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Sex differences in the relation of HDL cholesterol to progression of carotid intima-media thickness: The Los Angeles Atherosclerosis Study

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Abstract

Epidemiologic studies have revealed that the protective association of high-density lipoprotein cholesterol (HDL-C) with CHD is stronger in older men and younger women. We aimed to investigate sex differences in the relation of HDL-C to progression of carotid intima-media thickness (IMT) (an indicator of subclinical atherosclerosis) in middle age. IMT progression and serum HDL-C were determined for a cohort of 500 women and men aged 40–60 years over three examinations (1.5-year intervals). IMT at baseline was inversely associated with serum levels of HDL-C and the associations were comparable in women and men. However, in multivariate longitudinal growth models adjusting for potential confounders, IMT progression was inversely associated with serum levels of HDL-C in men, but directly associated in women ($p=0.0007$ for interaction). Our results suggest that although HDL-C was protective against progression of carotid atherosclerosis in middle-aged men, anti-atherogenic effects of HDL may diminish in women around the age of menopause.

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Keywords: Atherosclerosis; HDL; Intima-media thickness; Men; Middle age; Women

1. Introduction

Blood concentration of high-density lipoprotein cholesterol (HDL-C) has been identified as an inverse and apparently independent predictor of CHD and ischemic stroke [1,2]. Extent of human atherosclerosis also shows an inverse association with HDL-C [3]. Atherosclerosis is substantially reduced in susceptible mice crossed with transgenic mice characterized by elevated HDL-C [4].

However, there are a number of observations that raise questions about the role of HDL levels in the pathogenesis of atherosclerosis and atherothrombotic events [5–8]. Some interventions that increase HDL-C may have adverse effects on the risk of atherothrombotic events among women [9].

Thus, the role of HDL in the pathogenesis of atherosclerosis requires further investigation.

In large prospective studies, carotid intima-media thickness (IMT) is a strong predictor of cardiovascular disease events [10]. We thus examined the relation of HDL-C to progression of IMT in middle-aged women and men in randomly sampled cohort using an ultrasound protocol with high reproducibility [11,12].

2. Methods

2.1. Subjects

Participants were members of a cohort established in the Los Angeles Atherosclerosis Study (LAAS), which began in 1995. The cohort consists of women (aged 45–60 years) and men (aged 40–60 years) ($n=573$) with no history of cardiovascular heart disease (CHD) or stroke at

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baseline. Fully informed consent was obtained from all participants. The study protocol was approved by the Institutional Review Board of the Keck School of Medicine at University of Southern California. Three examinations were completed at 1.5-year intervals. A total of 500 men ($n = 268$) and women ($n = 232$) completed at least one follow-up examination and were included in the present study. Those with incomplete follow-up examination did not differ significantly on baseline variables except for older age.

Baseline demographic, behavioral, and physiological characteristics of cohort participants are shown in [Table S1](#) (<http://www.sciencedirect.com>). Men had higher body mass index (BMI) and were more frequently involved in vigorous exercise than women. Men were also more likely to be current smokers, alcohol consumers and users of lipid lowering medications in comparison with women. Men had higher levels of total serum cholesterol, LDL-C, triglycerides, and blood pressure and lower levels of HDL-C than women. About 72% of women had undergone menopause, 47% were taking hormone replacement therapy, 76% were current or former oral contraceptive users, and 35% had a history of hysterectomy.

2.2. Serum lipoprotein profile

Fasting blood was processed immediately after collection and frozen at -80°C . Total serum cholesterol, HDL-C and triglycerides were measured by autoanalyzer with the Roche direct HDL-cholesterol method which meets the 1998 NIH/NCEP guidelines [13]. Low-density lipoprotein cholesterol (LDL-C) was estimated for fasting samples only (>8 h) [14]. Non-HDL cholesterol was calculated as total serum cholesterol minus HDL-C.

2.3. Carotid IMT measurement

Procedures for image acquisition and processing and reproducibility of intima-media thickness (IMT) measurements have been reported previously [12]. Briefly, IMT was assessed using high-resolution B-mode ultrasound (ATL scanner, model UM4+, with 7.5 MHz linear transducer). IMT was measured at the posterior wall and averaged over the 1 cm segment of the common carotid 0.25 cm proximal to dilatation of the carotid bulb. Participants were scanned in two body positions (supine and lateral) and two sides (right and left), with two video frames processed at each measurement (total of eight frames). IMT was observed at three examinations with an approximate 1.5-year interval between examinations. The intra-observer coefficient of variation (CV) for IMT is 4.2%. The IMT protocol in LAAS reduces reproducibility error by more than 50% relative to several protocols used in other major studies [12]. A single sonographer and a single ultrasound image analyst were used throughout the study to avoid inter-observer variation.

2.4. Statistical analysis

The participants were grouped into sex-specific quintiles based on the distribution of average HDL-C over the three examinations. Individual linear growth lines were fitted using the MIXED procedure in SAS. Initial level of IMT and time to examination (0, 1.5 and 3 years) were specified as random effects, and initial IMT and IMT progression rate were allowed to covary [15]. The between-examination covariance matrix of the individual level residuals was unconstrained. Covariate-adjusted means were estimated at sex-specific means of covariates. Cross-sectional associations with initial (baseline) IMT were estimated as main effects, while associations between risk factors and IMT progression were estimated as interactions between factors and time of examination. Parameter estimates were obtained by maximum likelihood estimation methods. Tests for linear trend were estimated using the ordinal quintile variable as a continuous variable.

Three sets of potential confounding variables were included in the growth models. Model 1 was unadjusted. Model 2 was adjusted for age, race/ethnicity and body height. Model 3 was further adjusted for body mass index (BMI, kg/m^2), smoking status (current/former/never), the proportion of energy from carbohydrates, diabetes (type 1 or 2), systolic blood pressure, pulse pressure, seated heart rate, serum non-HDL cholesterol, and serum triglycerides. Among women, Model 4 was further adjusted for menopausal status, current HRT use and current or past contraceptive use. However, only significant confounders were entered in the models (see [supplementary data](#) at <http://www.sciencedirect.com>). Repeated measurements of covariates were averaged to reduce intra-individual variation.

The correlation between temporal changes (Exam 1–Exam 3) of the selected cardiovascular risk factors and HDL-C was analyzed for men and women. To further disentangle the confounder effects, the association of other risk factors (average over three exams) with HDL-C quintiles was analyzed with general linear modeling procedure (see [supplementary data](#) at <http://www.sciencedirect.com>).

To illustrate the statistical implication of the relation of HDL with IMT progression, ordinary least squares regression was conducted. IMT from examinations at baseline (Exam 1), at 3-year follow-up (Exam 3), and 3-year change in IMT (ΔIMT) were regressed on an average HDL-C from Exams 1, 2 and 3. The regression models (the intercepts are ignored since the dependent variables were already residualized by adjustment for covariates) are expressed as

- (1) $\text{IMT}_a(\text{exam } 1) = b_1 \text{ HDL-C}$,
- (2) $\text{IMT}_a(\text{exam } 3) = b_2 \text{ HDL-C}$,
- (3) $\Delta\text{IMT} = b_3 \text{ HDL-C}$

The rationale for this analysis is as following. If the slope was steeper for Exam 3 than for Exam 1 ($|b_2| > |b_1|$), change of IMT ($\Delta\text{IMT} = \text{IMT at Exam } 3 - \text{IMT at Exam } 1$) is negatively related to HDL-C, i.e., b_3 would be negative;

conversely, if the slope was flatter for Exam 3 than for Exam 1 ($|b_2| < |b_1|$), change of IMT ($\Delta\text{IMT} = \text{IMT at Exam 3} - \text{IMT at Exam 1}$) is positively related to HDL-C, i.e., b_3 would be positive.

All statistical analyses were performed with SAS (version 8.02, SAS Institute Inc., Cary, NC). All reported p -values are two-tailed with significance defined as $p < 0.05$.

3. Results

Baseline IMT was significantly greater among men than among women. The mean IMT and 95% CI were 687.2 (674.7, 699.7) μm in men versus 643.0 (631.5, 654.4) μm in women (p for sex difference < 0.0001 ; adjusted to age 50 years). However, the progression of IMT evaluated by change in IMT per year over 3 years was not significantly different between women and men. The age-adjusted mean annual IMT progression and 95% CI were 9.7 (7.7, 11.8) $\mu\text{m}/\text{year}$ for women and 10.5 (8.5, 12.5) $\mu\text{m}/\text{year}$ for men ($p = 0.34$ for sex difference).

Sex-specific unadjusted and adjusted baseline IMT and IMT progression by quintiles of HDL-C were estimated from the mixed models (Table 1). For baseline IMT, an inverse relation between IMT and HDL-C was apparent across HDL-C quintiles for both sexes without adjustment for potential confounders. However, these inverse trends were largely explained by covariates in Models 3 and 4, and no interaction with sex was observed (p for sex interaction in Model 3 = 0.72).

In contrast, marked sex differences in the relation between IMT progression and HDL-C were observed (p for sex interaction < 0.001). IMT progression in men was inversely associated with HDL-C in all unadjusted and adjusted models ($p = 0.02$). Linear contrast tests indicated that significantly faster progression of IMT was observed in men from the lower two HDL quintiles (< 1.07 mmol/L) than men from higher quintiles ($p = 0.0005$). In contrast, a direct association between HDL-C and IMT progression was observed among women in Models 3 and 4. Linear contrast analysis revealed a significantly slower progression of IMT in women from the lower two HDL-C quintiles (< 1.37 mmol/L) than women from higher quintiles ($p = 0.001$).

Table 1
Baseline IMT and IMT progression according to quintiles of HDL-cholesterol^a

	Quintiles of HDL-cholesterol					p for linear trend
	1 (lowest)	2	3	4	5 (highest)	
Men ($n = 268$)						
Median of HDL-C (range) (mmol/L)	0.86 (0.59–0.95)	1.03 (0.95–1.07)	1.12 (1.07–1.19)	1.25 (1.19–1.32)	1.42 (1.32–2.15)	
n	55	52	53	54	54	
Baseline IMT (μm)						
Model 1 ^b	685.39 \pm 13.41	690.34 \pm 14.01	670.39 \pm 14.01	697.21 \pm 14.54	639.15 \pm 15.15	0.059
Model 2 ^c	682.95 \pm 14.33	686.46 \pm 14.94	675.51 \pm 14.29	686.55 \pm 14.96	638.03 \pm 15.56	0.035
Model 3 ^d	683.42 \pm 12.55	681.40 \pm 12.09	690.49 \pm 12.08	681.82 \pm 11.89	676.10 \pm 12.15	0.71
IMT progression ($\mu\text{m}/\text{year}$)						
Model 1 ^b	10.96 \pm 2.14	14.99 \pm 2.32	10.05 \pm 2.26	11.56 \pm 2.35	4.42 \pm 2.42	0.024
Model 2 ^c	11.18 \pm 2.45	15.13 \pm 2.66	10.11 \pm 2.46	11.36 \pm 2.59	4.19 \pm 2.65	0.015
Model 3 ^d	12.19 \pm 2.33	14.03 \pm 2.34	9.92 \pm 2.29	10.18 \pm 2.24	5.47 \pm 2.26	0.018
Women ($n = 229$)						
Median of HDL-C (range) (mmol/L)	1.09 (0.75–1.19)	1.31 (1.19–1.37)	1.47 (1.37–1.55)	1.66 (1.55–1.83)	1.96 (1.83–3.43)	
n	46	46	45	47	45	
Baseline IMT (μm)						
Model 1 ^b	679.20 \pm 11.92	668.78 \pm 12.28	637.51 \pm 12.69	639.17 \pm 13.13	622.90 \pm 12.98	0.0004
Model 2 ^c	669.42 \pm 12.84	664.56 \pm 12.83	625.68 \pm 13.60	627.47 \pm 13.97	607.88 \pm 14.10	< 0.0001
Model 3 ^d	641.91 \pm 10.74	670.41 \pm 10.25	641.54 \pm 10.08	635.87 \pm 10.00	638.42 \pm 10.79	0.25
Model 4 ^e	642.06 \pm 11.04	671.84 \pm 10.21	642.47 \pm 10.21	636.35 \pm 10.04	639.20 \pm 11.00	0.23
IMT progression ($\mu\text{m}/\text{year}$)						
Model 1 ^b	9.11 \pm 2.21	6.63 \pm 2.32	11.33 \pm 2.44	12.81 \pm 2.47	9.36 \pm 2.46	0.37
Model 2 ^c	7.82 \pm 2.26	8.11 \pm 2.31	10.54 \pm 2.47	12.01 \pm 2.50	8.54 \pm 2.49	0.47
Model 3 ^d	4.49 \pm 2.34	5.75 \pm 2.31	9.69 \pm 2.29	15.42 \pm 2.27	11.86 \pm 2.42	0.0027
Model 4 ^e	3.73 \pm 2.37	5.56 \pm 2.28	9.98 \pm 2.27	15.56 \pm 2.23	12.08 \pm 2.43	0.0020

The Los Angeles Atherosclerosis Study, 1995–1999.

^a Results are expressed as covariate-adjusted means \pm standard error stratified by quintiles of HDL-C.

^b Model 1 was not adjusted.

^c Model 2 was adjusted for demographic variables (age, ethnicity) and body height.

^d Model 3 was further adjusted for BMI, smoking status, diabetic status, pulse pressure, SBP, LDL-C, and the interaction of SBP with LDL-C.

^e Model 4 for women was further adjusted for menopausal status, current HRT use and current or past contraceptive use.

Table 2
Regression of IMT and change in IMT on HDL-cholesterol^a

Dependent variables	Men			Women		
	β (S.E.)	STB ^b	<i>p</i>	β (S.E.)	STB ^b	<i>p</i>
(1) IMT at exam 1	-1.03 (0.68)	-0.10	0.13	-0.67 (0.35)	-0.13	0.06
(2) IMT at exam 3	-2.09 (0.85)	-0.17	0.02	-0.12 (0.43)	-0.02	0.78
(3) Δ IMT	-1.20 (0.43)	-0.18	0.006	0.69 (0.29)	0.16	0.02

The Los Angeles Atherosclerosis Study, 1995–1999.

^a The level of HDL-cholesterol is an average of repeated measurements from three examinations. The regression analysis was performed with adjustment for covariates.

^b STB, standardized regression weight; IMT, intima-media thickness; HDL, high-density lipoprotein.

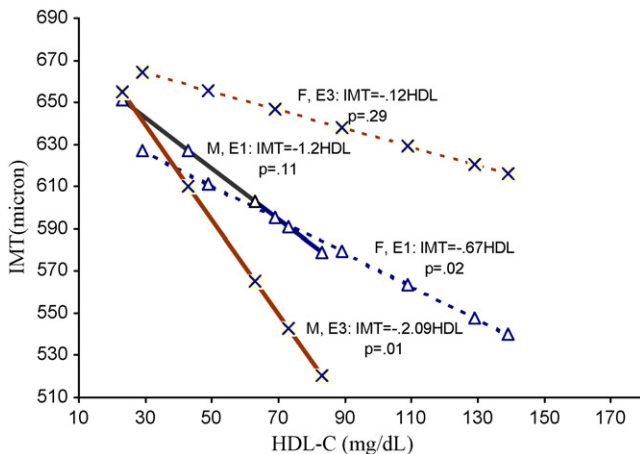


Fig. 1. Ordinal regression models were conducted for IMT from Exams 1 and 3 regressed on an average HDL-C from Exams 1, 2 and 3. The dependent variables were residualized by adjustment for covariates. The regression equations shown above the lines ignored intercepts. The slope and its significance are shown. For men, the slope became steeper from Exam 1 to Exam 3; for women, the slope became flatter. M = men, F = women, E1 = Exam 1, E3 = Exam 3.

To further investigate the sex interaction for the relation of IMT progression with HDL-C, IMT from Exams 1 and 3, and 3-year changes in IMT (Δ IMT) were regressed on the average HDL-C. In Table 2 and Fig. 1, the magnitude of the HDL-IMT inverse association increased over 3 years among men, while it diminished among women. Thus the temporal alteration of association magnitude resulted in the sex difference observed in the relation of HDL – Δ IMT progression (positive among women whereas negative among men). The correlation between temporal changes (over 3 years) of the

selected cardiovascular risk factors and HDL-C was shown for men and women in Table 3. Increase in HDL-C was weakly yet directly associated with increase in IMT and IMT at Exam 3 among women although change in HDL-C was inversely associated with change in BMI and LDL-C among women. In a separate analysis, hormone replacement therapy (HRT) use among women was significantly associated with higher HDL-C quintile levels ($X^2 = 16.08$, $p = 0.003$).

Although there was a positive association between HDL-C levels and IMT progression among women in multivariate models (Table 1, Models 3 and 4), this pattern was not apparent in models unadjusted for potential confounders (Table 1, Models 1 and 2). The association of other risk factors (potential confounders) and HDL quintiles were investigated for the potential reason of significance conversion. Table S2 (in supplementary data) shows that high HDL-C women possessed favorable risk factor profiles, including lower levels of BMI, sagittal-transverse abdominal diameter ratio, LDL-C, triglycerides, fasting insulin, fasting glucose, SBP and DBP, and higher levels of alcohol intake.

4. Discussion

This study shows that the protective effects of HDL on IMT progression diminished over time in middle-aged women but enhanced in middle-aged men (p for sex interaction <0.001). A pattern of enhanced inverse association between HDL-C and CHD risk with age among men has been reported previously. A protective association was observed for men over 50 years of age, but not for younger men in the Israeli Ischemic Heart Disease Study [7]. Prevention trials

Table 3
Correlation matrix for temporal changes of HDL, IMT and other selected risk factors among middle-aged men and women^a

	Δ HDL	Δ BMI	Δ LDL	Δ SBP	Δ IMT	IMT3
Δ HDL	1	-0.0071 (0.91 ^b)	-0.091 (0.21)	0.138 (0.04)	-0.0097 (0.88)	-0.055 (0.40)
Δ BMI	-0.185 (0.008)	1	0.184 (0.01)	0.175 (0.002)	0.031 (0.627)	0.054 (0.40)
Δ LDL	-0.270 (0.0002)	0.067 (0.37)	1	0.079 (0.28)	0.017 (0.81)	-0.271 (0.0002)
Δ SBP	0.061 (0.39)	0.157 (0.036)	-0.041 (0.58)	1	-0.058 (0.37)	0.063 (0.33)
Δ IMT	0.106 (0.13)	-0.040 (0.59)	-0.074 (0.32)	-0.106 (0.15)	1	0.581 (0.0001)
IMT3	0.117 (0.10)	-0.102 (0.15)	-0.05 (0.50)	0.011 (0.88)	0.559 (<0.0001)	1

The Los Angeles Atherosclerosis Study, 1995–1999.

^a The change score was the difference between the first (exam 1) and last examination (exam 3). The correlations for women are shown in the lower diagonal.

^b p -Values are shown in parentheses.

indicated a correlation between increased HDL-C levels and a reduced incidence of CHD in men [16,17]. Almost all available risk assessment tools including National Cholesterol Education Program Adult Treatment Panel III risk assessment assume high HDL-C as a favorable factor to reduce cardiovascular risk [18]. Although this may be true cross-sectionally, the protection of a higher level of HDL-C may diminish over time among women.

The pathophysiology underlying the declined HDL protection among middle-aged women is not known, and examining this mechanism is far beyond the scope of the present study. However, biochemical and animal studies may provide some clues for further investigation to disentangle the puzzle.

HDL particles are highly heterogeneous as well as functionally diverse [5,19–21]. The anti-atherogenicity of HDL is affected by both genetic-determined composition and environmental factors. For example, overexpression of apolipoprotein A-II in transgenic mice leads to elevated HDL-C but increased atherosclerosis [22]. HDL in mice susceptible to diet-induced atherosclerosis can become dysfunctional in preventing the formation or inactivating oxidized phospholipids by feeding an atherogenic diet [23]. During an acute-phase response, HDL may be converted from anti-inflammatory to pro-inflammatory particles [5]. One clinical study found that HDL from patients with established coronary disease exhibited pro-inflammatory properties *in vitro* [5].

Both HDL concentration and the dynamics of cholesterol transport through HDL can influence the anti-atherogenic effects of the HDL fraction [24]. Deficiency in several HDL-associated enzymes can result in increased atherosclerosis independent of total HDL levels [25–27]. Therefore, it is speculated that the altered composition or functionality of HDL in middle-age women may contribute to the dissociation of HDL with IMT in this population.

Furthermore, it has been proposed that the mechanism by which HDL is raised may determine the impact of the lipoprotein on atherosclerosis [25,28]. For example, hormone replacement therapy (HRT) raises HDL-C but may have an adverse effect on cardiovascular outcomes [9]. In fact, HRT does not seem to protect women with surgical menopause from IMT progression [29], although some researchers argued that estrogen-induced increasing HDL cholesterol explains the beneficial effect of estrogen therapy on the progression of carotid IMT in postmenopausal women [30]. More basic studies need to address whether HRT-derived HDL has diminished anti-atherogenic effects.

Although there was a positive association between HDL-C levels and IMT progression among women in multivariate models (Table 1, Models 3 and 4), this pattern was not apparent in models unadjusted for potential confounders (Table 1, Models 1 and 2). The true association with HDL-C was masked by the fact that women with high HDL-C tend to have favorable atherosclerotic profiles (Table S2). Thus, relations

between HDL-C and IMT progression become obvious only with careful control of confounding factors.

In addition, despite the correlation between IMT progression and cardiovascular events, it is not possible to extrapolate from the findings of this study to clinical events.

This cohort study illustrated how to interpret correctly the relationship between a predictor and the progression of a continuous outcome. The positive association between HDL-C and IMT progression in women may not necessarily indicate that HDL-C has already become directly associated with higher IMT in a cross-sectional view. Rather, it indicates a diminished inverse association between HDL-C and IMT as illustrated by Fig. 1 and Table 2. It also act as a dynamic indicator that those persons who were previously in a lower risk spectrum have begun to move toward a higher risk spectrum. As noted by other studies [31], the use of change in outcome over time as the sole outcome without considering cross-sectional relationships in an epidemiological study may result in spurious conclusions if not interpreted properly.

In summary, we examined the association of HDL-C with carotid IMT among middle-aged adults who were free of symptomatic cardiovascular disease at baseline. A significant sex interaction in the association between HDL-C and IMT progression was observed. The mechanism for the apparent weakening of HDL protection against atherosclerosis in women needs further investigation, especially any changes in the inflammatory and cholesterol reverse-transport properties of HDL that occur in middle age and perimenopause.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2007.03.045.

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