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Effects of menopause, gender and age on lipids and high-density lipoprotein cholesterol subfractions

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ABSTRACT

Objective: To distinguish the effects of menopause, gender and age on serum lipid risk markers for vascular disease, including high-density lipoprotein cholesterol (HDL-C) subfractions 2 and 3 (HDL_2-C and HDL_3-C). Q2

Methods: We undertook a cross-sectional database analysis of apparently healthy Caucasian pre- and postmenopausal women and men ($n = 515$, 518 and 800, respectively) not taking drugs affecting lipid metabolism (including contraceptive or post-menopausal steroids). Measurements of serum total cholesterol (TC), low-density lipoprotein (LDL-C), triglycerides (TG), HDL-C, HDL_2-C, HDL_3-C and non-HDL-C concentrations and the TC/HDL-C concentration ratio were considered.

Results: Men had lower TC than postmenopausal women ($p < 0.001$) and similar LDL-C. Compared with premenopausal women, postmenopausal women had a more atherogenic lipid profile with lower HDL_2-C (median 0.67 vs 0.60 mmol/L, $p < 0.001$) but no difference in HDL_3-C (0.96 vs 0.96 mmol/L, $p = 0.8$). Compared with either pre or postmenopausal women, men had a more atherogenic profile with lower HDL_2-C (0.36 mmol/L) and HDL_3-C (0.91 mmol/L, all $p < 0.001$). With standardization for confounding variables, including standardization to age of menopause (50 years), differences apparent in the non-standardized comparisons were generally sustained, although HDL_3-C levels were lower at menopause, HDL_2-C ceased to differ and LDL-C was lower in postmenopausal women than men.

Conclusions: Male gender is associated with a more atherogenic profile than female gender, with appreciably lower levels of the HDL_2-C subfraction. Among women, menopause is associated with a more atherogenic lipid profile, but has less effect than male gender.

1. Introduction

Q3 Growing recognition over past decades of the importance of cardiovascular disease [CVD] in women [1] has highlighted the impact of gender differences in CVD risk factors and led investigators to question whether loss of estrogen at menopause confers on women a male risk factor profile. An early analysis of data from the Framingham study showed a lower incidence of CVD events among premenopausal compared with age-matched postmenopausal women, which could not be attributed to the effect of menopause on CVD risk factors that included body weight and serum cholesterol [2]. More recent studies have shown that adverse effects of loss of ovarian function on CVD risk are particularly apparent in women who have undergone premature or surgical menopause [3,4]. However, in those studies that found higher CVD risk in postmenopausal women, it is not clear if higher risk after transition to menopause is a result of aging, estrogen deficiency or both [5].

An adverse effect of menopause on CVD risk is suggested by reports of a worsening of the lipid profile in the transition to postmenopausal status [6,7], with increases in total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C] and triglycerides [TG] and a net reduction in high-density lipoprotein cholesterol [HDL-C], with possible shifts in the distribution of cholesterol in HDL toward higher HDL subfraction 3 cholesterol [HDL_3-C] and lower HDL subfraction 2 cholesterol [HDL_2-C]. Menopause may, therefore,

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be associated with a lipid and lipoprotein profile that more closely resembles that in men, but few studies have distinguished the effects of menopause and gender from those of aging, particularly with regard to cholesterol concentrations in the HDL subfractions.

In 1993 we published an analysis of the effects of menopause on lipids and lipoproteins in a cross section of 542 apparently healthy pre- postmenopausal women not taking estrogens or other steroid hormones [6]. Recruitment into subsequent studies has increased these numbers to 1033, and we present here an updated analysis that incorporates these larger numbers, as well as data from 800 men with ages spanning both the pre- and postmenopausal female age ranges.

2. Materials and methods

2.1. Study design and subjects

This is a cross-sectional analysis of data from healthy Caucasian pre- and postmenopausal women who had volunteered for studies of oral contraceptives (OC) or hormone replacement therapies (HRT) (e.g. Refs. [8,9]) but who were not taking sex steroids at the time of the measurements included in this analysis. Also included are data from Caucasian men aged between 25 and 78, who were participating in an occupational cohort follow-up study (e.g. Ref. [10]). The lipid and lipoprotein profiles included in the present analysis were determined in the same laboratory, being recorded for the premenopausal women between 1984 and 1991, for the postmenopausal women between 1987 and 1998 and for the men, between 1971 and 1998. Measurements for the premenopausal women were made between days 21 and 27 of the menstrual cycle. Menopause was defined as amenorrhea for at least 12 months and elevated gonadotrophin levels (FSH > 40 IU/L). The studies from which the data derive received ethics committee approval and full informed consent was obtained from each participant.

All blood samples were obtained after a 12-h overnight fast. A venous blood sample was placed into plain tubes, allowed to clot and stored at 4 °C for assessment within 4 days. Age, height, weight and time since menopause were recorded, as well as, number of previous pregnancies, smoking status (non-smoker, <15 cigarettes/day, ≥15 cigarettes/day), alcohol consumption (non-drinker, ≤28 units/week, >28 units/week) and exercise habits (none, non-aerobic, aerobic). Body mass index (BMI) was calculated as weight (kg)/height^2 (m).

2.2. Laboratory assessments

Serum TC and TG concentrations were measured by fully enzymatic procedures. HDL-C and HDL_3-C concentrations were measured until 1979 by ultracentrifugation then in supernatants after sequential precipitation of apoB-containing lipoproteins with heparin and manganese ions [11] and cholesterol-rich HDL with dextran sulphate [12], respectively. HDL_2-C was calculated by subtracting the HDL_3-C from the HDL-C concentration. Ultracentrifugation and precipitation measurements agreed very closely [13]. LDL-C was calculated by the Friedewald equation [14]. Between-batch coefficients of variation (CV) were typically 1–2% for TC and TG, 2–4% for HDL-C, 8% for HDL_3-C and 11% for HDL_2-C. Quality control of the assays was monitored according to both internal and external quality assessment schemes throughout the periods of data collection and careful attention was given to ensuring consistency of measurements, with extensive comparisons being undertaken on the few occasions when methods were modified in any way.

2.3. Statistical analysis

Data were excluded from the present analysis if participants were: (1) taking lipid, blood pressure or uric acid-lowering medications; (2) had used any OC or HRT regimen in the preceding 3 months; (3) were taking glucose-lowering medication or had a fasting plasma glucose concentration of ≥7 mmol/L; (4) had evidence of endocrine dysfunction; or (5) had evidence of CVD. Non-HDL cholesterol was calculated as the difference between TC and HDL-C and the TC/HDL-C ratio was derived.

For the present analysis, statistical analyses were carried out in STATA (STATA 8.0, College Station, TX, USA). Group characteristics were summarized as means and standard deviations (SD) for normally distributed continuous variables, medians and interquartile ranges for non-normally distributed continuous variables and numbers and percentages for categorical variables. Significant variation between the three groups was explored using ANOVA, Kruskal–Wallis ANOVA or chi-square test, as appropriate. The analytical design we used followed that used in our previous 1993 analysis [6]. Accordingly, in a first analysis designed to illustrate the observed variation in lipid and lipoprotein concentrations with age, means and 95 percent confidence intervals for each lipid variable in 5-year age ranges were derived and plotted against age range in each of the three groups. Multiple linear regression analyses were then undertaken in each group separately with each lipid variable predicted by age, BMI, smoking <15 or ≥15 cigarettes per day, drinking ≤28 or >28 units of alcohol per week, taking non-aerobic or aerobic exercise and (for pre- and postmenopausal women) number of pregnancies. Coefficients were compared between groups to identify significant differences between groups in variation of each lipid variable with each demographic variable. Using coefficients from the multiple linear regression analyses in each group, data were then standardized to a nominal age of menopause of 50 years and a BMI of 25 kg/m^2, which was representative of the study group as a whole. Data were also standardized to zero smoking, alcohol, exercise and pregnancies. Missing values for demographic variables were imputed (STATA command: 'impute' – utilizing best subset regression). Statistically significant differences in lipid variables between the three groups were then evaluated using the standardized data.

3. Results

After exclusions, there were available for analysis: 515 premenopausal women (mean age 32.4, SD ± 6.4 years), 518 postmenopausal women (56.0 ± 6 years, mean age at menopause 48.9 years) and 800 men (46.6 ± 8.4 years). Demographic data for the three groups are shown in Table 1. Groups differed extensively in demographic characteristics, with BMI being lowest in premenopausal women and highest in men, and with premenopausal women and men generally smoking, drinking and exercising more than postmenopausal women, but with an appreciably higher proportion of heavy drinkers among the men. Compared with pre-, postmenopausal women had higher TC, TG, LDL-C, non-HDL-C levels and TC/HDL-C ratio, but lower HDL-C and HDL_2-C and no difference in HDL_3-C levels (Table 1). Compared with men, postmenopausal women had higher TC, HDL-C and HDL-C in both subfractions (more markedly for HDL_2-C) and lower TG concentrations and TC/HDL-C ratio. LDL-C and non-HDL-C did not differ between men and postmenopausal women. Compared with men, premenopausal women had lower levels of TC, TG, LDL-C non-HDL-C and TC/HDL-C ratio and higher HDL, HDL_2-C and HDL_3-C.

The age-related variation underlying these differences in group characteristics is illustrated in Figs. 1–3. Marked differences were

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