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Brief Communication

Oxidized LDL and the Risk of Coronary Heart Disease: Results from the MONICA/KORA Augsburg Study

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BACKGROUND: Oxidative stress plays a critical role in the initiation and progression of atherosclerosis. Oxidized LDL (oxLDL) is a marker of oxidative stress. We prospectively investigated whether increased serum oxLDL concentrations are associated with incident coronary heart disease (CHD).

METHODS: We conducted a prospective population-based case–cohort study within the MONICA/KORA Augsburg studies. Serum oxLDL concentrations were measured in 333 case individuals with incident CHD and in 1727 noncase individuals selected from a source population of 9300 middle-aged, healthy men and women. The mean (SD) follow-up time was 10.8 (4.6) years.

RESULTS: Baseline oxLDL concentrations were higher in case individuals than in noncase individuals ($P < 0.001$). After adjustment for age, sex, survey, smoking status, systolic blood pressure, physical activity, diabetes, body mass index, parental history of myocardial infarction, and alcohol consumption, the hazard ratio (HR) for comparing the first and third tertiles was 1.87 (95% CI, 1.33–2.64; $P < 0.001$). Additional adjustment for lipid parameters, inflammatory markers, and markers of endothelial dysfunction attenuated the association (HR, 1.29; 95% CI, 0.88–1.89; $P = 0.087$). We observed no significant interactions between oxLDL and sex or being overweight.

CONCLUSIONS: Increased oxLDL concentrations were associated with an increased risk for incident CHD. Nevertheless, because this effect became nonsignificant after adjustment for covariates, particularly the ratio of total cholesterol to HDL cholesterol, it may be mediated primarily by lipid parameters. Further studies are warranted to clarify this issue.

Despite the involvement of oxidized LDL (oxLDL)[4] in all stages of atherosclerosis from the initiation of fatty streaks to the development of plaque instability and rupture, the potential association of systemically measured oxLDL with coronary heart disease (CHD) is still a matter of controversy *(1, 2)*. oxLDL has been hypothesized to induce foam cell formation, an early yet critical step in the development of atherosclerosis *(3)*. Furthermore, oxLDL downregulates endothelial nitric oxide synthase, increases the formation of metalloproteinases, and induces apoptosis in human coronary endothelial cells *(4–6)*. Nevertheless, the data from epidemiologic studies remain controversial. Although several studies that investigated the association between circulating levels of oxidative biomarkers and CHD yielded fairly strong associations, others were not able to demonstrate any meaningful relationship with CHD *(7, 8)*. We sought to further elucidate whether oxLDL is a predictor of incident CHD in a large prospective population-based cohort study of middle-aged men and women.

We conducted a case–cohort study that used data from the population-based MONICA/KORA (Monitoring of Trends and Determinants in Cardiovascular Diseases/Kooperative Gesundheitsforschung in der Region Augsburg) Augsburg cohort study. The study design has previously been described *(9)*. In brief, 3 independent surveys were conducted with 13 427 participants (25–74 years of age). A combined end point that included incident fatal/nonfatal myocardial infarction (MI) and sudden cardiac death occurring before the age of 75 years was used as the outcome variable. Until 2000, the diagnosis of a major nonfatal MI event was based on the MONICA algorithm, which takes into account symptoms, cardiac enzymes, and electrocardiographic changes. After 2000, MI was diagnosed according to European Society of Cardiology and American College of Cardiology criteria. Deaths from MI were validated by autopsy reports, death certificates, chart review, and information from the last treating physician. Owing to the low incidence of CHD

[4] Nonstandard abbreviations: oxLDL, oxidized LDL; CHD, coronary heart disease; MONICA, Monitoring of Trends and Determinants in Cardiovascular Diseases; KORA, Kooperative Gesundheitsforschung in der Region Augsburg; MI, myocardial infarction; apo B-100, apolipoprotein B-100; sICAM-1, soluble intercellular adhesion molecule 1; HR, hazard ratio; AUC, area under the ROC curve.

in individuals <35 years old, the present study was limited to 10 718 persons between the ages of 35 and 74 years at baseline. After the exclusion of participants with self-reported prevalent CHD, the source population for the present study comprised 9300 individuals. For the case–cohort study, a random sample of the source population was selected and stratified by sex and survey. Participants with missing values for oxLDL or any of the covariables were excluded. The present analysis comprised 1079 men and 981 women.

Serum samples stored at −80 °C were used to analyze baseline oxLDL levels. Previous work by Holvoet et al. *(10)* has convincingly shown that oxidative biomarkers can be measured in samples that have been stored for >15 years. Serum oxLDL concentrations were measured by ELISAs from Mercodia. We used the monoclonal antibody 4E6, which is directed specifically against an epitope in the apolipoprotein B-100 (apo B-100) moiety of oxLDL that is formed from the substitution of aldehydes for lysine residues in apo B-100. The intra- and interassay CVs were <10%. All statistical analyses have been described in detail elsewhere *(9)*. Cox proportional hazards analysis was used to assess the association between oxLDL and incident CHD by using different adjustments for classic cardiovascular risk factors. Owing to the case–cohort design, SEs and *P* values were determined by the sampling-weight approach and robust variance estimates developed by Barlow et al. [see *(9)* and references therein]. All evaluations were performed with the statistical software package SAS (version 9.1 for Windows; SAS Institute).

Overall, 2060 participants (333 with incident CHD and 1727 without incident CHD) were included in this study. The mean (SD) follow-up time was 10.8 (4.6) years. The baseline demographic and laboratory characteristics are shown in Table 1. The geometric mean (antilogarithm of the SE for the logarithmic mean) for oxLDL was 103.3 (1.02) U/L in case individuals and 87.8 (1.01) U/L in noncase individuals.

We calculated Pearson partial correlation coefficients between the logarithm of the oxLDL concentration, inflammatory markers, and lipid markers and found a moderate but statistically significant correlation between the logarithm of the oxLDL concentration and the logarithm of interleukin-6, the logarithm of the C-reactive protein concentration, soluble E-selectin, and soluble intercellular adhesion molecule 1 (sICAM-1). We also found a strong, significant correlation between oxLDL and total cholesterol, HDL, the total cholesterol–HDL ratio, and LDL (see Table 1 in the Data Supplement that accompanies the online version of this brief communication at http://www.clinchem.org/content/vol57/issue8).

Table 2 shows the results of the Cox proportional hazards analysis, in which we assessed the association of baseline oxLDL concentrations with incident CHD. Interaction analyses using likelihood ratio tests showed no significant interactions between oxLDL and sex, smoking status, or being overweight (data not shown). In age-, sex- and survey-adjusted analyses (model 1), there was a strong, statistically significant association between increased oxLDL concentrations and incident CHD [hazard ratio (HR) for third tertile vs first tertile, 2.30; 95% CI, 1.67–3.16; *P* for trend, <0.001]. After further adjustment for traditional cardiovascular risk factors (model 2), as well as for the total cholesterol–HDL ratio (model 3) and for inflammatory markers (C-reactive protein and interleukin-6) plus markers of endothelial dysfunction (sICAM-1 and soluble E-selectin) (model 4), the effect was attenuated but still statistically significant (Table 2). With full adjustment for all of these parameters (model 5), the effect was weakened and became statistically nonsignificant (HR, 1.29; 95% CI, 0.88–1.89; *P* for trend, 0.087).

Furthermore, Table 2 shows that the predictive accuracy in the diagnosis of incident CHD, as quantified by the area under the ROC curve (AUC), was increased by oxLDL in the basic models, whereas the effect was lost in the fully adjusted model. This finding was confirmed by the 2 other measures of accuracy (integrated discrimination improvement, net reclassification index), which showed only a marginal additional contribution of oxLDL in predicting CHD events.

The present report represents the largest single study to date on the relationship of oxLDL concentration to incident CHD. Despite several reports linking oxLDL to stable CHD *(11)*, a higher Framingham risk score *(12)*, and the number and size of plaques *(13)*, further prospective data on the association of oxLDL with subsequent coronary events are sparse. In a previous prospective nested case–control study of initially healthy men, we compared plasma oxLDL concentrations for 88 cases and 258 matched controls *(7)*. Baseline plasma oxLDL concentrations were significantly higher in men who subsequently experienced a coronary event than in the matched controls [mean (SD), 110 (32) U/L vs 93 (28) U/L; $P \leq 0.001$], and the HR for future CHD events (comparing the first and third tertiles) was 2.79 (95% CI, 1.21–6.42) after adjustment for traditional cardiovascular risk factors, C-reactive protein, and conventional lipid markers. Two additional studies of patients without preexisting vascular disease yielded discrepant results. Wu et al. identified 266 male and 235 female patients from 2 populations who were matched with 2 controls each and reported a moderate but statistically significant association be-

Table 1. Baseline demographic, clinical, and laboratory characteristics of study participants with and without incident CHD during follow-up (n = 2060).[a]

Characteristic	CHD case individuals (n = 333)	Non-CHD individuals (n = 1727)	*P*
Age, years[b]	57.3 (0.44)	52.3 (0.26)	<0.001
Education <12 years, %	79.3 (2.6)	76.5 (1.5)	0.271
Smoking status, %			<0.001
Current smoker	38.8 (3.1)	23.4 (1.5)	
Former smoker	33.6 (3.0)	28.4 (1.6)	
Never smoker	27.5 (2.8)	48.3 (1.7)	
Frequency of exercise, %			<0.001
Inactive	72.3 (2.8)	61.1 (1.7)	
Alcohol consumption, %[c]			0.255
0/0 g/day	29.5 (2.9)	31.6 (1.6)	
<0–39.9/0–19.9 g/day	39.8 (3.1)	42.5 (1.7)	
≥40/≥20 g/day	30.7 (2.9)	25.9 (1.5)	
Body mass index, kg/m^2[b]	28.4 (0.23)	27.1 (0.10)	<0.001
Waist-to-hip ratio[b,d]	0.929 (0.005)	0.868 (0.002)	<0.001
Parental history of MI, %			0.015
Positive	23.2 (2.7)	20.0 (1.4)	
Unknown	26.7 (2.8)	21.1 (1.4)	
Negative	50.1 (3.1)	58.9 (1.7)	
History of hypertension or actual hypertension, %	65.1 (3.0)	41.2 (1.7)	<0.001
Systolic blood pressure, mmHg[b]	142.9 (1.15)	133.6 (0.47)	<0.001
Diastolic blood pressure, mmHg[b]	83.6 (0.68)	81.5 (0.27)	0.004
Prevalent diabetes, %	18.3 (2.4)	4.4 (0.7)	<0.001
Current HRT, %[e,f]	5.5 (1.4)	10.4 (1.1)	0.109
TC/HDL ratio[b]	5.74 (0.12)	4.51 (0.04)	<0.001
C-reactive protein, mg/L[g]	2.53 (1.06)	1.44 (1.03)	<0.001
IL-6, pg/mL[g]	3.15 (1.05)	2.00 (1.03)	<0.001
sICAM-1, ng/mL[b]	872.0 (19.1)	755.9 (6.9)	<0.001
sE-selectin, ng/mL[b]	65.9 (2.08)	55.3 (0.65)	<0.001
oxLDL, U/L[g]	103.3 (1.02)	87.8 (1.01)	<0.001

[a] Data for categorical variables are expressed as weighted percentages. Differences between groups were evaluated with the χ^2 test. The expression and statistical analysis of other data are as indicated. Sampling weights in analyses are as follows: cases = all cases/nonmissing cases; noncases = 1/sampling fraction, with sampling fraction being equal to the subcohort/full cohort without cases for each sex and survey.
[b] Data for normally distributed continuous variables are expressed as the weighted mean (SE). Differences between groups were evaluated with the *t*-test.
[c] Alcohol-consumption groups for men and women are presented as follows: (amount consumed daily for men)/(amount consumed daily for women).
[d] Measured only in participants of surveys 2 and 3 (case individuals, n = 229; noncase individuals, n = 1170).
[e] HRT, hormone-replacement therapy; TC/HDL ratio, total cholesterol–HDL ratio; IL-6, interleukin-6; sE-selectin, soluble E-selectin.
[f] Only for women ≥50 years of age (case individuals, n = 68; noncase individuals, n = 517) with no current use of oral contraceptives.
[g] Skewed continuous variables are expressed as the weighted geometric mean (antilogarithm of the SE of the logarithmic mean).

tween oxLDL concentration and CHD risk, which was attenuated and no longer predictive after adjustment for standard lipid variables *(8)*. In contrast, Nordin Fredrikson et al. showed in a small nested case–control study in 2003 that an increased circulating oxLDL concentration at baseline was associated with a higher risk of cardiovascular disease *(14)*; however, this small prospective study, which analyzed 26 individuals per group, lacked the power to control for all potential confounders and in particular did not adjust for lipid markers.

In the present prospective population-based study, we found oxLDL to be positively associated with the risk of incident CHD. This association became

Table 2. HRs for incident CHD according to baseline oxLDL concentrations.

	oxLDL tertile							
	T1	T2, HR (95% CI)	T3, HR (95% CI)	*P* for trend	AUC without oxLDL	AUC with oxLDL	IDI[a]	NRI
Model 1[b]	1.0	1.18 (0.83–1.67)	2.30 (1.67–3.16)	<0.001	0.799	0.808	0.007	0.086
Model 2[c]	1.0	1.08 (0.74–1.56)	1.87 (1.33–2.64)	<0.001	0.831	0.835	−0.001	0.026
Model 3[d]	1.0	0.99 (0.69–1.41)	1.50 (1.06–2.13)	0.009	0.816	0.818	0.001	0.031
Model 4[e]	1.0	1.03 (0.71–1.48)	1.88 (1.35–2.61)	<0.001	0.822	0.827	0.004	0.055
Model 5[f]	1.0	0.89 (0.60–1.30)	1.29 (0.88–1.89)	0.087	0.845	0.845	−0.003	0.015

[a] IDI, integrated discrimination improvement; NRI, net reclassification index; T1, tertile 1.
[b] Adjustment for age, sex, and survey.
[c] Adjustment for age, sex, survey, smoking status, systolic blood pressure, physical activity, diabetes, body mass index, parental history of MI, and alcohol consumption.
[d] Adjustment for age, sex, survey, and total cholesterol–HDL ratio.
[e] Adjustment for age, sex, survey, C-reactive protein, interleukin-6, sICAM-1, and soluble E-selectin.
[f] Adjustment for age, sex, survey, smoking status, systolic blood pressure, physical activity, diabetes, body mass index, parental history of MI, alcohol consumption, total cholesterol–HDL ratio, C-reactive protein, interleukin-6, sICAM-1, and soluble E-selectin.

nonsignificant after adjustment for various established cardiovascular risk factors and additional markers of subclinical inflammation. Although the incremental value in the AUC observed with oxLDL was small, it still could be clinically important, given the relative insensitivity of the AUC for detecting moderately sized effects. For example, even widely established cardiovascular risk factors, such as systolic blood pressure and cholesterol, are associated with small incremental gains in the AUC for predicting cardiovascular events *(15)*.

Recently, Zeibig et al. reported that oxLDL influences the mechanical stability of atherosclerotic plaques *(16)*. Repeated systemic administration of Fc-CD68, a new oxLDL-binding protein, significantly reduced the occurrence of spontaneous ruptures of established plaques in $ApoE^{-/-}$ mice by 20%, and drastically increased the collagen content of plaques. Pertinent to antigen-induced anti-oxLDL antibodies, recombinant anti-oxLDL antibodies were recently shown to reduce the progression of atherosclerosis and plaque inflammation in $ApoE^{-/-}$ and Apobec-$1^{-/-}$/LDL $receptor^{-/-}$ mice, respectively *(17, 18)*.

Several limitations of our study need to be addressed. First, oxLDL concentrations were significantly correlated with measures of subclinical inflammation; however, regression diagnostics revealed no colinearity in the final models. Second, the number of female cases was considerably lower than the number of male cases; however, no interactions between oxLDL tertiles were seen in likelihood ratio tests. Third, the total circulating oxLDL may be indicative not only of the extent of atherosclerosis but also of oxidative processes in remote tissues, such as adipose tissues. Nevertheless, no significant interactions of oxLDL with being overweight were found.

Our study also has several strengths, including its population-based prospective design, the large number of incident cases, the simultaneous measurement of oxidative and inflammatory factors, a long follow-up period, minimization of the likelihood of survival bias because both fatal and nonfatal coronary events were included, and careful adjustment for conventional and several emerging risk factors by using multivariable methods.

In this large prospective case–cohort study, increased oxLDL concentrations did not remain independently predictive of incident coronary events after adjustment for lipid markers and markers of inflammation/endothelial dysfunction. A considerable portion of the effects of oxLDL on CHD seems to be mediated by lipid parameters. Large studies are warranted to further evaluate this issue.

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