman RB, Durieux J: Microplate methods for determination of serum cholesterol, high density lipoprotein cholesterol, triglyceride and apolipoproteins. Lipids 28:151-155, 1993

24. Otvos JD: Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy, in Handbook of Lipoprotein Testing. Washington, DC, AACC, 1997, pp 497-508

25. Tilly-Kiesi M, Knudsen P, Groop L, et al: Hyperinsulinemia and insulin resistance are associated with multiple abnormalities of lipoprotein subclasses in glucose-tolerant relatives of NIDDM patients. Botnia Study Group. J Lipid Res 37:1569-1578, 1996

26. Peeples LH, Carpenter JW, Israel RG, et al: Alterations in low density lipoproteins in subjects with abdominal adiposity. Metabolism 38:1029-1036, 1989

27. Barakat HA, Carpenter JW, McLendon VD, et al: Influence of obesity, impaired glucose tolerance, and NIDDM on LDL structure and composition. Diabetes 39:1527-1533, 1990

28. Tall A, Granot E, Brocia R, et al: Accelerated transfer of cholesteryl esters in dyslipidemic plasma. Role of cholesteryl ester transfer protein. J Clin Invest 79:1217-1225, 1987

29. Tollefson JH, Albers JJ: Isolation, characterization, and assay of lipid transfer proteins. Methods Enzymol 129:797-816, 1986

30. The Writing Group for the PEPI Trial: Effects of estrogen or estrogen-progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial period. JAMA 272:199-208, 1995

31. Moorjani S, Dupont A, Labrie F, et al: Changes in plasma lipoprotein and apolipoprotein composition in relation to oral versus percutaneous administration of estrogen alone or in cyclic association with utrogestan in menopausal women. J Clin Endocrinol Metab 73:373-379, 1991

32. van der Mooren MJ, de Graaf J, Demacker PNM, et al: Changes in the low-density lipoprotein profile during 17β-estradiol-dydrogesterone therapy in postmenopausal women. Metabolism 43:799-802, 1994

33. Omu AE, Al-Qattan N: Effect of postmenopausal estrogen replacement therapy on lipoproteins. Int J Gynecol Obstet 52:155-161, 1996

34. Walsh BW, Li H, Sacks FM: Effects of postmenopausal hormone replacement with oral and transdermal estrogen on high density lipoprotein metabolism. J Lipid Res 35:2083-2093, 1994