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LDL Oxidation and Its Association With Carotid Artery Intima-Media Thickness and Other Cardiovascular Risk Factors in a Sample of Spanish General Population

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Abstract

We studied the association between oxidized low-density lipoproteins (OxLDLs) and early atherosclerosis, assessed by carotid artery intima-media thickness (cIMT), as well as with other known atherosclerosis risk factors in a sample of the general middle- and old-age population of Burgos (Spain). Circulating OxLDL showed a significant and independent association with the average cIMT of both carotid arteries but not with the absence or presence of ≥ 1 carotid atheroma plaques. Plasma OxLDL concentrations were associated with age, smoking, low-density lipoprotein-cholesterol, and triglycerides, independently of other variables. Our findings in an asymptomatic sample representative of the Spanish middle- and old-age population underscore the role of OxLDL in early atherosclerosis represented by the cIMT especially in older asymptomatic individuals, but this cannot be extended to more advanced atherosclerosis, represented by carotid plaques.

Keywords

oxidized LDL, intima-media thickness, early atherosclerosis, cardiovascular risk factors, carotid arteries

Introduction

Circulating oxidized low-density lipoproteins (OxLDLs) may play an important role in the progress of atherosclerosis, since they are found in atherosclerotic lesions.¹ The modification of low-density lipoproteins (LDLs), caused partially by the end products of lipid peroxidation, potentiates their atherogenic nature.² They are taken up by the scavenger receptors of the macrophages and lead to the formation of foam cells, which give rise to fatty streaks and plaques.

Oxidative modification of LDL can generate “fully oxidized LDL” (recognized by scavenger receptors) and “minimally modified LDL” (not recognized by scavenger receptors).^{3,4} Both of these have been detected in atherosclerotic plaques, but only the latter exists in substantial amounts in the circulation.⁵

The OxLDL may not only contribute to foam cell generation but also stimulates the synthesis of adhesion molecules by endothelial cells and induces inflammatory processes¹ through mediators such as inflammatory cytokines or C-reactive protein (CRP).⁶ Holvoet and his colleagues⁷ for the first time and thereafter Toshima et al.⁸ found elevated plasma levels of OxLDL in patients with coronary artery disease.

Inflammation is thought to be involved in the pathogenesis of atherosclerosis,^{9,10} but few population-based studies have investigated the relationship between early atherosclerosis development and OxLDL, particularly in southern Europe where atherosclerosis

is less severe than in other more studied Western populations.¹¹ Moreover, the reports of such association with noninvasive imaging of atherosclerosis have yielded heterogeneous results.¹²⁻¹⁴

We evaluated the relationship between established and more recent cardiovascular risk factors, such as OxLDL, and related them with carotid artery intima-media thickness (cIMT); a surrogate marker of early atherosclerosis and cardiovascular disease risk, in a sample of a middle- and old-age population of Burgos (Spain).

Material and Methods

Population Studied

A total of 171 middle- and old-aged (age range 40-93 years) patients were randomly selected from the records of the 200000

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referral population of “Gamonal Antigua” Health Care Centre of the city of Burgos. They were selected, so that their age and sex percentage distribution matched the Spanish population in the 2004 to 2010 census of this age interval. They were asked by telephone to attend an interview in which participation in this study was proposed. The number of individuals needed to detect with an α error of 0.05, a power of 60%, and a 0.2-mm difference from a normal cIMT of 0.65 ± 0.15 mm was 180. A 10% of losses of recruited participants were expected but only 9 were lost, thus leaving 171 patients for analysis.

Due to economic limitations, serum levels of OxLDL were measured in a random subset of 127 patients. Ultrasensitive CRP (UsCRP) levels and established cardiovascular risk factors, such as blood pressure, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides, and apolipoprotein levels were also determined.

Blood pressure was assessed by the mean of 2 systolic and diastolic readings after 10 minutes rest in supine position. Hypertension was defined as a systolic/diastolic blood pressure of $>140/95$ mm Hg or current use of antihypertensive drugs. Body mass index (BMI), waist-to-hip ratio, and demographic data were also recorded.

A standardized questionnaire on current and past personal or familial cardiovascular history, alcohol or tobacco consumption, sociodemographic variables, diabetes mellitus, or other previous disease was completed by each participant.

Informed consent was obtained from each patient. The study was approved by the Clinical Studies Committee of the referral hospital.

Analytical Methods

Venous blood samples were collected under standardized conditions and after ≥ 12 hours of fasting, allowing only water intake. Total cholesterol and triglycerides were determined enzymatically (Roche Diagnostics, Basel, Switzerland), LDL-C using the Friedewald formula and very low-density lipoprotein-cholesterol (VLDL-C) by dividing the triglycerides concentration by 2.18. Both LDL-C and VLDL-C were calculated in mmol/L. Apolipoproteins A and B were quantified by a commercially available rate nephelometry method (Siemens, Marburg, Germany).

The UsCRP was analyzed with a particle-enhanced immunoturbidimetric assay (Tina Quant C-reactive protein latex ultrasensitive assay; Roche Diagnostics). Serum levels of OxLDL were measured by a competitive enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden), using the same specific murine monoclonal antibody 4E6 (mAb-4E6), as in the assay described by Holvoet et al.¹⁵

It is based on a direct sandwich technique that, using this antibody (mAb-4E6), allows to measure very small amounts of OxLDL containing a conformational epitope in the apoB-100 moiety of LDL that is generated as a consequence of substitution of at least 60 lysine residues of apoB-100 with aldehydes. This number of substituted lysines corresponds to the minimal number of substituted lysines required for

Table 1. Anthropometric and Lipoprotein Parameters.

Variables	N	Descriptive Statistics ^a
Age, years	171	63 (20)
Weight, kg	171	72.1 \pm 11.8
BMI, kg/m ²	170	28.1 \pm 3.7
Waist-to-hip ratio	130	1.4 \pm 0.2
CVD personal history	170	18 (10.5%)
Smokers	170	57 (33.5%)
SBP, mm Hg	170	140.7 \pm 17.6
DBP, mm Hg	169	80.4 \pm 10.5
Total cholesterol, mmol/L	171	5.53 \pm 1.06
HDL cholesterol, mmol/L	171	1.51 \pm 0.4
LDL cholesterol, mmol/L	171	3.43 \pm 0.89
Triglycerides, mmol/L	168	1.08 (0.62)
Fasting glucose, mmol/L	171	5.2 (0.9)
UsCRP, mg/L	141	1.8 (2.8)
OxLDL, U/L	127	63.50 \pm 17.59
Right mean cIMT, mm	169	0.73 (0.23)
Left mean cIMT, mm	169	0.76 (0.22)
Overall mean cIMT, mm	169	0.74 (0.22)
Subjects with at least one plaque	171	41 (24%)

Abbreviations: N, number of subjects; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OxLDL, oxidized LDL; cIMT, carotid intima-media thickness; UsCRP, ultrasensitive C reactive protein.

^a Nominal variables are expressed as percentages and quantitative variables are expressed as mean \pm standard deviation except for age, triglycerides, glucose, UsCRP, and cIMT that are expressed as median (interquartile range).

scavenger-mediated uptake of OxLDL. The coefficient of variation for the assay was 7.4% to 8.3%.

The cIMT was determined by B-mode ultrasound in the far wall of the left and right common carotid arteries, 1 cm proximal to its bifurcation. The ultrasound equipment was a HP Image Point with a 10-MHz linear probe.

Patients lay supine with the neck in a neutral, fixed position, and the probe was applied to it parallel to its longitudinal axis, in an anterolateral plane (60°, with 0° being the horizontal). Each cIMT measurement was performed twice in both the left and the right carotid of each patient, and the average of the right and left cIMT was calculated. Measurements were conducted by the same investigator blind to the rest of the data, who has previous experience in making these measurements.

An atheroma plaque was defined echographically as a hyper-echogenicity or protrusion in the vascular lumen of the intima of at least twice the thickness of the adjacent intima media in all the segments of both common carotids, carotid bifurcations, external, and internal carotid arteries accessible to ultrasound.

Statistical Analyses

All variables followed a normal distribution, except for age, triglycerides, glucose, UsCRP, and cIMT, for which results were expressed as medians and interquartile ranges. The other results were expressed as mean \pm standard deviation (SD).

To assess the relationship between cIMT and OxLDL, a bivariate linear regression analysis was performed. The same analysis of cIMT and secondary independent variables such

Table 2. Risk Factors Affecting Average Intima-Media Thickness of Both Carotid Arteries.^a

	Univariate Analysis			Multivariate Analysis (Adjusted R ² .432)	
	n	r	P	B	CI 95% B
Oxidized LDL, U/L	126	.266	.003	0.002	0.001-0.003
Sex (male)	169		.004	0.071	0.033-0.109
Age, years	169	.640	<.001	0.006	0.005-0.008
Weight, kg	169	.172	.025	NE	
BMI, kg/m ²	168	.343	<.001	NE	
Waist-hip index	129	.420	<.001	NE	
Smoker	168		.791	NE	
SBP, mm Hg	168	.411	<.001	NE	
DBP, mm Hg	167	-.096	.219	NE	
Total cholesterol, mmol/L	169	-.046	.548	NE	
LDL cholesterol, mmol/L	169	-.008	.914	NE	
HDL cholesterol, mmol/L	169	-.153	.046	NE	
Triglycerides, mmol/L	169	.091	.239	NE	
Apolipoprotein A, mg/dL	164	-.069	.378	NE	
Apolipoprotein B, mg/dL	161	-.048	.549	NE	
UsCRP, mg/L	139	.149	.079	NE	

Abbreviations: r, correlation coefficient; B, regression coefficient; NE, does not enter the final model; BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UsCRP, ultrasensitive C reactive protein; CI, confidence interval.

^a Univariate and multivariate linear regression analyses; SBP, systolic blood pressure; DBP, diastolic blood pressure.

as age, gender, BMI, waist-to-hip ratio, systolic and diastolic blood pressure, HDL-C, LDL-C, plasma triglycerides, fasting glucose, smoking history, and current or past personal or familial cardiovascular history was followed.

To determine the association of OxLDL and cIMT after adjusting for secondary independent variables, a multivariate linear regression analysis was performed. A multivariate linear regression analysis was also applied to assess the importance of various contributing factors to the plasma OxLDL concentration.

Associations between circulating OxLDL, cIMT, and other risk factors for atherosclerosis were calculated with Spearman rank correlation coefficients. The statistics program SPSS 15.0 was used for the analysis, and a $P < .05$ was considered significant.

Results

The clinical characteristics and lipoprotein parameters of the study population are presented in Table 1. The age of patients ranged from 44 to 93 years (mean 64.2 years, SD 12.04). The average cIMT of both the carotid arteries (overall mean cIMT) ranged from 0.53 to 1.27 mm (median 0.76 mm, interquartile range 0.23) and 41 (24%) individuals had ≥ 1 plaques.

The OxLDL concentration in the population ranged from 30 to 125 U/L, with a mean of 63.5 and an SD of 17.59. Data showed a normal distribution, but OxLDL concentration mean was higher in men (68.17 U/L) than in women (59.44 U/L), and the differences were significant ($P < .01$).

Univariate analysis showed that OxLDL, older age, male gender, higher BMI, higher waist-to-hip ratio, and higher systolic blood pressure were associated with thicker cIMT.

In multivariate linear regression analysis, controlling for confounding factors, only OxLDL, older age, and male sex

were significantly associated with increased average cIMT of both the carotid arteries (Table 2).

Circulating OxLDL showed a significant and independent association with left ($r = .298$; $P < .01$), right ($r = .215$; $P < .05$), or average cIMT ($r = .266$; $P < .01$) of both the carotid arteries. This association remains in individuals with UsCRP ≤ 1 mg/L.

In Figure 1, we can see the direct correlation between OxLDL and average cIMT of both the carotid arteries as well as the increase in the average cIMT of both the carotid arteries over tertiles of OxLDL.

Mean cIMT in the highest OxLDL tertile group was significantly thicker than in the lowest tertile group, 0.65 (0.11) versus 0.73 (0.17) mm, $P < .05$.

There were no significant differences in mean OxLDL concentrations among individuals who did not have any plaque and those with ≥ 1 plaques (63.06 vs 65.11 U/L; $P = .59$). No significant differences were also found in mean UsCRP.

In the univariate analysis, OxLDL was significantly and positively associated with BMI, smoking history, total cholesterol, LDL-C, apolipoprotein B, and triglycerides and significantly and negatively associated with C-HDL and apolipoprotein A.

Univariate and multivariate analyses between the OxLDL and the other continuous variables are summarized in Table 3. In the multivariate analysis, plasma OxLDL concentrations were associated with age, smoking, LDL-C, and triglycerides independently of other variables.

Discussion

The entry of OxLDL in the arterial intima is an initiating event of atherosclerosis. Since cIMT is a marker of early atherosclerosis and is predictive of both stroke and myocardial

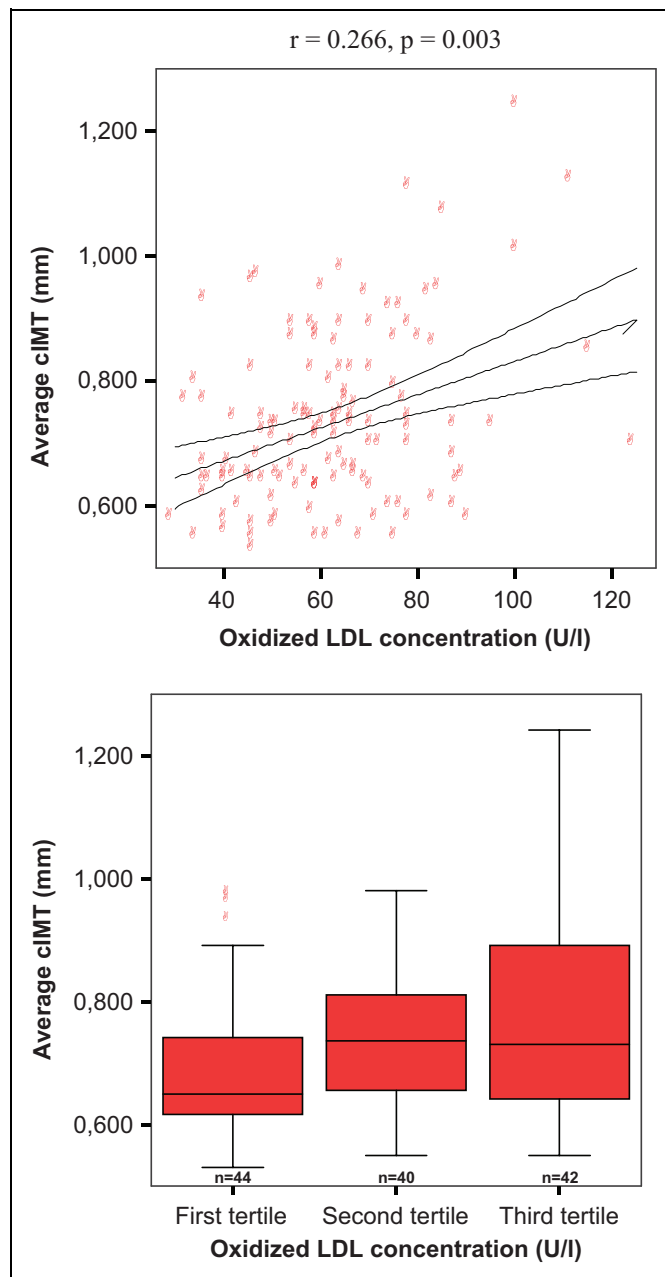


Figure 1. Scatterplot and boxplot showing the relation between oxidized LDL concentration and average carotid artery intima-media thickness (cIMT).

infarction, although not more than traditional risk factors,¹⁶ its association with OxLDL has been studied in particular age ranges and predominantly in males, but not in a sample representative of an asymptomatic middle- and old-age population of both sexes, as in our study. Atherosclerosis is universal, but populations of different countries have variable risk loads, and there are few data available for south European countries, which is why we initiated this study. Our main finding is that higher OxLDL concentrations are associated with increased cIMT values in this south European population before and after excluding confounding factors. Not unexpectedly, this also

happened with older age and male sex, variables that are known to be associated with increased atherosclerosis.¹⁷ This positive association of OxLDL and cIMT values has been found in middle-aged males^{12,13,18} and in asymptomatic patients diagnosed with familial combined hyperlipidemia¹⁹ but not in a sample of men and women that did not include older-aged patients (age range: 35-55 years).¹⁴

The independent association found between OxLDL and cIMT in older people suggests that the correlation of OxLDL with noninvasive measures of atherosclerosis should be better in older populations characterized by more generalized atherosclerotic disease.

We found no association between OxLDL and more advanced atherosclerosis represented by carotid atheroma plaques. In the AIR study, elevated OxLDL concentrations was associated with femoral but not with carotid plaques in men.¹² In agreement with previous studies of asymptomatic patients,^{20,21} we did not find a positive correlation of cIMT and CRP nor of CRP and OxLDL,^{21,22} and our patients from the general population with mainly normal CRP levels still showed an association between cIMT and OxLDL, which may suggest that OxLDL and CRP may act on different pathophysiological pathways in atherogenesis. High CRP values seem to be associated more with increased arterial stiffness.²³

As could be expected, we found a positive association of traditional cardiovascular risk factors such as age, triglycerides, and plasma LDL-C concentrations with plasma OxLDL, which has been reported previously in both coronary disease and healthy participants.^{24,25} We and others²⁶ also found smoking to be associated with higher OxLDL, but other authors could not confirm it.^{12,15} We also found a negative association in bivariate analysis between OxLDL and HDL, an antiatherogenic lipoprotein, but this association is not found to be independent, as in the study of Liu et al.¹⁹ Because HDL has antioxidant properties,^{27,28} we were not surprised to find it negatively correlated with OxLDL levels.

The limitations of this study are a small sample size, and that OxLDL was determined in approximately two-thirds of the sample. However, bias is not likely as the patients were randomly excluded, and although dedicated software may be more accurate in the measurement of cIMT, repeated manual measurements were made by the same experienced investigator following a fixed protocol to decrease variability.

Our findings in an asymptomatic sample representative of the Spanish middle- and old-age population underscore the role of OxLDL in early atherosclerosis as measured by cIMT especially in older asymptomatic individuals. However, this relationship cannot be extended to more advanced atherosclerosis represented by carotid plaques.

Authors' Note

The study was conducted in the Research Unit of General Yagüe Hospital in Burgos (Spain).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Table 3. Determinants of Circulating Oxidized LDL in a Sample of the Spanish General Population.

	Univariate Analysis			Multivariate Analysis (Adjusted R ² .243)	
	n	r	P	B	CI 95% B
Sex (male)	127		.004	NE	
Age, years	127	.144	.106	0.326	0.080-0.573
Weight, kg	127	.32	<.001	NE	
BMI	127	.25	<.001	NE	
Waist to hip ratio	127	.337	.001	NE	
Smoker	127		.007	9.94	4.088-15.792
SBP, mm Hg	126	.12	.181	NE	
DBP, mm Hg	127	.04	.66	NE	
Total cholesterol, mmol/L	127	.316	<.001	NE	
LDL cholesterol, mmol/L	127	.316	<.001	0.845	0.531-1.160
HDL cholesterol, mmol/L	127	-.176	.047	NE	
Triglycerides, mmol/L	127	.313	<.001	0.346	0.081-0.61
Apolipoprotein A, mg/dL	127	-.08	.37	NE	
Apolipoprotein B, mg/dL	126	.263	.003	NE	
UsCRP, mg/dL	127	-.083	.356	NE	

Abbreviations: r, correlation coefficient; B, regression coefficient; NE, does not enter the final model; BMI, body mass index; SBP, systolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UsCRP, ultrasensitive C reactive protein; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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