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Determinants and clinical significance of plasma oxidized LDLs in older individuals. A 9 years follow-up study

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Abstract

Oxidized LDLs (ox.LDLs) uptake by macrophages inside the arterial wall is a crucial step in atherosclerotic disease, and some studies suggest that high ox.LDLs plasma levels might be associated with cardiovascular disease (CVD). However, whether high ox.LDLs continue to be a CVD risk factors in older persons is unknown. We investigated the clinical correlates of plasma ox.LDLs, and their role in predicting long-term CVD/cardiac mortality in 1025 older community-dwelling individuals (mean age:75.5±7.4yrs; females:55%) from the InCHIANTI study. Kaplan-Meier curves were fitted to explore the relationship between tertiles of ox.LDLs (ox.LDL/LDL-C ratio) and time to CVD/cardiac death. Hazard Ratios (HR) were estimated by Cox regression analysis.

At multivariate analysis, ox.LDLs were independently associated with LDL-C, triglycerides, and HDL-C (adjusted r^2:0.42; P=0.001). The ox.LDL/LDL-C ratio (the extent of LDLs oxidation) was independently correlated with HDL-C, triglycerides, and beta-carotene (adjusted r^2:0.15, P=0.001). Among 1025 individuals, 392 died after 9 years, 166 from CVD. The HR for CVD/cardiac mortality was not significantly different across tertiles of ox.LDLs or ox.LDL/LDL-C ratio, both in the whole sample and in individuals with prevalent CVD.

We conclude that in an elderly population LDL-C, triglycerides, and HDL-C are the most important determinants of ox.LDLs levels, indirectly suggesting an association between small dense LDLs and LDLs oxidation. No association emerged between higher ox.LDLs levels and 9-years CVD/cardiac mortality, suggesting that in advanced age the prognostic information added by ox.LDLs on CVD/cardiac mortality might be negligible.

Keywords

Oxidized LDL; Mortality; Cardiovascular Disease; Aging

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Introduction

Atherosclerosis is the first cause of death in Western Countries. Current theories on the pathogenesis of atherosclerosis call for an interplay of inflammation and oxidative stress that progressively damage arterial walls. Oxidation of low-density lipoproteins (LDLs) and their subsequent uptake by macrophages inside the arterial wall are considered crucial steps in this process (1). Indeed, macrophages do not take up LDL particles unless they undergo in vivo modifications such as oxidation. Accordingly, cross-sectional studies suggest that the rate of LDLs oxidation might be associated with coronary heart disease (CHD) and carotid atherosclerosis (2, 3). There is some evidence that LDLs are oxidized in the extravascular space of the arterial wall (1, 4) but small part of oxidized LDLs (ox.LDLs) escape the uptake by macrophages or might leak from atherosclerotic plaques and re-enter the blood stream. Of consequence, the measurement of ox.LDLs might contribute to the estimation of cardiovascular risk (5).

Conditions associated with high ox.LDLs plasma levels include a specific lipoprotein profile, LDL particles size (6), diabetes mellitus (7), markers of systemic inflammation (8), and central obesity (9). Interestingly, LDL-cholesterol (LDL-C) and apo B levels have been consistently associated with ox-LDLs plasma levels (10). For this reason, it has been suggested that the ox.LDL/LDL-C ratio (i.e. the relative amount of ox.LDLs) is the best indicator of the risk associated with ox.LDLs levels (8). Although atherosclerosis is common in older people, and ageing itself has been associated with increasing levels of oxidative stress, data about circulating ox.LDLs in older populations are scarce (11). Indeed, only a few studies investigated the impact of increased ox.LDLs levels on the incidence of new cardiovascular events (5), mainly in selected samples of patients with CHD (12-14) or congestive heart failure (15, 16). Furthermore, the clinical value of circulating oxLDL assessment in older person is virtually unknown.

Using data for the InCHIANTI dataset, a population-based study of older people living in the community with a 9-year follow-up, we investigated the clinical correlates of plasma ox.LDLs and tested the hypothesis that baseline circulating ox.LDL levels might predict long-term cardiovascular mortality.

Materials and Methods

This study is part of the “Invecchiare in Chianti” (Aging in the Chianti area, InCHIANTI) study, a prospective population-based study of older persons, designed by the Laboratory of Clinical Epidemiology of the Italian National Research Council of Aging (INRCA, Florence, Italy). The study included participants randomly selected from the residents in two towns of the Chianti area (Greve in Chianti and Bagno a Ripoli, Tuscany, Italy). A detailed description of the sampling procedure and data collection method has been previously published (17). Briefly, in August 1998, 1270 persons ≥65 years old were randomly selected from the population registries. Additional subjects (*n.* 29) were randomly selected among those who were ≥90 years old, to obtain a sample of 30 men and 30 women in this age group. Of the initial 1299 subjects, 39 were not eligible (they had died or left the area). Clinical visits and assessments were performed in the studyclinic and were preceded by an interview conducted at the participants' homes. Trained interviewers administered structured questionnaires on dietary intakes, household composition, social networks, economical status, education, and general information on health and functional status. The Italian National Research Council on Aging (INRCA) Ethical Committee ratified the entire study protocol.

The analyses here presented are based on data from 1025 individuals aged over 65 in which metabolic parameters including oxidized LDLs, and inflammatory mediators had been measured at the time of baseline visit.

Clinical chemistry parameters

All parameters were measured on fresh serum or plasma drawn after 12 hours overnight fasting, after the patient has been sedentary in sitting or supine position for 15 minutes. Commercial enzymatic tests (Roche Diagnostics) were used for determining serum total cholesterol, triglycerides, and HDL-C concentrations. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald's formula as follows: LDL-C: TC – (TG/5) – HDL-C. As an indirect marker of LDL particle size we used the TG/HDL-C ratio, a validated parameter previously used for assessing the presence of small LDL particles (18, 19). According to data by Muruyama et al., we considered subjects with a TG/HDL-C ratio cut-off > 2.0 as having small LDL particles (18). Fasting insulin was determined using a commercial double-antibody, solid-phase radioimmunoassay (Sorin Biomedica, Milan, Italy). Fasting blood glucose was determined by an enzymatic colorimetric assay using a modified glucose oxidase-peroxidase method (Roche Diagnostics, GmbH, Mannheim, Germany) and a Roche-Hitachi 917 analyzer.

Insulin resistance was calculated according to the Homeostasis Model Assessment (HOMA) as follows: fasting insulin × fasting glucose/22.5. Metabolic syndrome was defined by the criteria of the NCEP-ATP III - AHA/NHLBI statement published in 2005(20). The glomerular filtration rate (GFR) was calculated by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (21).

Determination of plasma oxidized LDLs—Sampled blood was transferred into the tube avoiding haemolysis. Aliquoted, plasma specimens were frozen and stored at −80°C until used for testing. Oxidized LDLs were measured by ELISA (Kit Mercodia AB, Uppsala, Sweden). In this solid-phase two-site enzyme immunoassay two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. The detection limit is <1 mU/L. The intrassay and interassay coefficients of variation were 6.3% and 8%, respectively.

Plasma ox.LDL values were strongly correlated with LDL-C plasma concentrations (r: 0.58; P= 0.001); thus, LDL-C was the strongest determinant of ox.LDL absolute levels in our population. To overcome this, we adjusted the plasma values of ox.LDLs (U/L) by the plasma levels of LDL-C (mmol/L) by calculating their ratio (Units of ox.LDLs per mmol of LDL-C). The ratio was used to perform statistical analysis and express the results.

Inflammatory markers—Interleukin-6 (IL-6), interleukin-1 Beta (IL-1β), interleukin-18 (IL-18), Tumor Necrosis Factor-alpha (TNF-α), interleukin-1 Receptor Antagonist (IL-1RA), and high sensitivity C reactive protein (hs.CRP) were measured as previously described (22).

Plasma antioxidants—Plasma Alpha-tocopherol concentrations were measured by reversed-phase high-performance liquid chromatography (HPLC) as previously described (23). Tocopherol concentrations were expressed in μmol/L. Intra- and interbatch CVs were 3% and 4.2%, respectively. Beta-carotene has been measured by HPLC (24). Within-run and between-run coefficients of variation, respectively, were 4.5% and 5.4%. Plasma Selenium was measured by graphite furnace atomic absorption spectrometry in a Perkin Elmer Analyst 600 with Zeeman background correction (25). Within-run and between-run coefficients of variation, respectively, were 3.1% and 7.1%.

Anthropometry

Weight and height were measured by using standard techniques. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Waist circumference was measured to the nearest 0.5 cm by using a non-elastic plastic tape, at the level of the smallest area of the waist between the lower rib margin and the iliac crest.

Dietary variables

Data on dietary daily intake were collected by the questionnaire created for the European Prospective Investigation into Cancer and Nutrition (E.P.I.C.) study (26).

Mortality Follow-up

Participants were evaluated for the 3-year (2001 to 2003), 6-year (2004 to 2006) and 9-year follow-up visits (2007 to 2009). Mortality data of the original InChianti cohort were collected using data from the Mortality General Registry maintained by the Tuscany Region, and the death certificates that are deposited immediately after death at the Registry office of the municipality of residence. Cardiovascular mortality, based on underlying cause of death, was defined as any cardiovascular mortality and coded using the International Classification of Diseases, 9th Revision (ICD-9 codes: 390-459).

Other Measures

The prevalence of specific medical conditions was establishedusing standardized criteria that combined information from self-reported history, medical records, and a clinical medical examination. The following diseases were considered in the present study: coronary heart disease (CHD - acute myocardial infarction, chronic heart failure, cardiac arrest, acute and chronic ischemic heart disease), peripheral arterial disease (PAD), stroke, hypertension, and diabetes. Prevalent cardiovascular disease (CVD) was defined as the as presence of one of the following: coronary heart disease (as previously specified), peripheral arterial disease, and stroke. The ankle-brachial index (ABI) was measured in all subjects by using a Doppler stethoscope (Parks model 41-A; Parks Medical Electronics, Inc; Aloha, Oregon). Besides clinical information, in the presence of ABI value <0.9, the diagnosis of PAD was made.

Statistical analysis

Continuous variables were expressed as mean (SD) or median (interquartile range) when necessary. Means were compared by t test or ANOVA, while medians were compared by Mann-Whitney non-parametric test. Correlations between continuous variables were tested by Pearson's correlation. Due to the skewed distribution of values, cytokines, insulin, TG, and HOMA were log-transformed, in order to approximate a normal distribution, before entering regression analysis. Prevalence was compared by the χ^2 test. Multivariate linear regression analysis (method stepwise backward) was performed to test the independent association between ox.LDLs or ox.LDL/LDL-C ratio and other variables of interest.

The parameters included into the Ox.LDL model were: age, gender, LDL-C, HDL-C, TG, uric acid, HOMA, waist circumference, BMI, metabolic syndrome, daily Kcal intake, IL-1β, alpha-tocopherol, beta-carotene, and selenium.

The parameters included into the Ox.LDL/LDL-C ratio model were: age, gender, HDL-C, TG, HOMA, uric acid, WBC count, metabolic syndrome, waist circumference, smoking, CKD-EPI, TNF-α, log IL-18, log IL-1ra, beta-carotene, and selenium.

Kaplan-Meier curves were fitted to explore the relationship between tertiles of plasma ox.LDLs, or ox.LDL/LDL-C ratio, and time to CVD death defined by ICD-9-CM codes

390-459. Death rates per 1.000-person years were also calculated. Hazard Ratios (HR) for each tertile of ox.LDL, or ox.LDL/LDL-C ratio, were separately estimated, in the whole population and in subjects with documented CVD at baseline, by Cox proportional hazard regression analysis. The assumption of proportionality of all variables introduced in the models was assessed through the analysis of Schoenfeld residuals. The lower tertile (I) was considered as the reference category. The Cox models were adjusted for known risk factors for cardiovascular mortality including age, gender, diabetes, hypertension, and smoking; furthermore, CHD and stroke were included into the models for whole population analysis.

All analyses were performed by the SPSS for Windows statistical package, version 13.0 (SPSS, Inc, Chicago, IL).

Results

One thousand twenty five community dwelling older individuals were enrolled into the present study; the characteristics of the sample are reported in TABLE 1. Ox.LDLs values were normally distributed, with a mean of 42.14±11.72 U/L. On the whole population, 226 (22%) individuals resulted affected by CVD at baseline. Ox.LDLs levels (43.5 vs 41.8 U/L; P=0.07) as well as ox.LDL/LDL-C ratio (12.8 vs 12.1U/mmol; P=0.10) were slightly higher in subjects with CVD compared with controls.

Metabolic syndrome (MS) was more frequent in subjects with high ox.LDLs (>42.12 U/L) while history of CHD, stroke, PAD, diabetes, and smoking was similar across ox.LDL tertiles. By multivariate regression analysis we found that LDL-C was strongly related to ox.LDLs (β: 0.21; SE: 0.010; P=0.001), explaining more than one third of ox.LDLs total variability (adjusted r^2:0.36; P=0.001; age and gender covariates). When other variables associated with ox.LDLs at univariate analysis were forced into the model, also TG (β: 0.042; SE: 0.005; P=0.001), and HDL-C, (β: −0.11; SE: 0.026; P=0.001) resulted independently associated with ox.LDLs (adjusted r^2: 0.42; P=0.001). In TABLE 2 are described the characteristics of the sample according to low or high ox.LDL/LDL-C ratio (below/above mean value). Participants with high ox.LDL/LDL-C ratio had higher values of TG, fasting glucose, HOMA score, uric acid, creatinine, waist circumference, white blood cells (WBC) count, IL-1 receptor antagonist (IL-1ra) and IL-18, and by lower levels of HDL-C and beta-carotene. Participants with high ox.LDL/LDL.C ratio were also more likely to be smokers, have a MS diagnosis, and to report a history of diabetes or stroke (P=0.07)

The ox.LDL/LDL-C ratio correlated positively with TG, fasting glucose, HOMA score, uric acid, creatinine, CKD-EPI values, waist circumference, WBC count, IL-1ra, TNF-alpha, and IL-18, and negatively with HDL-C, beta-carotene and selenium (Table 3).

At multivariate regression analysis the ox.LDL/LDL-C ratio correlated with HDL-C (β: −0.032; SE: 0.008; P= 0.001), TG (β: 0.014; SE: 0.002; P= 0.001), and beta-carotene (β: −1.14; SE: 0.43; P= 0.008) (adjusted r^2: 0.15, P=0.001), independent of potential confounders.

We successively investigated the possible association between ox.LDLs levels (or ox.LDL/LDL-C ratio) and nine-years CVD mortality. Among the 1025 individuals enrolled into the present study, 392 had died after 9 years. The principal causes of death was the following: 42 % (166) were from CVD (among this 71 from cardiac causes), 20 % from neoplasm, 5 % from diseases of the respiratory system, 5 % from fracture and accidents due to natural and environmental factors, 4 % from diseases of nervous system, 4 % from diseases of gastro-intestinal tract, 3 % from endocrine/nutritional/metabolic diseases, and immunity disorders. In FIGURE 1 are reported the Kaplan-Meier survival curves for CVD mortality in the whole

population, according to ox.LDLs (A) or ox.LDL/LDL-C ratio tertiles (B). The adjusted HR for CVD mortality according to ox.LDLs tertiles was 1.09 (95%CI: 0.73-1.61) for tertile II, and 1.17 (95%CI: 0.79-1.73) for tertile III. As regards ox.LDL/LDL-C ratio, the HR was 0.84 (95%CI: 0.51-1.12) for tertile II, and 0.89 (95%CI: 0.55-1.18) for tertile III.

In FIGURE 2 are shown the Kaplan-Meier survival curves for CVD mortality in subjects with prevalent CVD across tertiles of plasma ox.LDLs (A) or ox.LDL/LDL-C ratio (B). As expected, mortality rates were much higher compared to the whole population. Furthermore, participants with CVD were older, more often males, and had greater prevalence of diabetes, hypertension, and other risk factors. According to ox.LDLs tertiles, the adjusted HR for CVD mortality in subjects with prevalent baseline CVD was 1.09 (95%CI: 0.65-2.12) for tertile II, and 1.12 (95%CI: 0.69-2.14)for tertile III. As regards ox.LDL/LDL-C ratio tertiles, the adjusted HR was 0.95 (95%CI: 0.54-1.77) for tertile II, and 1.07 (95%CI: 0.58-1.94) for tertile III.

Finally, Cox multivariate analysis was specifically focused on cardiac mortality (71 subjects; ICD-9 codes 410, 411, 414, 427.5, and 428). Adjusted HR for cardiac mortality according to ox.LDLs tertiles was 1.27 (95%CI: 0.54-2.32) for tertile II, and 1.33 (95%CI: 0.64-2.74) for tertile III, while for the ox.LDL/LDL-C ratio the HR was 0.59 (95%CI: 0.39-1.20) for tertile II, and 0.67 (95%CI: 0.43-1.37) for tertile III.

Discussion

We evaluated plasma ox.LDLs levels in a large sample of community dwelling older individuals from the InChianti study. The levels we measured in our sample were in line with those previously reported by using the same ELISA method. In healthy individuals, Nakhjavani reported a mean value of 42 U/L (7), while Nordin reported a mean value of 48 U/L (27).

The major determinants of ox.LDLs in this population were the levels of LDL-C, TG, and HDL-C. In agreement with other reports, LDL-C was by far the strongest determinant, explaining 36% of total variability. This result further supports the concept that the absolute rate of LDLs oxidation depends not only on the levels of oxidative stress, but also on the amount of substrate (8). Interestingly, a negative association between ox.LDLs levels and age was found in our population (r:−0.101; P=0.001), perfectly mirroring the relationship between LDL-C and age (r:−0.110; P=0.001). This finding suggests that after 65 years of age, although the oxidative stress might increase with ageing, circulating ox.LDLs tend to decrease as a consequence of the progressive reduction of the substrate.

TG levels were also positively associated with ox.LDLs levels. It is known that high TG values are strongly associated with the presence of small, dense LDL particles, the “pattern B” LDLs (28). Interestingly, Kondo et al. demonstrated that decreased LDLs size together with increased TG leads to increased ox.LDLs levels (29). Of consequence, we advance that the relationship between TG and ox.LDLs levels might be mediated by the small size of LDL particles. Supporting this hypothesis, we found that the TG/HDL-C ratio, a validated indicator of small LDLs size (18, 19), was associated with ox.LDLs levels. Indeed, the prevalence of high ox.LDLs in subjects with TG/HDL-C ratio below or above 2.0 was 39.5% and 66%, respectively (P=0.001); moreover, the risk of high ox.LDLs was associated with a TG/HDL-C ratio >2.0 (OR: 3.06; 95%I.C.:2.36-3.97).

HDL-C was also negatively associated with ox.LDLs, independent of TG values. HDLs might contribute in reducing LDL oxidation (30); indeed, HDLs contain enzymes (e.g. paraoxonase 1-3) able to destroy lipid hydro peroxides that oxidize LDLs, while Apo A-I removes lipid hydro peroxides from LDLs.

The mean value of the ox.LDL/LDL-C ratio was also similar to those previously reported in other studies (31, 32). Again, TG and HDL-C were the major determinants of the ox.LDL/LDL-C ratio explaining, together with beta carotene, 15% of its total variability. A TG/HDL-C ratio >2.0 was associated with high values of ox.LDL/LDL-C ratio (OR:1.69; 95%CI:1.31-2.18). Thus, our data suggest that TG and HDL-C are independent predictors of both absolute levels of ox.LDLs and of the extent of LDLs oxidation. Interestingly, beta carotene was negatively correlated with the ox.LDL/LDL-C ratio, suggesting that it might improve the oxidative status of circulating LDLs in this older population. Different antioxidants have been shown to inhibit *in vitro* LDL oxidation (33-35), and to reduce the susceptibility of LDLs to oxidation in humans (34, 36). Since plasma levels of beta carotene can be increased by diet or dietary supplementation without side effects, they may show promise in the prevention of atherosclerosis even in older individuals.

We also investigated the relationship between plasma ox.LDLs (or ox.LDL/LDL-C ratio) and nine years CVD/cardiac mortality. Indeed, it has been suggested that higher ox.LDLs levels might have a prognostic value in predicting future CVD or CHD (5, 11, 13, 14). Our results did not demonstrate any significant association between the absolute or relative amount of plasma ox.LDLs and nine years CVD/cardiac mortality; these results were found both in the whole population and in subjects with prevalent CVD at baseline. Actually, prospective data on the clinical significance of ox.LDLs are scarce, and most of the studies have been conducted on relatively small samples of patients with prevalent CHD (5, 12-14).

Only one study, conducted on 3033 subjects aged 70 to 79 years from the Healthy ABC study has been focused on the elderly population (11). Although these Authors could not demonstrate any association between ox.LDLs and CHD incidence (4 years follow up), the risk for MI was significantly increased in participants with higher ox.LDLs.

The reason for our negative results might be different. In one hand it is possible that in older individuals plasma ox.LDLs do not strictly reflect the real amount of ox.LDLs and/or the levels of oxidative stress inside the arterial wall. On the other hand, ox.LDLs might lose their predictive value with advancing age since individuals disposed to develop precocious atherosclerosis have already died, while ageing population might be progressively selected for resistance to risk factors. This phenomenon has been already observed for other cardiovascular risk factors including plasma lipids (37). Furthermore, we evaluated the risk for CVD/cardiac mortality, whereas previous studies investigated the incidence of all CVD events, an outcome that may be more specifically related to lipid abnormalities; this methodological aspect might also contribute to explain our findings. Finally, since the aim of the study was to test the ability of ox.LDL to predict 9-years CV death, we measured this variables only at baseline; nevertheless, information about ox.LDLs concentration over time might give further risk indications.

Some limitations of the study must be acknowledged. First, LDL-C was estimated by the Friedewald formula, and not directly. Second, we used an indirect indicator of LDL particles size, the TG/HDL-C ratio. Although this marker has been validated (18, 19), and is much easier to use in a large population based study, a direct measure such as polyacrylamide linear gradient gel electrophoresis or NMR would be much more precise.

We also would like to underline the strength of the study. To our knowledge, this is at present time the population-based longitudinal study on circulating ox.LDLs with the longest follow-up period.

Conclusion

We confirmed in an elderly population that, besides LDL-C, TG and HDL-C are two independent determinants of ox.LDLs levels, supporting the existence of an association between small, dense LDLs phenotype and LDLs oxidation. Most important, we could not demonstrate any association between higher ox.LDLs plasma levels and nine years CVD/cardiac mortality rates, both in the whole sample and in participants with prevalent CVD. This result suggests that in advanced age the prognostic information added by ox.LDLs on future CVD/cardiac mortality might be negligible.

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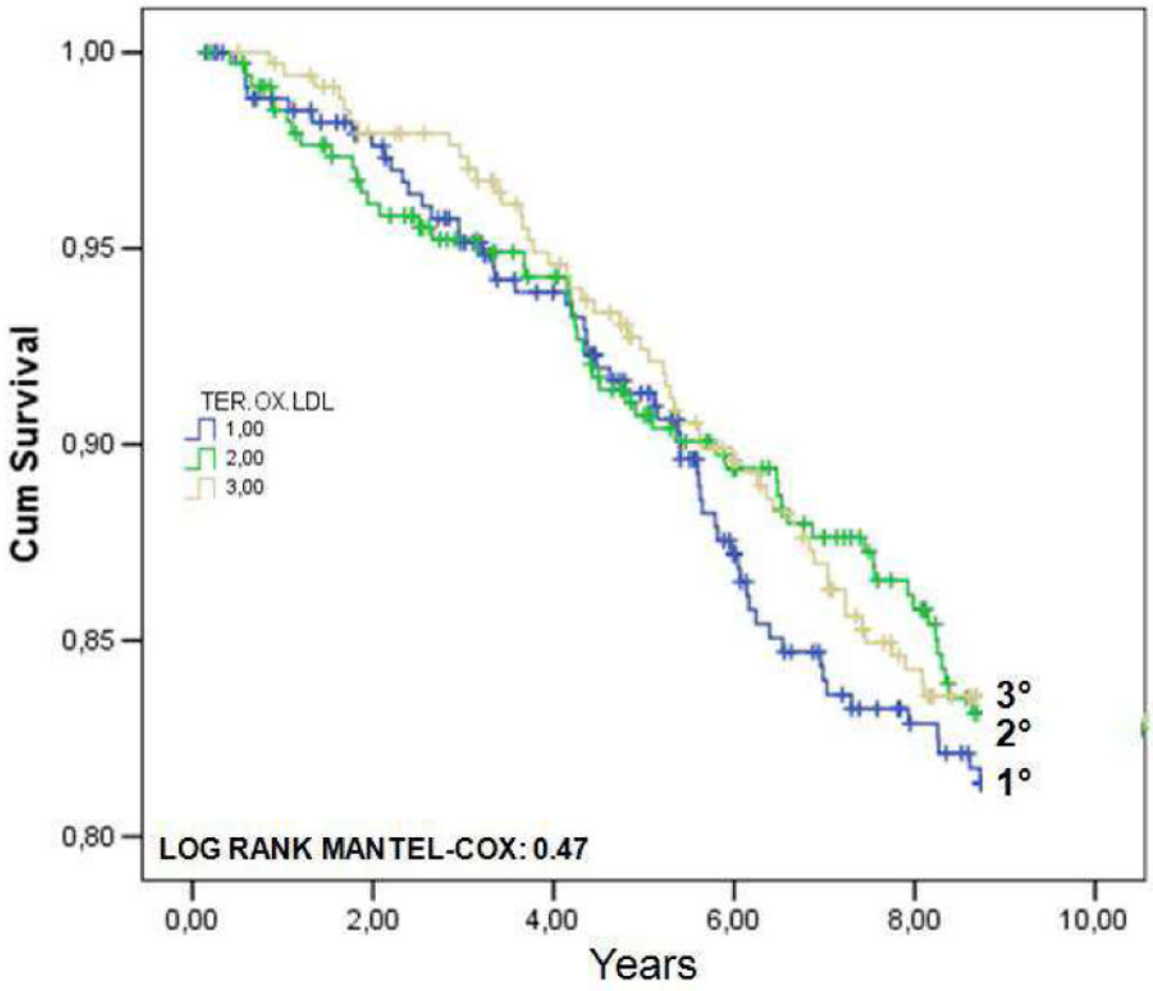
Highlights

- We studied 1025 older community-dwelling subjects with 9-years follow up
- LDL-C, TG, and HDL-C are the most important determinants of ox.LDLs levels
- The extent of LDLs oxidation was independently correlated with HDL-C, TG, and b-carotene.
- No association emerged between higher ox.LDLs levels and 9-years CVD/cardiac mortality.



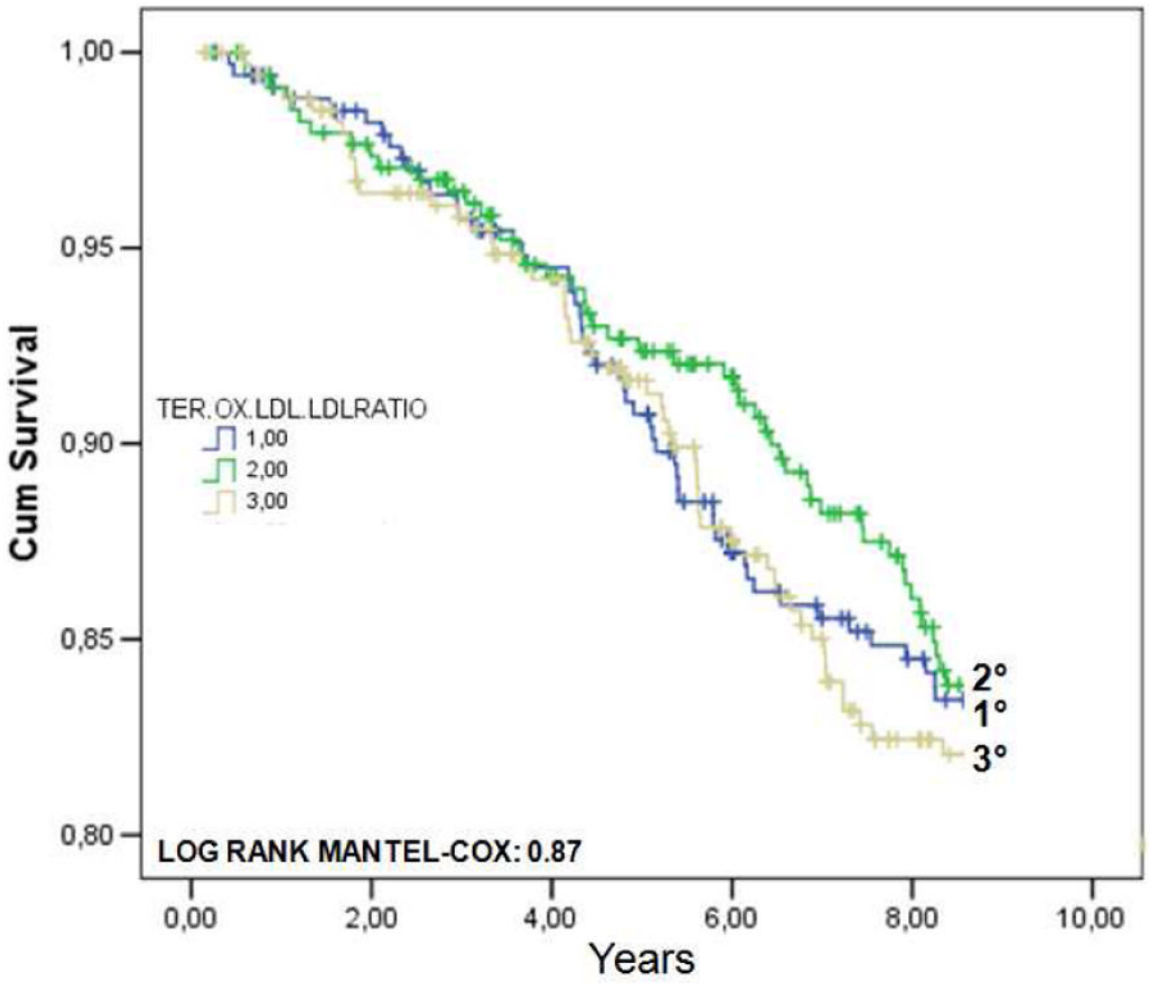
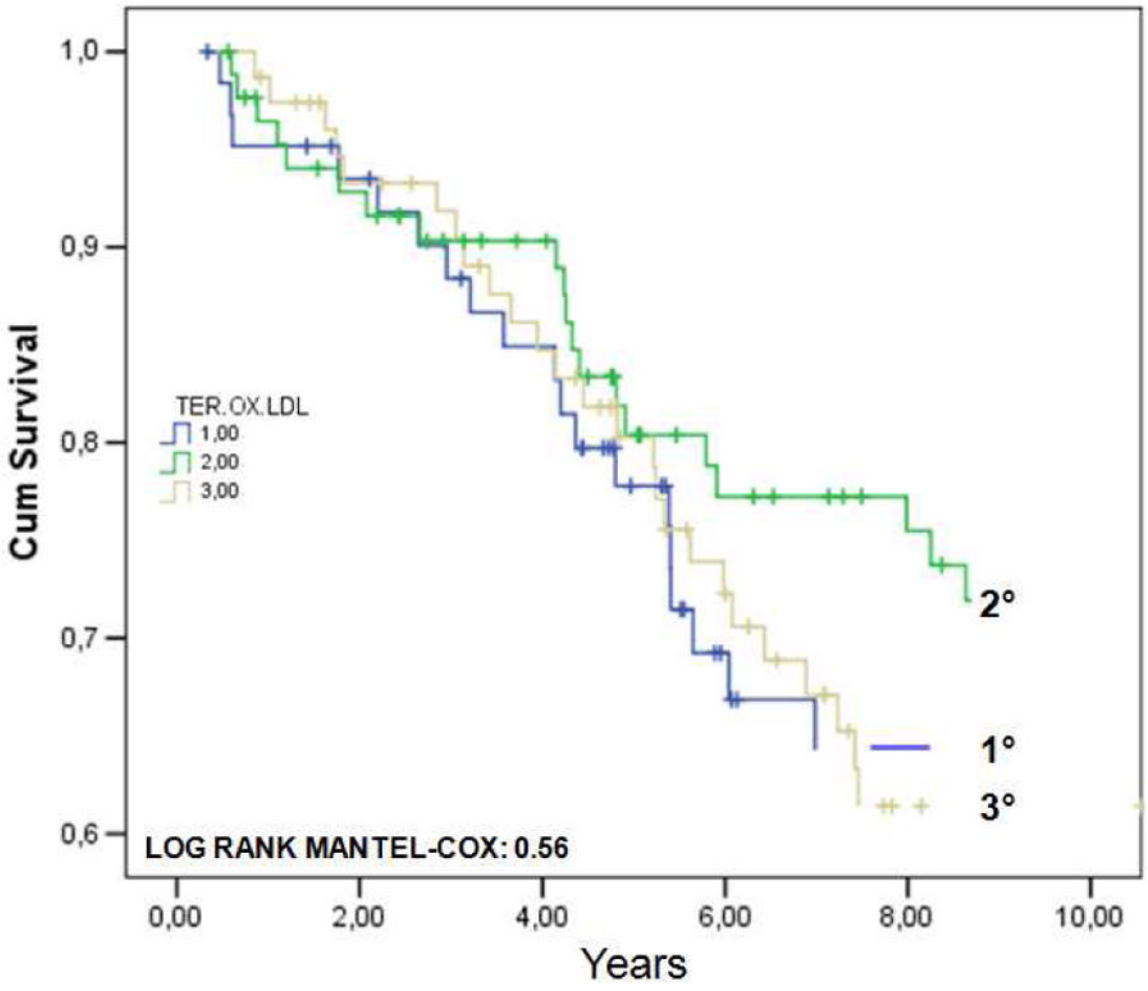


FIGURE 1.

Kaplan-Meier survival curves for cardiovascular (CVD) mortality in 1025 older individuals from the InChianti study, according to ox.LDLs (A) or ox.LDL/LDL-C ratio tertiles (B).



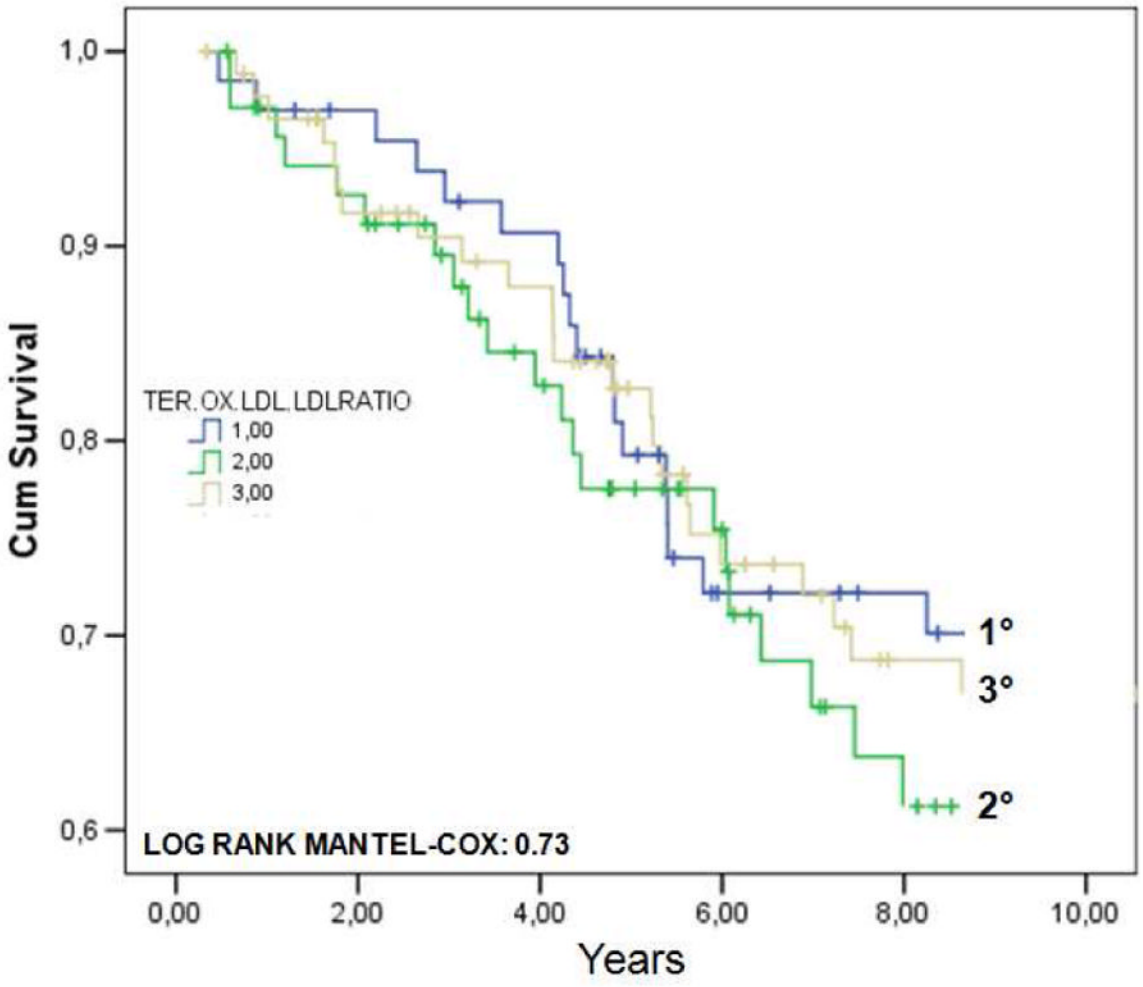


FIGURE 2.
Kaplan-Meier survival curves for cardiovascular (CVD) mortality in 226 older individuals with prevalent CVD at baseline, from the InChianti study, across tertiles of plasma ox.LDLs (A) or ox.LDL/LDL-C ratio (B).

TABLE 1

principal characteristics of 1025 community dwelling older individuals enrolled in the InChianti study.

PARAMETER	
Age (years)	75.5±7.4
Gender (female)	55%
Oxidized LDLs (U/L)	42.14±11.72 U/L
Total cholesterol (mg/dl)	217±40
LDL-Cholesterol (mg/dl)	136±34
HDL-Cholesterol (mg/dl)	56±15
Triglycerides (mg/dl) °	110 (83-150)
Fasting Glucose (mg/dl)	96±26
HOMA score °	2.25 (1.54-3.28)
Uric acid (mg/dl)	5.2±1.4
Creatinine (mg/dl)	0.93±0.24
Body Mass Index (kg/m^2)	27.4±4.0
Waist circumference (cm)	92.7±10.2
WBC count (×1000/mm3)	6.14±1.60
Ankle Brachial Index (ABI)	1.03±0.16
IL -1 β (pg/ml) °	0.12 (0.08-0.19)
IL-1 ra (pg/ml) °	132 (97-187)
TNF-α (pg/ml) °	5.07 (2.85-7.64)
IL-6 (pg/ml) °	1.47 (0.87-2.29)
IL-18 (ug/ml) °	386 (303-484)
Hs.CRP (mg/L) °	2.81 (1.32-5.84)
α Tocopherol (umol(L)	33.6±7.6
B Carotene (umol/L) °	0.35 (0.24-0.53)
Selenium (ug/L)	74.4±13
Diet (daily intake)	
- Kcalories	1910±563
- Cholesterol (mg)	268±96
- Saturated fatty acids (g)	22±7.5
- Alcohol (g) °	6.2 (0-20)
Smoking habit (past/current)	41%
Metabolic Syndrome (by NCEP-ATP III)	31%
Diabetes	11.9%
Hypertension (by NCEP-ATP III)	93%
Coronary Heart Disease (CHD)	7.6%
Stroke	6.6%

PARAMETER	
Peripheral Arterial Disease (PAD)	12.1%

HOMA: homeostasis model assessment; WBC: white blood cells; CHD: coronary heart disease

° Median (interquartile range)

TABLE 2

principal characteristics of 1025 community dwelling older individuals enrolled in the InChianti study according to the presence of low (<12.28 U/mmol) or high (≥12.28 U/mmol) values of ox.LDL/LDL-C ratio.

	ox.LDL/LDL-C ratio		
PARAMETER	**LOW**	**HIGH**	**P**
Age (years)	75.1±7	75.9±7.6	0.09
Gender (female)	58.5%	53%	0.07
Total cholesterol (mg/dl)	223±39	208±39	**0.001**
LDL-C (mg/dl)	142.5±33	126±34	**0.001**
HDL-C (mg/dl)	58±15	53±15	**0.001**
Triglycerides (mg/dl) °	101 (76-137)	124 (87-172)	**0.001**
Fasting Glucose (mg/dl)	94±25	98±27	**0.005**
HOMA score °	2.05 (1.36-3.03)	2.21 (1.543.32)	**0.001**
Uric acid (mg/dl)	5±1.4	5.4±15	**0.001**
Creatinine (mg/dl)	0.92±0.22	0.95±0.23	**0.05**
BMI (kg/m^2)	27.3±3.9	27.7±4.1	0.20
Waist circumference (cm)	92±10	93.1±10	**0.02**
ABI	1.02±.015	1.03±0.18	0.69
WBC count (×1000/mm3)	6.05±1.53	6.27±1.68	**0.04**
IL -1 β (pg/ml) °	0.12 (0.07-0.19)	0.12 (0.08-1.20)	0.91
IL-1 ra (pg/ml) °	127 (92-178.4)	137 (98.4-189)	**0.01**
TNF-α (pg/ml) °	5.02 (2.83-7.62)	5.32 (2.89-7.91)	0.23
IL-6 (pg/ml) °	1.22 (0.70-1.98)	1.29 (0.80-2.09)	0.14
IL-18 (ug/ml) °	358 277-461)	382 (302-484)	**0.002**
Hs.CRP (mg/L) °	2.32 (1.07-5.26)	2.50 (1.23-5.32)	0.17
α Tocopherol (umol(L)	33.8	33.2	0.21
B Carotene (umol(L) °	0.37 (0.27-0.55)	0.34 (0.21-0.50)	**0.005**
Selenium (ug/L)	74.8	73.8	0.17
Diet (daily intake)			
- Kcal	1892±544	1939±586	0.18
- Cholesterol (mg)	267±95	267±89	0.92
- Saturated fatty acids (g)	22.1±7.5	21.6±7.3	0.74
- Alcohol users	65%	63%	0.30
Smoking habit (smokers)	37%	45%	**0.01**
Metabolic Syndrome	27%	38%	**0.001**
Diabetes	10.2%	14%	0.07
CHD	7.2%	8.4%	0.30
Stroke	5.4%	7.8%	0.07
PAD	11.2%	13.4%	0.17

HOMA: homeostasis model assessment; BMI: body mass index; ABI: ankle brachial index; WBC: white blood cells; CHD: coronary heart disease;

° Median (interquartile range)

TABLE 3

correlation coefficients between ox.LDL/LDL-C ratio and other parameters in 1025 community dwelling older individuals.

PARAMETER	r	P
Age	0.032	0.30
Metabolic Syndrome	0.167	**0.001**
HDL-C (mg/dl)	−0.238	**0.001**
Triglycerides (mg/dl)	0.249	**0.001**
Fasting Glucose (mg/dl)	0.096	**0.002**
HOMA score	0.087	**0.006**
Uric acid (mg/dl)	0.137	**0.001**
AST (U/L)	0.013	0.98
ALT (U/L)	0.037	0.23
Creatinine (mg/dl)	0.108	**0.001**
GFR by CKD-EPI (ml/m/1.73 m^2)	−0.132	**0.001**
BMI (kg/m^2)	0.053	0.10
Waist circumference (cm)	0.101	**0.002**
WBC count (/mm3)	0.079	**0.01**
Kcal/day	0.058	0.06
IL -1 β (pg/ml)	0.013	0.67
IL-1 ra (pg/ml)	0.066	**0.03**
TNF-α (pg/ml)	0.060	**0.05**
IL-6 (pg/ml)	0.037	0.23
IL-18 (ug/ml)	0.075	**0.01**
hs.CRP (mg/L)	0.011	0.73
α Tocopherol (umol(L)	0.20	0.52
B Carotene (umol(L)	−0.12	**0.001**
Selenium (ug/L)	−0.07	**0.05**

HOMA: homeostasis model assessment; GFR: glomerular filtration rate; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; BMI: body mass index; WBC: white blood cells