

# Hormone Therapy, Lipoprotein Subclasses, and Coronary Calcification

## *The Healthy Women Study*

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**Background:** The Women's Health Initiative (WHI) clinical trial found no reduction in coronary heart disease events among hormone therapy (HT) users despite an improvement in lipid levels. We hypothesized that a lack of benefit of HT on atherosclerosis would be better explained by the lipoprotein subclasses than by standard lipid levels. To test this hypothesis, we evaluated differences in coronary calcification, lipids, and lipoprotein subclasses among HT users and nonusers in a longitudinal study of the menopause.

**Methods:** Lipoprotein subclasses determined by nuclear magnetic resonance spectroscopy and coronary artery calcification (CAC) determined by electron beam computed tomography were compared between HT users (49%) and nonusers among 243 women, approximately 8 years postmenopausal, from the Healthy Women Study.

**Results:** The distribution of CAC scores was not significantly different between HT users and nonusers. As

expected, HT users had higher levels of large high-density lipoprotein (HDL) particles and large very low-density lipoprotein (VLDL) particles. However, despite lower low-density lipoprotein (LDL) cholesterol levels among HT users, there were no significant differences between HT users and nonusers in any LDL subclass measures, including particle size or concentration. Regardless of HT use, women with CAC had higher levels of large VLDL and small LDL particles, higher LDL particle concentration, and smaller mean LDL size compared with women with no detectable CAC.

**Conclusions:** Compared with nonusers, HT users had higher levels of VLDL particles (triglycerides) and did not have a better LDL subclass distribution, which may explain the failure of HT to be associated with a difference in CAC in our study or with a reduction in coronary heart disease risk in randomized clinical trials.

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**T**HE FAILURE OF HORMONE therapy (HT) to reduce coronary heart disease (CHD) events in the Women's Health Initiative (WHI) clinical trial was surprising, given the improvement in lipid levels among estrogen + progestin users.<sup>1,2</sup> Because a 1% decline in low-density lipoprotein cholesterol (LDL-C) has been associated with a 2% reduction in CHD and a 1-mg/dL (0.0259-mmol/L) increase in high-density lipoprotein cholesterol (HDL-C) has been associated with a 2% to 3% decrease in CHD.<sup>3</sup> Therefore, a 12.7% reduction in LDL-C and 7% increase in HDL-C among estrogen + progestin users in the WHI would be expected to produce a 30% to 35% reduction in CHD incidence compared with placebo, rather than the slight increase in risk that was observed.<sup>2</sup> The estrogen alone arm of the WHI was recently reported and also did not demonstrate an overall benefit of es-

trogen alone vs placebo for decreasing incidence or mortality due to CHD, although there was a suggestion of benefit (ie, reduction in CHD events) in the 50- to 59-year age group.<sup>4</sup> Several other recent studies have also failed to demonstrate the benefits of HT on CHD risk,<sup>5</sup> progression of carotid intimal medial wall thickness,<sup>6</sup> and coronary stenosis among women with CHD.<sup>7,8</sup>

There are several possible explanations for the lack of benefit of HT on cardiovascular disease.<sup>2</sup> First, the benefits of HT may be blunted by an adverse effect on specific atherogenic or thrombogenic lipoproteins. Second, HT increases C-reactive protein levels, which may reflect proinflammatory changes that increase CHD risk. Finally, HT may reduce the extent of coronary atherosclerosis but increase the risk of thrombosis, resulting in an increase in coronary events among women with even moderate amounts of coronary atherosclerosis. Animal models, such as studies in pri-

mates, are consistent with the hypothesis that estrogen or estrogen+progestin therapy may slow the development of coronary atherosclerosis. However, these animal studies have primarily examined the early progression of coronary atherosclerosis.

In the present article, we report an investigation of the first hypothesis, that the apparent benefits of HT on lipid levels may be blunted by an adverse effect on specific lipoprotein subclasses or properties (ie, particle size or concentration). Measures of lipoprotein subclasses (number, distribution, and size) may provide additional information on CHD risk, especially for LDL. For example, the LDL peak particle size (the size of the most abundant LDL subclass) has been shown to be associated with CHD, both cross-sectionally and prospectively.<sup>9-13</sup> High levels of LDL particles have been shown to predict incident CHD, independent of lipid levels.<sup>11,14,15</sup> A few small studies have shown that HT may adversely shift the lipoprotein subclass distribution, especially LDL, toward smaller particles.<sup>16,17</sup> Therefore, in a longitudinal study of the menopause, the Healthy Women Study (HWS), we evaluated differences in lipoprotein subclasses and coronary artery calcification (CAC) by HT use, to answer the following 3 questions: (1) Do HT users have less CAC than nonusers 8 years after menopause? (2) Are levels of lipoprotein subclass measures different between HT users and nonusers? (3) Are associations between lipoprotein subclasses and CAC different for HT users and nonusers? To our knowledge, this is the first study to examine these associations with longitudinal data on women from premenopause through postmenopause.

## METHODS

The HWS, a longitudinal study of the effects of menopause on cardiovascular risk, has been described in detail.<sup>18</sup> In 1983-1984, 541 premenopausal women, aged 42 to 50 years, were recruited from driver's license lists in Allegheny County, Pennsylvania. Eligibility criteria included menstrual bleeding within the past 3 months, no surgical menopause, diastolic blood pressure less than 100 mm Hg, and not using lipid-lowering, antihypertensive, or psychotropic medications, thyroid hormone, estrogens, or insulins. The response rate was approximately 90%, with 60% of eligible women participating. At the baseline (premenopausal) study visit, cardiovascular risk factors, including fasting serum lipid levels, were measured. When each woman reported amenorrhea and/or the initiation of HT for 12 successive months, she was scheduled for her "1-year postmenopause" follow-up visit (1y-post), which was repeated at 2 (2y-post), 5 (5y-post), and 8 (8y-post) years after menopause.<sup>18</sup>

At 8y-post, 348 women (approximately 80% of those eligible for an 8y-post visit) had electron beam tomographic scans to measure CAC. Of these women, 316 had stored plasma from 8y-post, which was used for lipoprotein subclass determination. Hormone therapy use and smoking were defined as yes/no and were collected at every visit, but this analysis was stratified by HT use as of 8y-post. Women using lipid-lowering medications (n=24), missing HT information (n=46), or with a triglyceride level greater than 400 mg/dL (4.5 mmol/L) (n=3) at 8y-post were excluded from the analysis, reducing the sample to 243 women. **Table 1** gives the characteristics of HT users vs nonusers at 8y-post. The University of Pittsburgh institutional review board approved the project, and all participants provided written informed consent.

**Table 1. Characteristics of HT Users vs Nonusers at 8 Years After Menopause (the Healthy Women Study)\***

Characteristics	Nonusers (n = 125)	HT Users (n = 118)
Age at scan, y	61.6 ± 1.7	61.7 ± 1.8
Systolic BP, mm Hg	121.0 ± 19.2	122.8 ± 16.3
Diastolic BP, mm Hg	71.8 ± 8.3	71.8 ± 8.0
BMI	27.3 ± 5.7	26.9 ± 4.7
Waist circumference, cm	83.1 ± 14.0	83.5 ± 11.5
Fasting glucose, mg/dL	93.7 ± 23.4	91.9 ± 18.0
Race (nonwhite)	6 (5)	11 (9)
Education (≥college degree)	59 (47)	67 (57)
Smokers: 8 y-post	23 (19)	8 (7)†
Smokers: premenopause	41 (33)	19 (16)†
Diabetic	4 (3)	3 (3)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); BP, blood pressure; 8 y-post, 8 years after menopause; HT, hormone therapy.

SI conversion factor: To convert glucose to millimoles per liter, multiply by 0.0555.

\*Data are given as mean ± SD value or number (percentage) of subjects.

†P≤.05.

Total cholesterol, HDL-C, and triglyceride concentrations were determined by conventional enzymatic methods from fasting (12-hour) blood samples. Low-density lipoprotein cholesterol level was estimated by the Friedewald equation. Lipoprotein subclasses were determined by nuclear magnetic resonance (NMR) spectroscopy (LipoScience Inc, Raleigh, NC), as previously described.<sup>19</sup> In brief, lipoprotein particles were quantified by their proton NMR signals, which differ according to particle diameter. The total plasma signal was deconvoluted into derived signal amplitudes for each lipoprotein subclass, using data from previously modeled reference subclasses. The quantity of each subclass is proportional to its signal amplitude, which is multiplied by a standard lipid amount to provide the results in either milligrams per deciliter of cholesterol for HDL and LDL, or milligrams per deciliter of triglycerides for very low-density lipoprotein (VLDL). The lipoprotein subclasses were categorized as large (60- to 200-nm), medium (35- to 60-nm), and small (27- to 35-nm) VLDL; large (21.3- to 23.0-nm), medium (19.8- to 21.2-nm), and small (18.3- to 19.7-nm) LDL; and large (8.2- to 13-nm) and small (7.3- to 8.2-nm) HDL. Mean particle sizes were calculated for LDL, HDL, and VLDL by weighting the concentration of each subclass by its standard reference diameter. The LDL particle concentration, which includes the total number of small, medium, and large LDL particles and is similar to a plasma apolipoprotein B concentration, was reported as nanomoles of particles per liter.

Electron beam tomography, with an Imatron C-150 Scanner (Imatron, South San Francisco, Calif) was used to obtain 30 to 40 contiguous 3-mm-thick transverse images of the heart, as previously described.<sup>20</sup> Coronary artery calcification scores were calculated according to the Agatston method. The reproducibility of the electron beam tomographic scans from this laboratory had an intraclass correlation of 0.99.<sup>20</sup>

Significance was defined as a P≤.05 (2-tailed test). Because CAC scores were skewed, with many scores of 0, CAC was analyzed in 4 categories (0, 1-10, 11-100, and 101-1175) or as a dichotomous variable (0 vs >0). Differences (and 95% confidence intervals on the differences) between HT users and nonusers were evaluated with *t* tests for normally distributed variables, the Mann-Whitney (Wilcoxon rank sum) test for non-normally distributed variables, and the  $\chi^2$  statistic for categorical variables. We formally tested differences between HT us-

**Table 2. Premenopausal and Change in Mean Lipid and Lipoprotein Levels for HT Users vs Nonusers (the Healthy Women Study)**

Enzymatically Determined Lipids	Nonusers (n = 125)	HT Users (n = 118)	Difference* (95% CI)
Premenopause (baseline)			
Total cholesterol, mg/dL	178.8	183.7	4.9 (−2.1 to 11.9)
HDL-C, mg/dL	60.4	61.9	1.5 (−2.2 to 5.1)
LDL-C, mg/dL	103.1	105.7	2.6 (−3.8 to 9.1)
Triglycerides, † mg/dL	66.0	64.5	−1.5 (−8.0 to 5.0)
Change from premenopause to 8 y-post			
Total cholesterol, mg/dL	37.7	28.8	−8.9 (−15.9 to 2.0)‡
HDL-C, mg/dL	0.1	2.1	2.0 (−1.4 to 5.4)
LDL-C, mg/dL	30.4	17.3	−13.1 (−19.3 to 6.9)‡
Triglycerides, mg/dL	31.5	46.9	15.4 (3.6 to 27.2)‡

Abbreviations: CI, confidence interval; 8 y-post, 8 years after menopause; HDL-C, high-density lipoprotein cholesterol; HT, hormone therapy; LDL-C, low-density lipoprotein cholesterol.  
SI conversion factors: To convert to millimoles per liter, multiply by 0.0259 for cholesterol and by 0.0113 for triglycerides.

\*Point estimate of difference (HT users – nonusers).

†Median.

‡ $P \leq .05$ .

ers and nonusers in the associations of CAC with lipoprotein subclass measures by including the interaction between HT use and the predictor variables in logistic regression models that included both groups. Final regression models adjusted for age, smoking, and enzymatically determined lipid levels. Finally, the models were repeated using linear regression with  $\ln(\text{CAC} + 1)$  as the dependent variable. For statistical analysis SAS software (version 8.2; SAS Inc, Cary, NC) was used, except for the 95% confidence intervals and best estimates of the differences between medians<sup>21</sup> for nonnormally distributed variables in **Tables 2, 3, and 4**, which were calculated with the R software package, version 1.8.1.<sup>22</sup>

## RESULTS

At 8y-post, the participants' mean age was 62 years (range, 57-67 years). Current use of HT was reported by 49%, of whom 77% used combined estrogen + progestin therapy, 14% used oral estrogens, and 87% had been HT users at 5y-post. Hormone therapy users and nonusers were similar in age, blood pressure, body mass index, and waist circumference at 8y-post, but HT users were less likely to smoke and were more likely to quit smoking between baseline and 8y-post (Table 1).

The distribution of CAC (**Figure**) was not significantly different between HT users and nonusers ( $P = .08$ ). Slightly more HT users had CAC scores of 0 compared with nonusers (63% vs 50%, respectively), but this was not significant ( $P = .11$ ) after adjustment for baseline smoking, which was much less common among HT users. In addition, when all women with electron beam tomographic scans ( $n = 296$ ) were included (regardless of whether they had NMR-determined lipoprotein measurements), the unadjusted difference between

users (51%) and nonusers (43%) was small and nonsignificant ( $P = .17$ ). We also examined differences according to the timing of HT initiation, comparing CAC levels between "early" users (who initiated HT use by 1y-post) and "late" users (who initiated HT use between 1y-post and 5y-post). We found no significant difference in CAC between early and late HT users (data not shown).

As previously reported,<sup>18</sup> premenopausal lipid levels were not significantly different between women who later used or did not use postmenopausal HT (Table 2). From premenopause to postmenopause, HT users had smaller increases in total and LDL-C levels, but larger increases in triglyceride levels compared with nonusers (Table 2). Correspondingly, at 8y-post, HT users had lower mean LDL-C levels ( $P \leq .05$ ), higher median triglyceride levels ( $P \leq .05$ ), and slightly higher mean HDL-C levels (not significant) compared with nonusers (Table 3). Among the NMR-determined lipoprotein subclass measures, at 8y-post, HT users had higher levels of both large HDL and large VLDL ( $P \leq .05$  for both). However, there were no significant differences between HT users and nonusers in any other lipoprotein subclass measures, including small LDL particles, LDL particle concentration, or mean LDL particle size.

Among nonusers, those with any CAC had higher levels of total cholesterol, LDL-C, and triglycerides and lower levels of HDL-C ( $P \leq .05$ ) compared with those with CAC scores of 0 (Table 4). However, among HT users, 8y-post total cholesterol and LDL-C levels were actually lower in those with any CAC, although the differences were not significant for any 8y-post lipid levels. Among the lipoprotein subclass measures, both HT users and nonusers with CAC had higher levels of small LDL and large VLDL and smaller mean LDL particle size ( $P \leq .05$  for all) compared with those with no detectable CAC. Coronary artery calcification was also associated with higher LDL particle concentration, although this was only significant among nonusers.

We used logistic regression to formally test whether associations between CAC and small LDL levels, large VLDL levels, mean LDL particle size, and LDL particle concentration differed by HT use (data not shown). In agreement with Table 3, no interaction term was significant, which shows similar associations between CAC and the lipoprotein subclass measure for HT users and nonusers. Results were unchanged in multiple linear regression models, with  $\ln(\text{CAC} + 1)$  as the dependent variable (data not shown).

## COMMENT

In summary, among healthy women 8y-post, the distribution of CAC was not significantly different between HT users and nonusers, despite lower smoking rates among HT users. At 8y-post, HT users had lower LDL-C levels but higher triglyceride levels compared with nonusers. Hormone therapy users also had higher large HDL and large VLDL levels, but were not significantly different from those for nonusers in any of the LDL subclass measures. Coronary artery calcification was associated

**Table 3. Mean Lipid and Lipoprotein Levels at 8 Years After Menopause by HT Use (the Healthy Women Study)**

Lipid/Lipoprotein	Nonusers (n = 125)	HT Users (n = 118)	Difference* (95% CI)
Enzymatically determined lipids			
Total Chol, mg/dL	216.5	212.5	-4.0 (-13.0 to 5.0)
HDL-C, mg/dL	60.5	64.0	3.5 (-1.0 to 7.9)
LDL-C, mg/dL	133.7	123.0	-10.7 (-19.1 to -2.2)†
TG, mg/dL‡	95.0	115	17.0 (5.0-30.0)†
NMR-determined lipoprotein subclass measures			
Small HDL, mg/dL Chol	18.7	17.9	-0.8 (-2.6 to 1.0)
Large HDL, mg/dL Chol	39.1	47.1	8.0 (3.0 to 13.1)†
Mean HDL size, nm	9.04	9.12	0.08 (-0.04 to 0.21)
Small LDL, mg/dL Chol‡	31.7	32.2	0.0 (-7.3 to 5.1)
Medium LDL, mg/dL Chol‡	16.5	16.7	0.0 (0.0 to 0.0)
Large LDL, mg/dL Chol	91.1	90.3	-0.81 (-12.6 to 11.0)
Mean LDL size, nm	21.04	20.99	-0.05 (-0.21 to 0.12)
LDL particle concentration, nmol/L	1775	1828	53 (-75 to 179)
Small VLDL, mg/dL TG‡	32.0	28.5	-3.9 (-8.6 to 0.8)
Medium VLDL, mg/dL TG‡	30.3	28.5	-0.2 (-6.2 to 5.6)
Large VLDL, mg/dL TG‡	5.1	12.3	2.1 (0.0 to 5.9)†
Mean VLDL size, nm	45.85	47.66	1.8 (-0.8 to 4.4)

Abbreviations: Chol, cholesterol; CI, confidence interval; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HT, hormone therapy; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; NMR, nuclear magnetic resonance; TG, triglycerides; VLDL, very low-density lipoprotein. SI conversion factors: To convert to millimoles per liter, multiply by 0.0259 for Chol and by 0.0113 for TG.

\*Point estimate of difference (HT users - nonusers).

† $P \leq .05$ .

‡Median.

with traditional lipid levels among nonusers but not among HT users. Associations between CAC and the lipoprotein subclass measures were similar for HT users and nonusers; the strongest risk factors were higher levels of small LDL and large VLDL, smaller mean LDL particle size, and higher LDL particle concentration.

### CORONARY CALCIFICATION

The CAC distribution was not significantly different by HT use, and the relatively small difference in the proportion of women with no detectable CAC was not significant after adjusting for smoking, which was much more prevalent among nonusers and was strongly associated with CAC in the HWS.<sup>20</sup> There was also no significant difference in CAC according to whether HT use began "early" (by 1y-post) vs "late" (between 1y-post and 5y-post) in the postmenopausal years, but low power for this comparison prevented a definitive test of the hypothesis.

Few studies of HT use have examined CAC as an outcome. An observational study by Schisterman et al<sup>23</sup> found no decrease in CAC for HT users after adjusting for potential confounders. In contrast, Akhrass et al<sup>24</sup> has reported that women using HT were less likely to have CAC scores of 400 or greater after adjusting for cardiovascular disease risk factors. However, the risk factors in that cross-sectional observational study were all self-reported yes/no questions and answers, which may have reduced the authors' ability to reliably adjust for potential confounders. Our results are consistent with recently reported randomized clinical trial results,<sup>1,5-8</sup> which have demonstrated no benefit of HT on atherosclerosis or cardiovascular disease events.

### LDL-C LEVELS AND THE LDL SUBCLASS DISTRIBUTION

In our study, LDL subclass measures were not significantly different between HT users and nonusers. However, it is noteworthy that the lower LDL-C levels ( $P \leq .05$ ) among HT users were not reflected in lower levels of small LDL, lower LDL particle concentration, or larger mean LDL particle size, relative to nonusers. As previously noted, other studies have reported an adverse effect of HT on the LDL subclass distribution, shifting it toward smaller LDL particles.<sup>16,17,25</sup> For both HT users and nonusers, higher levels of small LDL, smaller mean LDL particle size, and higher LDL particle concentration were associated with CAC, which is in agreement with previous reports<sup>26</sup> and other studies that did not evaluate HT effects. The absence of a significant difference in the LDL subclass distribution (despite lower LDL-C levels) may be one explanation for the failure of HT to be associated with less coronary calcification in the present study, or with reduced atherosclerosis and cardiovascular disease in clinical trials.

Another explanation for the failure of HT to reduce atherosclerosis may be that the effect of HT on levels of LDL-C is too small. As our longitudinal data (Table 2) show, the lower mean postmenopausal LDL-C level among HT users relative to nonusers is due to a smaller postmenopausal increase in LDL-C compared with nonusers, rather than to an actual decrease in LDL-C from premenopausal levels. Specifically, although HT users experienced a smaller postmenopausal increase in mean LDL-C level compared with nonusers, their 8y-post LDL-C levels were still approximately 16% higher than their premenopausal LDL-C levels (Table 2). This is a relatively weak effect in compari-

**Table 4. Mean Lipid and Lipoprotein Levels by CACS (the Healthy Women Study)**

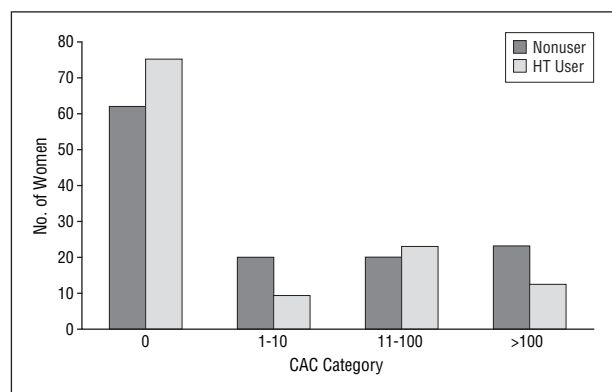
Lipid/Lipoprotein	CACS for Nonusers (n = 125)			CACS for HT Users (n = 118)		
	0 (n = 62)	>0 (n = 63)	Diff* (95% CI)	0 (n = 74)	>0 (n = 44)	Diff* (95% CI)
<b>Lipids</b>						
Total Chol, mg/dL	207.6	225.2	17.6 (3.5 to 31.8)†	215.0	208.2	-6.8 (-17.7 to 4.0)
HDL-C, mg/dL	63.9	57.2	-6.7 (-12.6 to -0.8)†	66.2	60.3	-5.9 (-13.0 to 1.0)
LDL-C, mg/dL	124.7	142.7	18.0 (4.7 to 31.3)†	124.7	120.2	-4.5 (-14.5 to 5.7)
TG, † mg/dL	87.0	104.0	16.0 (1.0 to 31.0)†	115.0	126.0	12.0 (-9.0 to 33.0)
<b>Lipoprotein subclasses</b>						
Small HDL, mg/dL Chol	19.3	18.1	-1.2 (-3.6 to 1.2)	17.8	18.1	0.3 (-2.4 to 3.0)
Large HDL, mg/dL Chol	41.0	37.2	-3.8 (-10.6 to 3.0)	48.5	44.8	-3.7 (-11.6 to 4.2)
HDL size, nm	9.14	8.94	-0.2 (-0.4 to 0.0)†	9.17	9.04	-0.1 (-0.3 to 0.1)
Small LDL, mg/dL Chol‡	19.9	37.7	14.5 (3.4 to 25.4)†	26.9	36.3	10.9 (0.0 to 26.9)†
Medium LDL, mg/dL Chol‡	17.9	11.4	0.0 (-8.2 to 0.0)	19.7	6.5	0.0 (-9.8 to 0.0)
Large LDL, mg/dL Chol	88.9	93.2	4.3 (-12.6 to 21.2)	95.3	81.7	-13.6 (-30.8 to 3.6)
LDL size, nm	21.15	20.93	-0.22 (-0.44 to 0.00)†	21.10	20.81	-0.29 (-0.54 to -0.05)†
LDL particle concentration, nmol/L	1599	1949	350 (181 to 519)†	1778	1911	133 (-54 to 319)
Small VLDL, mg/dL TG‡	29.2	37.0	9.3 (2.4 to 17.3)†	27.0	29.8	2.1 (-4.5 to 8.7)
Medium VLDL, mg/dL TG‡	28.5	30.4	3.3 (-5.1 to 11.3)	27.0	36.2	4.7 (-4.6 to 15.0)
Large VLDL‡, mg/dL TG	1.7	10.4	3.7 (0.5 to 10.6)†	8.9	28.0	10.2 (2.3 to 25.9)†
VLDL size, nm	45.6	46.1	0.6 (-3.4 to 4.5)	46.1	50.3	4.2 (0.9 to 7.5)†

Abbreviations: CACS, coronary artery calcification score; Chol, cholesterol; CI, confidence interval; Diff, difference; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; VLDL, very low-density lipoprotein. SI conversion factors: To convert to millimoles per liter, multiply 0.0259 for Chol and by 0.0113 for TG.

\*Point estimate of difference (CACS >0 - CACS = 0).

†P ≤ .05.

‡Median.



**Figure.** Distribution of coronary artery calcification (CAC) ( $P = .08$ ). HT indicates hormone therapy.

son with the reductions in LDL-C levels seen with the use of therapy with statins, and may not have been large enough to influence subsequent levels of coronary calcification or other measures of atherosclerosis.

### LARGE HDL AND LARGE VLDL

Hormone therapy users had higher levels of large HDL compared with nonusers, but the relationship between large HDL level and CAC was weak and not significant. Reports of associations between various HDL subclasses and CHD have been mixed.<sup>13,27,28</sup> Compared with nonusers, HT users had a larger premenopausal to postmenopausal increase in triglyceride levels (Table 2). Very low-density lipoprotein is the primary carrier of triglycerides, and large VLDL particles accumulate preferen-

tially as triglyceride levels increase.<sup>29</sup> Therefore, HT users also had higher postmenopausal levels of large VLDL particles compared with nonusers. Coronary artery calcification was positively associated with higher levels of large VLDL particles for both HT users and nonusers and remained significantly associated for the entire group after adjustment for HT use, age, smoking, and lipid levels. Other studies have found that higher levels of large VLDL are associated with CAD,<sup>13,30</sup> but it is not clear whether large VLDL particles directly cause atherogenesis. The association may be due to the role of VLDL particles in lipoprotein metabolism and/or thrombosis. The production of large VLDL particles appears to be regulated independently from that of smaller VLDL particles, and large VLDL particles may be the precursor to atherogenic triglyceride-rich remnant particles that have a delayed clearance time.<sup>29</sup> In addition, large VLDL particles contributes to the production of small, dense LDL particles,<sup>31</sup> so that LDL size is highly correlated with plasma VLDL and triglyceride levels. Therefore, the association with higher levels of large VLDL may reflect the metabolic state that is favorable to the increased production of small, dense LDL particles.

### LIMITATIONS

The generalizability of these results is limited because our participants were healthy, predominantly white women with a moderate to high socioeconomic status. This was also an observational study of HT use, rather than a randomized clinical trial, and selection bias (the healthy-user effect) was suggested by the higher smoking rates and lower quitting rates among nonusers in our study.

This healthy-user effect may account for the relatively small difference in those with CAC scores of 0, but the changes in lipoprotein subclasses were generally unfavorable, which is not expected with a healthy-user bias. Because this was an observational study, we did not assign a single type and dosage of HT. However, most women were using combined estrogen + progestin, and their use of HT was assessed by trained clinic personnel at repeated study visits. In addition, clinical trials have demonstrated null results with a variety of HT (combination and estrogen-only) preparations.<sup>1,4,5,7</sup>

## CONCLUSIONS

Despite lower LDL-C levels among HT users, levels of CAC were similar between HT users and nonusers, especially after adjusting for smoking. Higher levels of large VLDL (triglycerides), no difference in the LDL subclass distribution, and the relatively weak effect of HT on LDL-C levels (ie, in comparison with the use of statins), may account for the failure of HT to be associated with less coronary calcification in our study, or with a reduction in CHD risk in randomized clinical trials. Further evaluation of the effects of HT on atherosclerosis should focus on the distribution and composition of lipoprotein particles, especially measures of the LDL subclass distribution and LDL particle concentration.

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