Atherosclerosis

Is Plasma Oxidized Low-Density Lipoprotein, Measured With the Widely Used Antibody 4E6, an Independent Predictor of Coronary Heart Disease Among U.S. Men and Women?

Tianying Wu, MD, PhD,* Walter C. Willett, MD, DrPH,†‡ Nader Rifai, PhD, Iris Shai, PhD,* JoAnn E. Manson, MD, DrPH,‡§ Eric B. Rimm, ScD†‡

Boston, Massachusetts

OBJECTIVES

Our aim was to examine whether circulating oxidized low-density lipoprotein (oxLDL) is a predictor of coronary heart disease (CHD) independent of lipid markers and to compare oxLDL, apolipoprotein B100 (apoB), and total cholesterol (TC)/high-density lipoprotein-cholesterol (HDL-C) ratio as predictors of CHD.

BACKGROUND

Measurement of circulating oxLDL with antibody 4E6 has been widely used in many studies; however, few large prospective studies have examined whether this marker is a predictor of CHD independent of lipids and compared oxLDL with other important lipid predictors.

METHODS

After 6 years of follow-up among 18,140 men from the HPFS (Health Professionals Follow-up Study) and 8 years among 32,826 women from the Nurses’ Health Study who provided blood samples at baseline, we identified incident nonfatal myocardial infarction or fatal CHD in 266 men and 235 women. Each case was matched with two control subjects by age, smoking, and time of blood draw. The oxLDL was measured via enzyme-linked immunosorbent assay with antibody 4E6 against oxidized apoB.

RESULTS

Among both men and women, oxLDL was significantly related to risk of CHD in multivariate analysis before adjustment for any lipid markers. However, when oxLDL, LDL cholesterol, HDL-C, and triglycerides were mutually adjusted, oxLDL was no longer predictive. When oxLDL and apoB were mutually adjusted, only apoB was predictive of CHD. Similar results were found when oxLDL and TC/HDL-C ratio were mutually adjusted.

CONCLUSIONS

Our results suggest that circulating oxLDL, measured with antibody 4E6, is not an independent overall predictor of CHD after adjustment of lipid markers and is less predictive in development of CHD than apoB and TC/HDL-C ratio.

© 2006 by the American College of Cardiology Foundation

Oxidative stress and oxidized low-density lipoprotein (oxLDL) have been proposed to play an important role in the development of atherosclerosis (1,2). The oxLDL is present in atherosclerotic lesions from humans (3,4) and animals (5); however, the importance of circulating oxLDL as a predictor of subsequent clinical coronary events has not been confirmed.

See page 980

Reliable markers of oxLDL for epidemiologic studies are limited. Biopsy samples from atherosclerotic lesions for measuring oxLDL are difficult to obtain and commonly used in vitro measurements of oxLDL (6,7) are time consuming and not practical for large studies with stored samples. Recently, direct measurement of circulating oxLDL in plasma by either an in-house enzyme-linked immunosorbent assay (ELISA) or a commercial ELISA kit provided by Merodia (Winston-Salem, North Carolina) with the monoclonal antibody 4E6 have been used in many case-control, cross-sectional (8–17) and some prospective studies (18–21). These studies suggest that oxLDL is positively associated with coronary heart disease (CHD) and related risk factors. However, large prospective studies including men and women designed to evaluate this marker as an independent predictor of CHD are lacking.

Measurement of LDL-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides are traditionally recommended lipid screening tests for CHD; therefore, it is important to examine whether oxLDL can predict CHD independent of these lipid markers. Several prospective studies have suggested that apolipoprotein B100
The Nurses’ Health Study I

Study population and collection of blood samples. The Nurses’ Health Study I cohort, established in 1976, consists of 121,700 female registered nurses 30 to 55 years of age who provided information on medical history and lifestyle factors at baseline and on subsequent biennial questionnaires. In 1989 and 1990, we collected blood samples from 32,826 cohort members who were then 43 to 69 years of age. Whole blood was shipped, processed, and stored by the methods described previously. The women who returned blood samples were not substantially different from the remaining women in the cohort. We identified 28,601 women among those who returned samples who were free of cardiovascular disease and cancer for our nested case-control study. The study was approved by the institutional review board of Brigham and Women’s Hospital, Boston.

METHODS

Study population and collection of blood samples. HPFS (HEALTH PROFESSIONALS FOLLOW-UP STUDY). The HPFS is a prospective cohort study of diet, CHD, and cancer conducted among 51,529 U.S. male health professionals who were 40 to 75 years old at baseline in 1986. Health information, including reports of diagnosed heart disease, was obtained at baseline and assessed biennially by follow-up questionnaires. In 1993 and 1994 we sent venipuncture kits to participants, and 18,140 men returned specimens on ice with an overnight courier. Multiple aliquots of plasma were stored in liquid nitrogen freezers for future nested case-control analyses. The men who returned blood samples were not substantially different from the remaining men in the cohort except that the prevalence of current smoking was slightly lower among those who returned samples who were free of cardiovascular disease and cancer for future nested case-control studies.

THE NURSES’ HEALTH STUDY I. The Nurses’ Health Study I cohort, established in 1976, consists of 121,700 female registered nurses 30 to 55 years of age who provided information on medical history and lifestyle factors at baseline and on subsequent biennial questionnaires. In 1989 and 1990, we collected blood samples from 32,826 cohort members who were then 43 to 69 years of age. Whole blood was shipped, processed, and stored by the methods described previously. The women who returned blood samples were not substantially different from the remaining women in the cohort. We identified 28,601 women among those who returned samples who were free of cardiovascular disease and cancer for our nested case-control study. The study was approved by the institutional review board of Brigham and Women’s Hospital, Boston.

Ascertainment of cases and selection of control subjects. The assessment of nonfatal myocardial infarction (MI) and fatal CHD and selection of control subjects are described in detail elsewhere (26,27). Briefly, when an MI was reported on a biennial follow-up questionnaire, we sought permission to review medical records. For confirmation of cases of MI, we used World Health Organization criteria, which require symptoms plus either diagnostic electrocardiographic changes or elevated cardiac enzymes. The MIs of undetermined date were not included in this analysis.

The end point for this study comprised incident cases of nonfatal MI and fatal CHD that occurred before February 2000 for men and before June 1998 for women. We confirmed 266 cases in men and 235 cases in women among the “at risk” participants. Each case was matched by year of age, month of blood draw, and smoking status (never, past, current) to 2 control subjects who were free of CHD at the time of the case diagnosis. All women were also matched for fasting status (lower/higher than 8 h) before blood draw. All assays were conducted without knowledge of the case/control status. Matched case-control triplets were handled identically and together and assayed in the same analytical run.

Assessment of medical history, lifestyle, and dietary factors. Questions related to disease and lifestyle factors were sent out every 2 years, and a semiquantitative food frequency questionnaire was sent out every 4 years. For nutrients and other lifestyle characteristics, such as body mass index (BMI) and physical activity, we used the information from the time when subjects’ blood was collected (1994 for men and 1990 for women). The validity and reproducibility of self-reported lifestyle characteristics and semiquantitative food frequency questionnaire have been previously reported (28–32).

Measurement of oxLDL, apoB, other lipids, and inflammatory markers. The oxLDL was measured by a sandwich ELISA kit with the monoclonal antibody 4E6 (MERCodia). The antibody 4E6 is especially against an epitope in the apoB-100 moiety of oxLDL that is formed from substitution of lysine residues of apoB-100 with aldehydes (18). The coefficient variations of the intra- and inter-assay for measurement of oxLDL were <8%. Total apoB was measured by an immunoturbidimetric technique on the Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, Indiana). Total cholesterol, HDL-C, LDL-C, and triglycerides were measured by standard method with reagent from Roche Diagnostics and Genzyme (Cambridge, Massachusetts). Soluble tumor necrosis factor (TNF)-alpha receptors types 1 and 2 (sTNF-R1 and sTNF-R2) and C-reactive protein was measured by latex-enhanced immunoturbidimetric assay from Denka Seiken (Tokyo, Japan) on the Hitachi 911 system. Interleukin-6 was measured by ELISA (R&D Biometrics, C原型)
Systems, Minneapolis, Minnesota). The coefficient variations for apoB, other lipids, and inflammatory markers were <4% to 9%. The laboratory used for analysis is certified by the Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute Lipid Standardization Program.

Stability of oxLDL in whole blood stored on ice up to 36 h. As described earlier, participants sent whole blood shipped on ice via overnight courier from across the country. To assess the stability of oxLDL, we conducted a pilot study that mimicked these conditions and found that there was no degradation or increase in the measurement of oxLDL when assessed at baseline, 24 h, and 36 h after whole blood remained on ice (33).

Statistical analysis. Baseline characteristics. For baseline variables, we calculated means, medians, or proportions for cases and control subjects. We assessed statistical significance by chi-square tests for categorical variables. We used Wilcoxon signed-rank tests for non-normally distributed variables and 2 sample t tests for normally distributed variables and the age-adjusted, partial Spearman rank-correlation coefficient to assess the associations between continuous variables and the age-adjusted, partial Spearman rank-correlation coefficient to assess the associations between oxLDL and other variables.

Association of oxLDL with risk of CHD. We categorized participants into quintiles according to the distribution of plasma levels of oxLDL in the control participants. We used conditional logistic regression analyses with and without adjustment for traditional cardiovascular risk factors, including family history of MI, alcohol intake, BMI, physical activity, and history of hypertension or high cholesterol.

Comparison of oxLDL with apoB and other lipids. To examine whether oxLDL captures information beyond apoB or TC/HDL-C ratio, we compared the full model that included oxLDL and either apoB or TC/HDL-C ratio to the reduced model without oxLDL with a likelihood ratio test with 4 df. Oxidized LDL and other lipid markers (apoB and TC/HDL-C ratio) were included as quintiles with 4 dummy variables. With a similar approach, we examined whether apoB or TC/HDL-C ratio adds additional information beyond oxLDL. To pool the relative risk (RR) estimates for men and women, we used the weighted average of estimates with the DerSimonian and Laird random effects model (34).

Because of the linear relationship of the oxLDL, apoB, and TC/HDL-C with risk of CHD, to reduce residual confounding, we also fit the aforementioned models with oxLDL, apoB, and TC/HDL-C ratio as continuous, linear variables.

RESULTS

Baseline characteristics. We compared characteristics at blood draw between cases and control subjects (Table 1). Levels of oxLDL at baseline were significantly higher among cases than control subjects and also significantly correlated with all lipid parameters. The correlation between oxLDL and apoB was 0.59 in men and 0.44 in women; for TC/HDL-C ratio, the correlations were 0.40 in men and 0.65 in women (Table 2). The oxLDL was modestly correlated with inflammatory markers in women but not in men.

Association of oxLDL with risk of CHD and comparison of oxLDL with apoB and TC/HDL-C ratio. As compared with the lowest quintile, the highest quintile of oxLDL was significantly associated with an increased risk of CHD in both crude and multivariate-adjusted models (Table 3) for both men and women. However, the RRs were substantially attenuated and no longer significant after adjustment for LDL-C, HDL-C, and triglycerides.

When oxLDL and apoB were adjusted simultaneously, oxLDL was no longer predictive; whereas the association of apoB with CHD risk remained significant after adjustment for oxLDL. The multivariate RR between extreme quintiles for oxLDL was 1.31 (95% confidence interval [CI] 0.67 to 2.57) in men and 1.75 (95% CI 0.77 to 4.01) in women after adjustment of apoB. As shown in Table 4, in both men and women, the results of the likelihood ratio test (LRT) suggested that the full model with oxLDL and apoB was significantly or marginally significantly different from the model without apoB (with only oxLDL) (pLRT = 0.004 for men and pLRT = 0.06 for women). However, the full model was not significantly different from the model without oxLDL (with only apoB) in men (pLRT = 0.5) or women (pLRT = 1.0). These results indicate that apoB added information beyond oxLDL, whereas oxLDL had little predictive capacity beyond apoB in either men or women.

To directly compare oxLDL with apoB, we also entered both oxLDL and apoB in the multivariate model simultaneously as continuous variables. In men, the odds ratio (OR)/1 SD increase was 1.05 (95% CI 0.85 to 1.30; p = 0.7) for oxLDL and 1.66 (95% CI 1.33 to 2.09; p < 0.0001) for apoB. In women, the OR/1 SD increase was 1.04 (95% CI 0.77 to 1.40; p = 0.8) for oxLDL and 1.73 (95% CI 1.25 to 2.41; p < 0.0001) for apoB. Again, this suggested that apoB was a stronger predictor than oxLDL.

We performed similar analyses to compare oxLDL versus TC/HDL-C ratio and found similar patterns. The risk of oxLDL between extreme quintiles after adjustment for TC/HDL-C ratio was reduced to 1.70 (95% CI 0.93 to 3.13) in men and 1.17 (95% CI 0.51 to 2.68) in women (Tables 3 and 4). However, the RR of TC/HDL-C ratio between extreme quintiles was 2.82 (95% CI 1.48 to 5.38) in men and 4.30 (95% CI 1.76 to 10.52) in women after adjustment for oxLDL (Table 4). With the likelihood ratio test, we also found that oxLDL did not add any information beyond TC/HDL-C ratio, but TC/HDL-C ratio added significant information beyond oxLDL (Table 4).

Similarly, we entered oxLDL and TC/HDL-C ratio as continuous variables simultaneously in the model and found similar results. In men, the OR/1 SD increase was 1.25
We found that the measurement of circulating oxLDL with the 4E6 antibody, the most widely used antibody for this assay, predicted the risk of CHD in a large prospective setting including men and women. However, this association was not independent of standard lipid variables. Most importantly, this association was not independent of two powerful predictors, apoB and TC/HDL-C ratio. Conversely, apoB or the TC/HDL-C ratio predicted CHD independent of oxLDL. Even in the pooled analysis, the independent association between oxLDL and CHD was weak after accounting for lipid variables. These comparisons were not done in previous studies using the same antibody via ELISA.

The strong positive association found in multivariate analyses for oxLDL predicting CHD without adjustment for lipid markers was similar to that from several previous studies that have used antibody 4E6 via either the in-house ELISA or the commercially available ELISA kit provided by Merckodia. In several case-control and cross-sectional studies, oxLDL was associated with CHD (9) or stable CHD (17), higher CHD risk on the basis of the Framingham score (12), metabolic syndrome and a greater disposition to atherothrombotic coronary disease (13), glucose tolerance (15), or greater intima thickness and presence of plaque (14). At least 3 cohort studies and 1 nested-case control study, which used antibody 4E6, have found that oxLDL was associated with number and the size of plaques (20), with MI cases among men (19), with cardio-

### Table 1. Baseline Characteristics and Biochemical Parameters

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Control Subjects</th>
<th>p Value</th>
<th>Cases</th>
<th>Control Subjects</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 266)</td>
<td>(n = 532)</td>
<td></td>
<td>(n = 235)</td>
<td>(n = 470)</td>
<td></td>
</tr>
<tr>
<td><strong>Demographic, History, and Behavioral Variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>57.7 ± 8.4</td>
<td>57.7 ± 8.3</td>
<td>0.06</td>
<td>63.3 ± 6.5</td>
<td>63.6 ± 6.6</td>
<td>0.0006</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.2 ± 3.5</td>
<td>25.7 ± 3.5</td>
<td>0.07</td>
<td>26.7 ± 6.3</td>
<td>25.1 ± 4.6</td>
<td>0.0006</td>
</tr>
<tr>
<td>Physical activity (METS/week)</td>
<td>22.8 ± 42.1</td>
<td>26.6 ± 43.6</td>
<td>0.1</td>
<td>11 ± 17.4</td>
<td>11.5 ± 18.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>5.6 ± 15.6</td>
<td>7.0 ± 17.3</td>
<td></td>
<td>0.9 ± 9.6</td>
<td>1.95 ± 10.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of hypertension (%)</td>
<td>42 ± 31</td>
<td></td>
<td></td>
<td>57 ± 28</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of high cholesterol (%)</td>
<td>49 ± 41</td>
<td></td>
<td></td>
<td>43 ± 29</td>
<td></td>
<td>0.0002</td>
</tr>
<tr>
<td>History of diabetes (%)</td>
<td>8 ± 5</td>
<td></td>
<td></td>
<td>14 ± 3</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family history of MI (%)</td>
<td>15 ± 11</td>
<td></td>
<td></td>
<td>22 ± 13</td>
<td></td>
<td>0.0015</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>37 ± 37</td>
<td></td>
<td></td>
<td>34 ± 34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>46 ± 47</td>
<td></td>
<td>&lt;0.001</td>
<td>34 ± 35</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>12 ± 11</td>
<td></td>
<td></td>
<td>32 ± 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of aspirin (%)</td>
<td>39 ± 35</td>
<td></td>
<td></td>
<td>45 ± 31</td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>Postmenopausal hormone use (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Postmenopausal status (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>86 ± 83</td>
<td></td>
<td>&lt;0.0001</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma Biomarkers (Mean, Median, SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidized LDL (U/l)</td>
<td>76.5 ± 25</td>
<td>70.1 ± 24</td>
<td>&lt;0.001</td>
<td>38.7 ± 12</td>
<td>34.4 ± 12</td>
<td>0.002</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>215 ± 40</td>
<td>205 ± 38</td>
<td>&lt;0.001</td>
<td>236 ± 40</td>
<td>226 ± 40</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>152 ± 117</td>
<td>119 ± 121</td>
<td>&lt;0.001</td>
<td>128 ± 91</td>
<td>110 ± 76</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>42 ± 11</td>
<td>46 ± 12</td>
<td>&lt;0.001</td>
<td>52 ± 15</td>
<td>60 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>136 ± 36</td>
<td>127 ± 31</td>
<td>&lt;0.001</td>
<td>143 ± 34</td>
<td>133 ± 37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>5.4 ± 1.4</td>
<td>4.7 ± 1.4</td>
<td>&lt;0.001</td>
<td>4.9 ± 1.5</td>
<td>4.0 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dl)</td>
<td>104 ± 24</td>
<td>92 ± 22</td>
<td>&lt;0.001</td>
<td>130 ± 36</td>
<td>115 ± 31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>1.68 ± 5.1</td>
<td>1.08 ± 6.4</td>
<td>&lt;0.0001</td>
<td>3.1 ± 6.4</td>
<td>2.2 ± 4.8</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.86 ± 15.0</td>
<td>1.53 ± 11.1</td>
<td>0.009</td>
<td>1.99 ± 12.3</td>
<td>1.63 ± 7.3</td>
<td>0.002</td>
</tr>
<tr>
<td>sTNFR1 (pg/ml)</td>
<td>1514 ± 501</td>
<td>1505 ± 541</td>
<td>0.8</td>
<td>1452 ± 595</td>
<td>1265 ± 355</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sTNFR2 (pg/ml)</td>
<td>2992 ± 868</td>
<td>2944 ± 870</td>
<td>0.5</td>
<td>2795 ± 981</td>
<td>2487 ± 771</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means for continuous variables and proportions for categorical variables, except alcohol, physical activity, C-reactive protein, triglycerides, and interleukin (IL)-6, which are shown as medians. Age, smoking status and month of blood drawing were matched factors. Body mass index (weight divided by height squared).

HDL = high-density lipoprotein; LDL = low-density lipoprotein; METS = metabolic equivalent hours (1 MET corresponds to the energy used for 1 h of quiet sitting, running 1 h corresponds to about 6 MET); MI = myocardial infarction; sTNF-R1 and sTNF-R2 = soluble tumor necrosis factor receptor types 1 and 2.
transplanted vasculopathy in men and women (18), and acute CHD events in men (21). However, many of these studies were small and lacked the power to control for all potential confounders and, especially, did not finely adjust for lipid markers. Most importantly, none of them determined whether oxLDL was independent of apoB, and only for lipid markers. Most importantly, none of them determined whether oxLDL was independent of apoB, and only one study (21) adjusted for TC/HDL-C ratio, but their analysis included only men and adjusted for physical activity and obesity only as dichotomized variables, leaving the possibility of residual confounding.

Inflammatory processes produce oxidative products. However, oxLDL can also induce production of monocyte chemotactic protein-1 and monocyte-colony stimulating factor and lead to inflammatory response in vascular cells (35–37). Inflammation, reflected by circulating inflammatory markers such as C-reactive protein, are associated with incidence of CHD in large prospective studies (27,38). However, on the basis of our data, the association for oxLDL is not explained by inflammation, because the risk of oxLDL did not change after adjustment of any inflammatory markers.

The oxLDL is thought to be a mixture of heterogeneously modified particles. Many components in LDL can be oxidized, including apoB, phospholipids, LDL-cholesterol, and unsaturated fatty acids. Therefore, several antibodies against different epitopes on LDL have been generated, including the most widely used antibody 4E6 against oxidized apoB (as used in this report), antibody FOH1a/DLH3 (39), and antibody E06 (40,41) against oxidized phospholipids. Circulating oxLDL measured via the antibodies, FOH1a/DLH3, or E06 was not associated with apoB (40–42), and oxLDL measured via the antibody E06 was found to be significantly associated with lipoprotein(a) (Lp[a]) (41). However, we and several others (14–

### Table 2. Age-Adjusted Spearman Rank Correlation Coefficients Between Oxidized LDL and Selected Cardiovascular Risk Factors Among Control Subjects (n = 532 Men and n = 470 Women) at Baseline

<table>
<thead>
<tr>
<th>Oxidized LDL</th>
<th>Men r</th>
<th>Women r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>0.07</td>
<td>0.17*</td>
</tr>
<tr>
<td>Physical activity</td>
<td>0.04</td>
<td>-0.15*</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>0.03</td>
<td>-0.12*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.51‡</td>
<td>0.49‡</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.21‡</td>
<td>0.45‡</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.11‡</td>
<td>-0.41‡</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.55‡</td>
<td>0.53‡</td>
</tr>
<tr>
<td>Total cholesterol/HDL cholesterol ratio</td>
<td>0.40‡</td>
<td>0.65‡</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>0.59‡</td>
<td>0.44‡</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>0.08*</td>
<td>-0.05</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.03</td>
<td>0.17‡</td>
</tr>
<tr>
<td>sTNF-R1</td>
<td>-0.002</td>
<td>0.14†</td>
</tr>
<tr>
<td>sTNF-R2</td>
<td>-0.04</td>
<td>0.12*</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*p < 0.05; †p < 0.01; ‡p < 0.001.

Abbreviations as in Table 1.

### Table 3. Relative Risk of Coronary Heart Disease Across Quintiles of Oxidized LDL Among 798 Men From the Health Professional Follow-Up Study and 705 Women From the Nurses’ Health Study

<table>
<thead>
<tr>
<th>Quintile of Oxidized LDL</th>
<th>p for Linear Trend</th>
</tr>
</thead>
</table>

#### Men

<table>
<thead>
<tr>
<th>Quintile</th>
<th>Crude matched* (95% CI)</th>
<th>Model 1—multivariate adjusted† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.98 (0.57–1.72)</td>
<td>1.01 (0.57–1.79)</td>
</tr>
<tr>
<td>2</td>
<td>1.61 (0.98–2.65)</td>
<td>1.70 (1.01–2.87)</td>
</tr>
<tr>
<td>3</td>
<td>1.88 (1.12–3.13)</td>
<td>2.10 (1.22–3.63)</td>
</tr>
<tr>
<td>4</td>
<td>2.39 (1.42–4.05)</td>
<td>2.56 (1.46–4.50)</td>
</tr>
<tr>
<td>5</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

#### Women

<table>
<thead>
<tr>
<th>Quintile</th>
<th>Crude matched* (95% CI)</th>
<th>Model 1—multivariate adjusted† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.10 (0.58–2.08)</td>
<td>0.93 (0.47–1.85)</td>
</tr>
<tr>
<td>2</td>
<td>2.18 (1.23–3.88)</td>
<td>1.81 (0.97–3.38)</td>
</tr>
<tr>
<td>3</td>
<td>2.52 (1.43–4.44)</td>
<td>1.78 (0.94–3.36)</td>
</tr>
<tr>
<td>4</td>
<td>4.16 (2.26–7.65)</td>
<td>2.84 (1.42–5.66)</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

*Matched on age, smoking, and time of blood draw; †Model 1: adjusted for body mass index, physical activity, alcohol consumption, history of high blood pressure, high cholesterol and diabetes, family history of myocardial infarction, and use of aspirin. §Model 1 for men was additionally adjusted for fasting status; model 1 for women was additionally adjusted for postmenopausal hormone use and menopausal status. ApoB = apolipoprotein B; CI = confidence interval; other abbreviations as in Table 1.
In conclusion, we found that circulating oxLDL, measured by the most widely used antibody 4E6, can predict the risk of CHD but not independent of the two strongest risk factors, apoB and TC/HDL-C ratio, or other conventional lipid markers, including LDL-C, HDL-C, and triglycerides. Apolipoprotein B100 and TC/HDL-C ratio are more predictive in development of CHD than oxLDL. Measurement of oxLDL with multiple antibodies is needed in future large-scale, prospective studies to address the long-desired question of whether oxLDL is an independent risk factor of CHD.

Reprint requests and correspondence: Dr. Tianying Wu, Department of Nutrition, Harvard School of Public Health, 655 Huntington Avenue, Boston, Massachusetts 02115. E-mail: tianying@hsph.harvard.edu.

REFERENCES


Table 4. OxLDL in Comparison With Mutually Adjusted ApoB or Total/HDL Cholesterol Ratio in Multivariate Models

<table>
<thead>
<tr>
<th>Marker</th>
<th>Mutually Adjusted Marker 1</th>
<th></th>
<th></th>
<th>Mutually Adjusted Marker 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>pLRT</td>
<td>RR (95% CI)</td>
<td>pLRT</td>
<td>RR (95% CI)</td>
<td>pLRT</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OxLDL</td>
<td>1.31 (0.67–2.57)</td>
<td>0.5</td>
<td>2.80 (1.44–5.44)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OxLDL</td>
<td>1.70 (0.93–3.13)</td>
<td>0.1</td>
<td>2.82 (1.48–5.38)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OxLDL</td>
<td>1.75 (0.77–4.01)</td>
<td>1.0</td>
<td>2.67 (1.09–6.53)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OxLDL</td>
<td>1.17 (0.51–2.68)</td>
<td>0.2</td>
<td>4.30 (1.76–10.52)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OxLDL</td>
<td>1.17 (0.51–2.68)</td>
<td>0.2</td>
<td>4.30 (1.76–10.52)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multivariates included: body mass index, physical activity, alcohol consumption, history of high blood pressure, high cholesterol and diabetes, family history of myocardial infarction, and use of aspirin. The p value of the likelihood ratio test (LRT) is for adding the corresponding marker to the model; it compares the model with corresponding marker with the model without the corresponding marker with 4 df. Each marker is added to the model in quintiles with 4 dummy variables.

RR = relative risk in the highest compared with the lowest quintile of each marker; Ox = oxidized; other abbreviations as in Tables 1 to 3.

17) found that oxLDL measured via the antibody 4E6 is significantly associated with apoB but not with Lp(a). These data do highlight the importance of antibody selection, because different antibodies might reflect different components of oxLDL.

Two cross-sectional studies (41,42), with the antibody FOH1a/DLH3 and the antibody E06 to detect oxLDL, found that oxLDL was significantly associated with CHD after adjustment for apoB. Because there is 1 apoB particle/LDL particle, it is important to adjust apoB even if antibodies FOH1a/DLH3 and E06 are not specifically against apoB. These two studies indirectly support our contention that antibodies other than the most widely used antibody 4E6 could be alternative choices. Alternatively, a combination of antibodies should be evaluated simultaneously in future prospective studies.

We think our data have clinical implications for several reasons. Our study is the largest prospective study including men and women, and we compared oxLDL with other lipid markers after finely controlling multiple CHD risk factors. Given that so many studies that had used this 4E6 antibody found a strong relationship between this marker and CHD without finely controlling lipid variables in their analyses, our results for the first time raise the questions of whether oxLDL but not independent of the two strongest risk factors, apoB and TC/HDL-C ratio, or other conventional lipid markers, including LDL-C, HDL-C, and triglycerides. Apolipoprotein B100 and TC/HDL-C ratio are more predictive in development of CHD than oxLDL. Measurement of oxLDL with multiple antibodies is needed in future large-scale, prospective studies to address the long-desired question of whether oxLDL is an independent risk factor of CHD.

In conclusion, we found that circulating oxLDL, measured by the most widely used antibody 4E6, can predict the risk of CHD on level of oxLDL at baseline. However, other antibodies for measuring oxLDL were not available from these studies at the time of this analysis. We recognize that the risk of CHD in our cohorts might be lower than in other populations. However, major cohort studies, such as the Framingham Heart Study, have also not relied on national samples. The advantages of our cohorts are that our participants had a high level of education and can provide high-quality and valid information on self-administered forms. Data validity is the chief prerequisite for generalizing results. The range of lifestyle and biochemical variables in our cohorts is quite broad. In previous analyses we observed that the associations for CHD and other diseases are very similar to those found in other broad-based U.S. populations.

In conclusion, we found that circulating oxLDL, measured by the most widely used antibody 4E6, can predict the risk of CHD but not independent of the two strongest risk factors, apoB and TC/HDL-C ratio, or other conventional lipid markers, including LDL-C, HDL-C, and triglycerides. Apolipoprotein B100 and TC/HDL-C ratio are more predictive in development of CHD than oxLDL. Measurement of oxLDL with multiple antibodies is needed in future large-scale, prospective studies to address the long-desired question of whether oxLDL is an independent risk factor of CHD.

Reprint requests and correspondence: Dr. Tianying Wu, Department of Nutrition, Harvard School of Public Health, 655 Huntington Avenue, Boston, Massachusetts 02115. E-mail: tianying@hsph.harvard.edu.

REFERENCES


