Circulating oxidized LDL: determinants and association with brachial flow-mediated dilation

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Abstract Circulating oxidized LDL (oxLDL) levels are strongly correlated to LDL-cholesterol (LDL-c) and apolipoprotein-B100 (apoB100), making it difficult to disentangle their independent contributions to cardiovascular risk. We explored the determinants of oxLDL and the relation between oxLDL and flow-mediated dilation (FMD) of the brachial artery to investigate whether the oxLDL/LDL-c and oxLDL/apoB100 ratios are more informative than the separate variables. FMD of the brachial artery and plasma concentrations of oxLDL, LDL-cholesterol, and apoB100 were measured in 624 men and women (age range 50 to 87 years), participating in a population-based cohort study. OxLDL was strongly correlated with apoB100 ($r = 0.82$, $P < 0.001$) and LDL-c ($r = 0.67$, $P < 0.001$). Other major independent determinants of oxLDL were sex, HDL-cholesterol, and LDL particle size. LDL-c and apoB100 concentrations were not significantly associated with FMD. After adjustment for age, sex, glucose tolerance status, and Framingham risk score, the oxLDL/apoB100 ratio was negatively related to FMD ($P = 0.017$). This association was weaker for the oxLDL/ LDL-c ratio ($P = 0.062$) and absent for oxLDL level ($P = 0.27$). In contrast to oxLDL, the oxLDL/apoB100 ratio, and to a lesser extent the oxLDL/LDL-c ratio, are related to a functional measure of atherosclerosis. Therefore correction of oxLDL for LDL particle number may improve the clinical usefulness of oxLDL measurement.— van der Zwan, L. P., T. Teerlink, J. M. Dekker, R. M. A. Henry, C. D. A. Stehouwer, C. Jakobs, R. J. Heine, and P. G. Scheffer. **Circulating oxidized LDL: determinants and association with brachial flow-mediated dilation. *J. Lipid Res.* 2009.** 50: **342–349.**

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Atherosclerosis is considered a process involving the interplay of inflammation and oxidative stress. Oxidation of LDL and the subsequent uptake by macrophages in the vascular wall are important steps in the development of atherosclerosis (1). A small part of the oxidized LDL (oxLDL) particles escapes uptake by macrophages and returns to the blood stream or may leak from atherosclerotic plaques. Thus, measuring circulating levels of oxLDL may contribute to the estimation of cardiovascular disease (CVD) risk. In support of this notion, concentrations of oxLDL were elevated in patients with stable and unstable angina and acute myocardial infarction (2). In apparently healthy middle-aged men, oxLDL was found to be a strong predictor for acute coronary heart disease events (3).

The concentration of oxLDL depends not only on the degree of oxidative stress, but also on the amount of substrate for oxidation (i.e., the number of LDL particles). Indeed, oxLDL levels were consistently found to be strongly correlated to LDL-cholesterol (LDL-c) and apolipoprotein-B100 (apoB100) in several studies (3–8), making it difficult to disentangle their separate contributions to CVD. Therefore, to establish the additional value of oxLDL in the assessment of CVD risk, it may be necessary to account for the number of LDL particles. LDL-c and apoB100 have been used for this purpose, but their merits have never been compared.

Impaired flow-mediated dilation (FMD) of the brachial artery has been shown to predict future cardiovascular events (9, 10). Previous studies have investigated the relationship between oxLDL and FMD in specific patient populations (11–13), but this relation has not yet been evaluated in a large-population-based sample. We have used FMD data from the Hoorn Study, a community-based cohort study among elderly men and women, to assess the suitability of the oxLDL/LDL-c and oxLDL/apoB100 ratios to correct for LDL particle number. We have explored the determinants of oxLDL and the relations between oxLDL and the oxLDL/LDL-c and oxLDL/apoB100 ratios

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with FMD to establish whether these ratios provide information beyond the separate variables.

MATERIALS AND METHODS

Subjects

The present investigation was conducted in the 2000 follow-up examination of the Hoorn Study (14) and the Hoorn Screening Study (15), both of which were population-based studies in Caucasians. From the study population (n = 822), we excluded persons using lipid-lowering medication (n = 134) and with missing data on primary variables of interest (n = 64), leaving 624 individuals (300 men and 324 women), of whom 233 had normal glucose metabolism, 159 impaired glucose metabolism, and 232 type 2 diabetes according to WHO-99 criteria (16). The local ethics committee approved the study and all participants gave their written informed consent.

Plasma lipids and apoB100

LDL-c was directly determined by the "N-geneous" assay (GenZyme, Cambridge, MA). Intra- and interassay coefficients of variation (CV) were 0.7% and 2.7%, respectively. Triglyceride concentrations up to 13.5 mmol/L do not interfere with this assay. Total and HDL-cholesterol (HDL-c) and triglycerides were measured by standard enzymatic methods (Roche, Mannheim, Germany). ApoB100 concentrations were determined nephelometrically using an "Immage 800" immunochemistry system (Beckman Coulter Inc., Fullerton, CA) with intra- and interassay CV of 4.9% and 5.1%, respectively.

In vivo oxLDL

A competitive ELISA (Mercodia, Uppsala, Sweden) was used to determine oxLDL concentrations in EDTA-plasma. The 4E6-monoclonal antibody of the assay is directed against apoB100 with at least 60 lysines substituted by aldehydes (2) and is highly specific for oxLDL (17). Intra- and interassay CV were 6.7% and 7.0%, respectively. To estimate the extent of LDL oxidation, the ratio of oxLDL to LDL-c and the ratio of oxLDL to apoB100 were calculated. These estimates were expressed as U/mmol LDL-c and U/g apoB100, respectively.

LDL particle size and in vitro oxidizability

LDL was isolated by ultracentrifugation between densities 1.019 and 1.063 kg/L. LDL particle size was determined by high-performance gel-filtration chromatography as described previously, using thyroglobulin (17.0 nm) and fibrinogen (22.2 nm) as calibrators (18). Intra- and inter-assay CV were 0.1% and 0.2%, respectively.

The susceptibility of LDL to in vitro oxidation was determined by measurement of conjugated dienes, after addition of copper ions as pro-oxidant, as previously described (19). The resistance of LDL to oxidation was expressed as lag time (min). The intra- and interassay CV were 1.6% and 3.6%, respectively.

Glucose metabolism parameters

HbA1c was analyzed by ion exchange high-performance liquid chromatography (Bio-Rad, Veenendaal, The Netherlands). Fasting glucose was measured enzymatically (Roche, Mannheim, Germany) and fasting insulin with a double-antibody radioimmunoassay (Linco Research, St. Louis).

TABLE 1. Subject characteristics in tertiles of oxidized LDL (oxLDL)

	Unit	Overall	1st tertile	2nd tertile	3rd tertile	$P_{\text{for trend}}$
OxLDL, range	U/L		<57.6	57.6–71.3	>71.3	
Number		624	208	208	208	
Age	years	69.0 (7.3)	69.1 (7.6)	69.7 (7.4)	68.3 (6.6)	0.22
Female sex	%	52	46	53	57	0.019
ApoB100	g/L	1.04 (0.23)	0.85 (0.15)	1.03 (0.15)	1.26 (0.19)	<0.001
LDL-c	mmol/L	3.8 (0.9)	3.1 (0.7)	3.7 (0.6)	4.4 (0.8)	<0.001
HDL-c	mmol/L	1.39 (0.40)	1.50 (0.43)	1.42 (0.40)	1.25 (0.34)	<0.001
Triglycerides[a]	mmol/L	1.3 (1.0–1.8)	1.1 (0.8–1.4)	1.3 (1.0–1.7)	1.8 (1.3–2.4)	<0.001
Total cholesterol	mmol/L	5.8 (1.0)	5.1 (0.8)	5.8 (0.8)	6.5 (0.9)	<0.001
LDL particle size	nm	21.49 (0.45)	21.60 (0.38)	21.55 (0.42)	21.32 (0.51)	<0.001
LDL oxidizability	min	72.0 (9.7)	72.7 (9.9)	71.4 (9.0)	71.9 (10.2)	0.41
Glucose metabolism						
- Impaired	%	25.5	26.9	20.2	29.3	0.57
- Type 2 diabetes	%	37.2	34.6	38.0	38.9	0.36
HbA1c	%	6.1 (0.8)	6.0 (0.7)	6.0 (0.6)	6.2 (0.9)	0.005
Fasting glucose[a]	mmol/L	6.0 (5.5–6.9)	6.0 (5.5–6.7)	6.0 (5.5–7.0)	6.2 (5.6–7.2)	0.018
Insulin[a]	pmol/L	60 (42–88)	55 (40–78)	61 (43–91)	65 (45–94)	0.002
C-reactive protein[a]	mg/L	2.3 (1.1–4.8)	1.8 (1.0–3.4)	2.3 (1.0–4.5)	2.7 (1.4–6.5)	<0.001
Serum albumin	g/L	42 (40–43)	42 (40–43)	41 (40–43)	41 (40–43)	0.20
BMI	kg/m^2	27.6 (4.3)	26.9 (4.3)	27.6 (4.1)	28.3 (4.4)	<0.001
Waist circumference	cm	96.1 (12.5)	94.4 (12.3)	95.6 (12.2)	98.2 (12.7)	0.002
DBP	mmHg	83 (11)	83 (10)	83 (11)	83 (11)	0.47
SBP	mmHg	142 (21)	141 (18)	143 (23)	143 (22)	0.38
Alcohol intake						
<1 g/day	%	29	29	26	31	0.63
1–40 g/day	%	64	62	66	63	0.78
>40 g/day	%	8	9	7	6	0.18
Current smoking	%	17	13	16	20	0.050
(Micro-)albuminuria	%	15	14	15	15	0.87
Prior CVD	%	44	41	44	48	0.13

ApoB100, apolipoprotein-B100; BMI, body mass index; CVD, cardiovascular disease. Values are displayed either as means (SD), medians (interquartile range), or percentages.

[a] Variables were log-transformed prior to linear trend analysis.

Measurement of endothelium-dependent and endothelium-independent dilation

Ultrasound examination of the right brachial artery was performed according to the guidelines of the International Brachial Artery Reactivity Task Force (20). Baseline diameter, blood flow (peak systolic velocity), FMD, and nitroglycerin-mediated dilation (NMD) were determined by one single observer (RMAH) as previously described (21). The intraobserver CV were 4.3% for diameter, 14.7% for FMD, and 10.3% for NMD (21). Of the 624 participants, qualitatively satisfactory ultrasound examinations were obtained in 484 individuals. Poor definition of the arterial wall due to obesity and inability to remain motionless due to muscoskeletal disorders were the main reasons for missing ultrasound data (21).

Other measurements

Plasma C-reactive protein (CRP) was measured with a highly sensitive in-house sandwich enzyme-linked immunosorbent assay (22). Data on smoking habits and alcohol consumption were obtained by a questionnaire. Microalbuminuria was defined as urinary albumin-creatinine ratio ⩾2 mg/mmol. Prior CVD was defined as Minnesota Code 1.1–1.3, 4.1–4.3, 5.1–5.3, or 7.1 on the electrocardiogram or coronary bypass operation or angioplasty, or an ankle-brachial blood pressure index <0.9 in either leg, peripheral arterial bypass, or amputation for atherosclerotic disease. The Framingham risk score was calculated (23).

Statistical analyses

Data are presented as mean (SD) or median (interquartile range). Skewed variables were log-transformed prior to trend analyses and multiple linear regression analyses. In regression models for FMD, we considered age, sex, glucose tolerance status, baseline diameter, and the increase in peak systolic velocity as standard correction variables. In models for NMD, age, sex, glucose tolerance status, and baseline diameter were included. $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using SPSS software, version 15 (SPSS Inc., Chicago, IL).

RESULTS

Subject characteristics

The characteristics of the subjects by tertiles of circulating oxLDL levels are shown in **Table 1**. With increasing oxLDL concentrations, the percentage of women, apoB100, LDL-c, triglycerides, total cholesterol, HbA1c, fasting glucose, insulin, CRP, body mass index, and waist circumference increased significantly, while a decreasing trend was observed for HDL-c and LDL-size. Current smoking status tended to a significant positive relationship with oxLDL. There were no significant linear trends for age, LDL in vitro oxidation, serum albumin, blood pressure, alcohol consumption, microalbuminuria, and prior CVD across the oxLDL tertiles.

Adjustment of oxLDL for particle number

The trends with oxLDL shown in Table 1 were confirmed by linear regression analysis after adjustment for age and sex (**Table 2**). The associations of oxLDL with apoB100 and LDL-c were particularly strong, reflecting the fact that the total amount of LDL is an important determinant of oxLDL concentration (Table 2 and **Fig. 1**). To correct oxLDL levels for LDL particle number, the oxLDL/LDL-c and oxLDL/apoB100 ratios were calculated.

TABLE 2. Age and sex adjusted associations with oxLDL, and the oxLDL/LDL-c and oxLDL/apoB100 ratios

		OxLDL		OxLDL/LDL-c		OxLDL/apoB100	
Variable	Units or categories	st. β	*P*	st. β	*P*	st. β	*P*
Age	Years	−0.085	0.033	−0.082	0.042	−0.031	0.45
Sex	Female vs. male	0.110	0.006	−0.054	0.18	−0.037	0.36
OxLDL	U/L			0.323	<0.001	0.322	<0.001
ApoB100	g/L	0.815	<0.001	−0.033	0.42	−0.275	<0.001
LDL-c	mmol/L	0.671	<0.001	−0.443	<0.001	−0.222	<0.001
HDL-c	mmol/L	−0.389	<0.001	−0.348	<0.001	−0.220	<0.001
Triglycerides[a]	mmol/L	0.541	<0.001	0.390	<0.001	0.156	<0.001
Total cholesterol	mmol/L	0.715	<0.001	−0.276	<0.001	−0.229	<0.001
LDL particle size	nm	−0.367	<0.001	−0.503	<0.001	−0.240	<0.001
LDL oxidizability (lagtime)	min	−0.003	0.95	0.081	0.044	0.064	0.11
Glucose metabolism	Impaired vs. normal	0.102	0.022	0.062	0.16	0.060	0.18
	Diabetes vs. normal	0.107	0.016	0.265	<0.001	0.172	<0.001
HbA1c	%	0.147	<0.001	0.202	<0.001	0.123	0.002
Fasting glucose[a]	mmol/L	0.141	<0.001	0.243	<0.001	0.141	<0.001
Insulin[a]	pmol/L	0.147	<0.001	0.288	<0.001	0.166	<0.001
C-reactive protein[a]	mg/L	0.148	<0.001	0.236	<0.001	0.127	<0.002
Serum albumin	g/L	−0.041	0.31	−0.059	0.15	−0.170	<0.001
BMI	kg/m^2	0.143	<0.001	0.139	<0.001	0.118	0.003
Waist circumference	cm	0.181	<0.001	0.185	<0.001	0.145	<0.001
Diastolic blood pressure	mmHg	0.045	0.26	0.064	0.11	0.027	0.50
Systolic blood pressure	mmHg	0.046	0.26	0.072	0.078	0.012	0.76
Alcohol intake	1–40 g/day	−0.008	0.85	−0.134	0.003	−0.142	0.002
	>40 g/day	−0.003	0.95	0.040	0.38	−0.026	0.57
Current smoking	Yes vs. no	0.077	0.055	0.051	0.20	0.046	0.25

Regression coefficients are presented as standardized β.
[a] Variables were log-transformed prior to analyses.

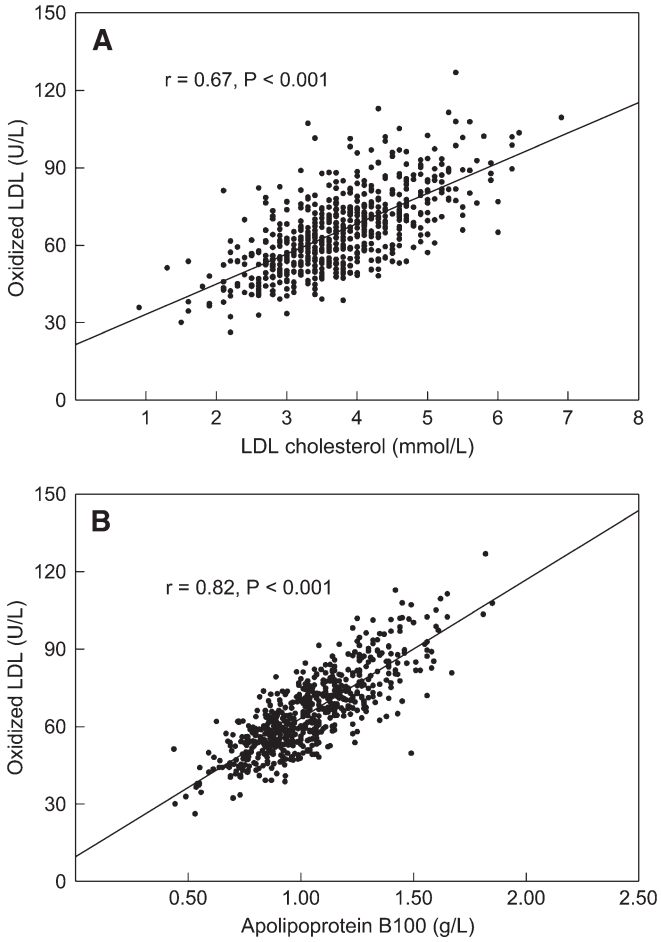

Fig. 1. The association between oxidized LDL and LDL cholesterol (A) and the association between oxidized LDL (oxLDL) and apolipoprotein-B100 (apoB100) (B).

Determinants of oxLDL

The variables associated with oxLDL/LDL-c and oxLDL/apoB100 ratios in linear regression analyses were identical to the variables associated with oxLDL, with a few exceptions (Table 2). The positive association with female sex was lost, and moderate alcohol intake (1–40 g/day) was negatively associated with both ratios. Furthermore, the oxLDL/apoB100 ratio was negatively associated with serum albumin.

To establish which variables other than LDL-c and apoB100 were independent determinants of (adjusted) oxLDL, we explored multivariate linear regression models with oxLDL, the oxLDL/LDL-c ratio, and the oxLDL/apoB100 ratio as dependent variables (**Table 3**). In all models, an identical set of potential predictor variables, excluding apoB100 and LDL-c, was used. Determinants other than age and sex were selected because of significant univariate associations and/or biological plausibility. Categories of glucose metabolism were used as a proxy for all variables associated with glucose metabolism. Because triglycerides were strongly associated with LDL particle size ($r = -0.67$; $P < 0.001$), only the latter was included in the models. LDL particle size was the only independent variable shared by all three models. Female sex and low HDL-cholesterol were additional major determinants of oxLDL. Additional independent determinants of the oxLDL/LDL-c and oxLDL/apoB100 ratios were high CRP and low serum albumin, respectively. Furthermore, a weak negative association between moderate alcohol intake and the oxLDL/apoB100 ratio was observed. Age, categories of glucose metabolism, waist circumference, and current smoking did not significantly contribute to any of the models. The full models explained 20%, 28%, and 11% of the variability of oxLDL, oxLDL/LDL-c, and oxLDL/apoB100, respectively. If LDL-c or apoB100 were added as independent variables to the regression model for oxLDL, the explained variance increased to 63 and 71%, respectively.

OxLDL and vascular function

Neither LDL-c nor apoB100 were associated with FMD in unadjusted analysis or after adjustment for the standard variables age, sex, baseline diameter, glucose tolerance status, and increase in peak systolic velocity (all $P > 0.5$). Mean FMD values did not significantly differ between subjects with levels of oxLDL below or above the median value (**Table 4** and **Fig. 2A**). In contrast, if the study population

TABLE 3. Multivariable linear regression models for oxLDL, the oxLDL/LDL-c ratio, and the oxLDL/apoB100 ratio

		OxLDL		OxLDL/LDL-c		oxLDL/apoB100	
Independent variable	Units of increase or categories	st. β	*P*	st. β	*P*	st. β	*P*
Age	Years	−0.060	0.12	−0.052	0.16	−0.042	0.31
Sex	Female vs. male	**0.30**	**<0.001**	0.059	0.16	0.028	0.54
Glucose metabolism	Impaired vs. normal	0.032	0.45	−0.029	0.47	0.016	0.73
	Diabetes vs. normal	−0.078	0.10	0.061	0.18	0.083	0.10
Waist circumference	cm	0.066	0.14	−0.019	0.66	0.046	0.33
LDL particle diameter	nm	**−0.25**	**<0.001**	**−0.43**	**<0.001**	**−0.16**	**0.003**
HDL-cholesterol	mmol/L	**−0.24**	**<0.001**	−0.039	0.43	−0.072	0.20
C-reactive protein	mg/L, log transformed	0.024	0.58	**0.11**	**0.006**	−0.010	0.82
Serum albumin	g/L	−0.020	0.61	−0.059	0.12	**−0.18**	**<0.001**
Current smoking	Yes vs. no	0.047	0.21	0.030	0.40	0.027	0.50
Alcohol intake	1–40 g/day	0.054	0.20	−0.052	0.19	**−0.088**	**0.047**
	>40 g/day	0.017	0.70	0.036	0.40	−0.004	0.93
R^2 model		0.20		0.28		0.11	

Regression coefficients are presented as standardized β; independent variables significantly contributing to the models are printed in bold.

TABLE 4. Brachial artery characteristics according to levels of oxidized LDL (oxLDL)

OxLDL	Below median <64 U/L	Above median >64 U/L	*P*
N	244	240	
Diameter (μm)			
Baseline	4,687 (768)	4,599 (702)	0.20
After FMD	4,866 (789)	4,768 (709)	0.18
After NMD	5,131 (803)	5,030 (707)	0.18
Absolute change in diameter (μm)			
After FMD	177 (171)	171 (145)	0.70
After NMD	453 (214)	448 (217)	0.62
Percentage change in diameter (%)			
After FMD	3.96 (3.74)	3.87 (3.36)	0.99
After NMD	10.17 (5.77)	10.07 (5.87)	0.85
Peak systolic velocity (cm/s)			
Baseline	58 (13)	57 (12)	0.40
After reactive hyperemia	105 (25)	105 (26)	0.68
% Increase	84 (39)	88 (48)	0.62

Results are expressed as mean (SD). FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation. *P* values were calculated by Mann-Whitney analyses.

was dichotomized according to the oxLDL/LDL-c ratio or oxLDL/apoB100 ratio, the mean FMD was approximately 15% lower in subjects with values of these ratios above the median (Fig. 2B, C). To investigate whether the relationship between (adjusted) oxLDL and FMD was independent of traditional risk factors, several multiple linear regression models were explored (**Table 5**). After adjustment for standard variables, oxLDL was negatively related to FMD, but this association lost significance upon adjustment for prior CVD or Framingham risk score. The oxLDL/LDL-c ratio was negatively related to FMD with borderline significance, in the standard model and after additional adjustment. In contrast, the negative association between the oxLDL/apoB100 ratio and FMD remained highly significant after adjustment for prior CVD, Framingham risk score, microalbuminuria, and waist circumference. Mean NMD values did not significantly differ between subjects with levels of oxLDL below or above the median value (Table 4). In linear regression models, neither oxLDL nor the oxLDL/LDL-c and oxLDL/apoB100 ratios were associated with NMD (all $P > 0.3$), and the negative associations between these variables and FMD were not attenuated after adjustment for NMD (Table 5, models 5 and 7).

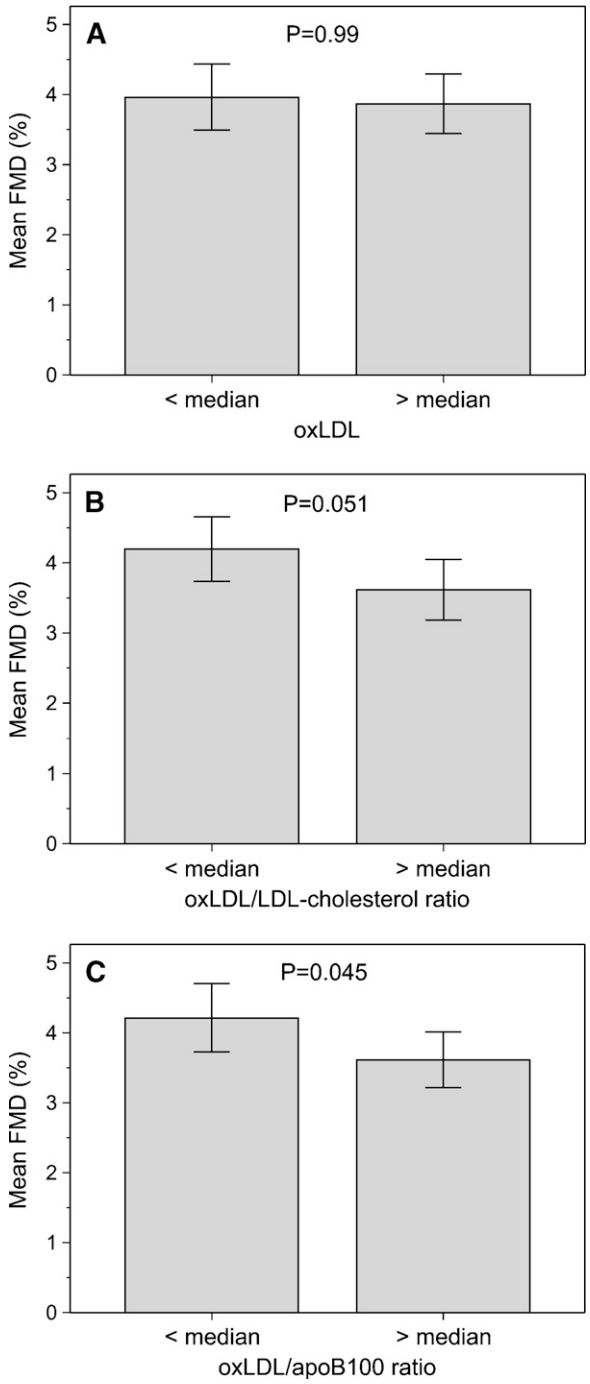

Fig. 2. Flow-mediated dilation (FMD) of the brachial artery according to low (below the median) or high (above the median) values of oxLDL (A), the oxLDL/LDL-cholesterol (LDL-c) ratio (B), or the oxLDL/ apoB100 ratio (C). FMD data are presented as means with 95% confidence intervals. Significance of differences was tested by Mann-Whitney test.

DISCUSSION

The present study shows that the total amount of LDL, either expressed as LDL-c or as apoB100 level, is by far the strongest determinant of oxLDL levels. In contrast to LDL-c, apoB100 and oxLDL concentrations; the oxLDL/LDL-c ratio; and, more prominently, the oxLDL/apoB100 ratio, independent of traditional risk factors, were related to endothelium-dependent dilation of the brachial artery. Correction of oxLDL for LDL particle number may thus improve the clinical usefulness of oxLDL measurement.

OxLDL concentrations not only depend on the level of oxidative stress, but also on the amount of substrate for oxidation (i.e., the total amount of LDL or the number of LDL particles). Because it is difficult to exactly determine the number of LDL particles, both LDL-c and apoB100 concentrations have been used as estimates (3–8). Adjustment of oxLDL for either LDL-c or apoB100 may be essential for a correct interpretation of the data, particularly in intervention studies. For example, Van Tits et al. (4) observed no effect of statin use on the oxLDL/

TABLE 5. Multivariable linear regression models with FMD as dependent variable and oxLDL, oxLDL/LDL-c ratio, or oxLDL/apoB100 ratio as predictor variables

Independent variable	Beta (95% confidence interval)	*P*-value
OxLDL, per 1 SD (15.4 U/L)		
Standard model	**−12.8 (−25.3 to −0.2)**	**0.048**
Model 1: standard + prior CVD	−12.1 (−24.7 to 0.5)	0.060
Model 2: standard + Framingham risk score	−8.0 (−22.3 to 6.3)	0.27
Model 3: standard + (micro)albuminuria	**−13.2 (−25.7 to −0.6)**	**0.040**
Model 4: standard + waist circumference	−11.1 (−23.8 to 1.7)	0.088
Model 5: standard + nitroglycerin-mediated dilation	**−11.8 (−23.2 to −0.6)**	**0.039**
Model 6: fully adjusted	−7.2 (−21.4 to 6.9)	0.32
Model 7: fully adjusted + nitroglycerin-mediated dilation	−8.1 (−20.7 to 4.6)	0.21
OxLDL/LDL-c ratio, per 1 SD (3.8 U/mmol)		
Standard model	−11.9 (−24.3 to 0.5)	0.060
Model 1: standard + prior CVD	−10.6 (−23.1 to 1.8)	0.093
Model 2: standard + Framingham risk score	−11.8 (−24.2 to 0.6)	0.062
Model 3: standard + (micro)albuminuria	−10.4 (−22.8 to 2.1)	0.10
Model 4: standard + waist circumference	−10.6 (−23.1 to 1.9)	0.097
Model 5: standard + nitroglycerin-mediated dilation	**−18.0 (−29.1 to −6.9)**	**0.002**
Model 6: fully adjusted	−8.3 (−20.8 to 4.2)	0.19
Model 7: fully adjusted + nitroglycerin-mediated dilation	**−14.6 (−25.8 to −3.4)**	**0.011**
OxLDL/apoB100 ratio, per 1 SD (9.1 U/g)		
Standard model	**−14.9 (−27.2 to −2.6)**	**0.018**
Model 1: standard + prior CVD	**−14.3 (−26.6 to −2.0)**	**0.023**
Model 2: standard + Framingham risk score	**−14.9 (−27.2 to −2.7)**	**0.017**
Model 3: standard + (micro)albuminuria	**−14.0 (−26.3 to −1.7)**	**0.025**
Model 4: standard + waist circumference	**−13.8 (−26.1 to −1.5)**	**0.028**
Model 5: standard + nitroglycerin-mediated dilation	**−16.9 (−27.9 to −5.9)**	**0.003**
Model 6: fully adjusted	**−12.8 (−25.1 to −0.5)**	**0.040**
Model 7: fully adjusted + nitroglycerin-mediated dilation	**−14.8 (−25.7 to −3.8)**	**0.008**

Standard model: determinant under consideration + age, sex, glucose tolerance status, baseline diameter, and increase in peak systolic velocity. Fully adjusted model: standard model + prior CVD, Framingham risk score, (micro)albuminuria, and waist circumference. Regression coefficients are expressed as absolute change in diameter (in μm) per 1 SD increase of the independent variable to facilitate direct comparison. Significant associations are printed in bold.

apoB100 ratio, although a significant decrease in unadjusted oxLDL concentration was observed. Thus, adjustments of oxLDL by LDL-c or apoB100 have been described in several studies, but, to our knowledge, a direct comparison of both adjustments has been performed in only one study (8). Both approaches may have some limitations. LDL-c is often estimated with the Friedewald formula, which may lead to reduced accuracy and precision. However, even if LDL-c is measured directly, as we did, it is not a perfect measure for LDL particle number, because LDL particles are heterogeneous and vary in cholesterol content (24). Due to the fact that each LDL particle contains exactly 1 apoB100 molcule, the apoB100 concentration may be a better estimator for the LDL particle number. Still, up to approximately 10% of apoB100 molcules is present in VLDL particles (25), resulting in a slight overestimation of LDL particle number. As illustrated in Fig. 1, the scatter in the relation between oxLDL and LDL-c was higher compared with the relation between oxLDL and apoB100. In agreement with this, the age and sex adjusted association between oxLDL and apoB100 was stronger than with LDL-c (Table 2). Altogether, apoB100 seems more suitable than LDL-c for adjustment of oxLDL.

In sex- and age-corrected analyses, many clinical and biochemical variables were significantly associated with oxLDL, and the oxLDL/LDL-c and oxLDL/apoB100 ratios (Table 2). In multivariate linear regression models with oxLDL, the oxLDL/LDL-c ratio, or the oxLDL/apoB100 ratio as dependent variable, most of these associations lost significance, and only a few independent determinants were identified. LDL particle diameter was by far the strongest predictor in all three models, in agreement with the observation that small LDL particles are more prone to oxidation than larger LDL particles (26). HDL-c was a negative determinant of oxLDL, consistent with the antioxidative properties of HDL (27). Likewise, the negative association between serum albumin and the oxLDL/apoB100 ratio may reflect the antioxidative properties of albumin. This association may, however, also reflect the relation between low albumin levels and low-grade inflammation (28). The strong positive association between CRP, a marker of inflammation (29), and the oxLDL/LDL-c ratio confirms that inflammation plays a role in the process of LDL oxidation.

Type 2 diabetes and variables related to glucose metabolism were positively related to oxLDL (Table 2), consistent with a role of LDL oxidation in the elevated cardiovascular morbidity and mortality in diabetes (30, 31). However, diabetes was not an independent determinant in the multivariate regression models. This may be explained by the fact that diabetic patients generally have low HDL-c levels and small LDL particles, both of which were associated with high oxLDL levels.

Alcohol consumption is related to CVD. Excessive alcohol consumption is proatherogenic, while moderate con-

sumption of alcohol may have an antiatherogenic effect. Paradoxically, Schroder et al. (32) observed a positive relation between oxLDL concentrations and moderate alcohol consumption. In the present study, moderate alcohol consumption was negatively associated with the oxLDL/LDL-c and oxLDL/apoB100 ratios. However, after full adjustment, this association was only borderline significant in the model for oxLDL/apoB100. Likewise, current smoking was positively associated with oxLDL with borderline significance in univariate analysis, but did not contribute to the multivariate models.

To investigate whether adjustment of oxLDL levels for LDL particle number may be relevant in clinical studies, we explored the impact of adjustment on the relation between oxLDL and FMD. Neither LDL-c nor apoB100 were significantly related to FMD, and oxLDL showed a borderline significant negative association. In contrast, the oxLDL/apoB100 ratio showed a significant, negative association with FMD, even after adjustment for other risk factors or NMD. These results strongly suggest that this ratio yields more information than the separate variables. Therefore, using the oxLDL/apoB100 ratio rather than the oxLDL level is not equivalent to statistical adjustment of the relation between oxLDL and FMD for apoB100.

Although our study was not designed to unravel the mechanisms linking elevated levels of oxLDL and FMD, our data allow drawing some tentative conclusions. Formation of oxLDL is promoted by oxidative stress, but oxLDL itself has also been identified as a potent stimulus for vascular free radical formation (33). This may lead to a vicious circle, making it virtually impossible to separate cause and effect. The inverse relation between oxLDL levels and FMD may indicate that oxidative stress is a common antecedent of diminished vasodilation and LDL oxidation. A second potential mechanism is that oxLDL itself, by inflicting damage to the endothelium, has a direct adverse effect on vasodilation. It should be noted that both mechanisms are not mutually exclusive but may act in concert. Our data, showing that the level of oxidative stress, as indicated by the oxLDL/apoB100 ratio, is a stronger determinant of FMD than the absolute concentration of oxLDL, supports a major role for the first mechanism.

The lack of a significant association between oxLDL/apoB100 and NMD suggests that the effect is mainly endothelium dependent. This is corroborated by the fact that the strength of the association between oxLDL/apoB100 and FMD was not attenuated by adjustment for NMD. Nitric oxide is the most important endothelium-derived vasodilator in conduit arteries, and it is well known that scavenging of nitric oxide by reactive oxygen species, in particular superoxide, reduces the bioavailability of nitric oxide in the vascular wall. Overall, the results of this study support the notion that the oxLDL/apoB100 ratio is an indicator of vascular production of reactive oxygen species, which by scavenging nitric oxide, limit vasodilation. However, our data do not fully rule out the possibility that oxLDL itself also has an adverse effect on vasodilation.

Our study had some limitations. First, the study population was limited to elderly Caucasians, and therefore the results may be different in other ethnic and age groups. Second, exclusion of subjects using lipid-lowering medication probably has led to selection of subjects with a low cardiovascular risk profile. This may have resulted in an underestimation of the strength of associations between oxLDL and CVD risk factors and vascular function.

Third, the assay used to measure oxLDL specifically detects epitopes of oxidized apoB100. However, oxLDL is not a single homogeneous entity, and assays detecting epitopes on other constituents of oxLDL may yield different results. A major strength of our study is that LDL-c was measured directly and not estimated by Friedewald formula. In addition, we had a large array of clinical and biochemical variables at our disposal to adjust for possible confounding.

Our data confirm that LDL-c and more prominently the number of LDL particles, estimated as apoB100, is a strong determinant of circulating oxLDL levels. The oxLDL/LDL-c and oxLDL/apoB100 ratios, which express the level of LDL oxidation independent of particle number, were shown to provide information beyond oxLDL, LDL-c, and apoB100, as evidenced by the independent association of these ratios with endothelium-dependent dilation of the brachial artery.

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