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Oxidized phospholipids on apoB-100-containing lipoproteins: a biomarker predicting cardiovascular disease and cardiovascular events

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Abstract

Oxidative stress is a well-known etiologic factor in the development of cardiovascular disease. Oxidation of lipoproteins, and in particular of low density lipoprotein, is a necessary if not obligatory mechanism for the generation of macrophage-derived foam cells, the first major initiating factor in the development of an atherosclerotic plaque. Oxidation of lipoproteins does not result in the generation of a single, defined molecular species, but of a variety of oxidation-specific epitopes, such as oxidized phospholipids and malondialdehyde-lysine epitopes. Unique monoclonal antibodies have been developed to bind these well-defined epitopes, and have been used in *in vitro* assays to detect them on circulating lipoproteins present in plasma. This article will summarize the accumulating clinical data of one oxidation-specific biomarker, oxidized phospholipids (OxPL) on apoB-100 lipoproteins. Elevated levels of OxPL/apoB predict the presence and progression of coronary, femoral and carotid artery disease, are increased following acute coronary syndromes and percutaneous coronary intervention, and predict the development of death, myocardial infarction, stroke and need for revascularization in unselected populations. OxPL/apoB levels are independent of traditional risk factors and the metabolic syndrome, and enhance the risk prediction of the Framingham Risk Score. The OxPLs measured in this assay reflect the biological activity of the most atherogenic lipoprotein(a) (Lp(a)) particles, reflected in patients with high plasma Lp(a) levels with small apo(a) isoforms. The predictive value of OxPL/apoB is amplified by Lp(a) and phospholipases such as lipoprotein-associated phospholipase A₂ and secretory phospholipase A₂, which are targets of therapy in clinical trials. This assay has now been validated in over 10,000 patients and efforts are underway to make it available to the research and clinical communities.

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Keywords

atherosclerosis; biomarker; lipoproteins; outcome; oxidation; oxidative stress; oxidized phospholipid

Oxidative stress is considered to be a key mechanism for the initiation and progression of atherosclerosis and the development of cardiovascular disease (CVD) [1,2]. Excessive oxidative stress occurs in response to many underlying cardiovascular risk factors, such as hypercholesterolemia, hypertension, diabetes mellitus and underlying genetic predisposition. Oxidative stress and subsequent oxidation of low-density lipoprotein (LDL) to produce oxidized (Ox)LDL is created through the reaction of reactive oxygen species, such as superoxide anion, and hydrogen peroxide and peroxynitrite. These oxidative species are generated during cellular metabolic pathways by lipoxygenases, myeloperoxidase, nitric oxide synthase, NADPH oxidase, xanthine oxidase and other oxidases, with polyunsaturated fatty acids, lipoproteins and amino acids, causing their modification to proinflammatory and atherogenic particles. One LDL particle is composed of approximately 600 molecules of free cholesterol, 1600 molecules of cholesteryl esters, 700 molecules of phospholipids and 185 molecules of triglycerides [3]. The polyunsaturated acyl chains of cholesteryl esters, polyunsaturated acyl and triglycerides are vulnerable to oxidation, as is the sterol of free cholesterol and cholesteryl esters. LDL contains one molecule of apoB-100, made up of 4536 amino acid residues with many exposed lysines, which can be directly oxidized or modified by lipid oxidation products [4]. Owing to its molecular composition, LDL is particularly susceptible to oxidation and OxLDL is considered among the strongest proinflammatory components of vulnerable plaques [5].

Oxidation of LDL leads to the generation of a variety of oxidation-specific epitopes (OSE), such as the oxidized phospholipid (OxPL) and malondialdehyde epitopes on LDL. Oxidation of LDL is thought to occur primarily in the vessel wall rather than in plasma, which is strongly enriched in antioxidants. These OSE are biologically active and upregulate adhesion molecules to attract monocytes into the vessel wall, mediate proinflammatory responses in cytokines and upregulate proinflammatory genes, promote macrophage retention and apoptosis [6,7], and are cytotoxic. These OSE are proatherogenic owing to their ability to enhance the unregulated uptake of OxLDL in macrophages through specific pathways generating activated macrophage foam cells (Figure 1). The accumulation of foam cells leads to fatty streak formation. Foam cell necrosis and/or apoptosis and continued accumulation of oxidized lipids in the extracellular space eventually lead to atheroma formation.

Oxidation-specific epitopes are also potent immunogens and lead to activation of T cells and B cells, resulting in the generation of autoantibodies to specific epitopes, which have been described in both humans and animals [8–10]. In mice, natural antibodies, secreted from OxLDL-specific B-1 cell lines, bind to OSE, block uptake of OxLDL by macrophages, recognize apoptotic cells, and are deposited in atherosclerotic lesions, suggesting a role of the innate immunity system in protecting hosts against these proinflammatory antigens (Figure 2).

Oxidized phospholipids play an important role in atherosclerosis and accumulate in human and mouse lesions. Specific OxPLs have been identified as major regulators of many cell types present in the vessel wall, including endothelial cells, smooth muscle cells, macrophages, dendritic cells and platelets [11,12]. Furthermore, several receptors and signaling pathways associated with OxPL action have been identified and demonstrated to be upregulated in human lesions [12,13]. OxPLs mediate plaque destabilization, being

present in higher quantities (70-fold) in plaque than plasma [5,14]. OxPL are key components of OxLDL, apoptotic cells and atherosclerotic lesions, and are important contributors to early events in atherogenesis by activating proinflammatory genes, leading to inflammatory cascades in the vessel wall [11,12], which reflect features of chronic inflammatory disease [1,15].

We have cloned a series of immuno-dominant IgM antibodies binding to OxLDL from apoE^{-/-} mice [10,16,17]. E06 is a well-characterized murine monoclonal antibody that binds to the phosphocholine (PC) head group of oxidized but not native phospholipids [18]. E06 is encoded by non-mutated germline genes and is 100% identical in the variable region of the heavy-chain and κ -light-chains sequences of the T15 natural antibody, which provides the optimal protection to mice against lethal infection with *Streptococcus pneumoniae* [19]. E06/T15 binds to PC exposed on OxPLs on Cu-OxLDL, as well as OxPL present on apoptotic cells, but also to PC coupled to teichoic/lipoteichoic acid on the cell wall of bacteria such as *S. pneumoniae*. Indeed, E06 recognizes OxPL on an equimolar basis when simply present as a PC salt or as PC on OxPL such as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-PC attached to a variety of different peptides, as well as PC on OxPL covalently linked (via its sn-2 oxidized side chain) to a variety of synthetic peptides irrespective of amino acid sequence [18]. E06 inhibits OxLDL uptake by macrophages, preventing recognition by scavenger receptors, and inhibits a number of other proinflammatory properties of OxPL generated via acute lung injury and infections [20,21]. E06 also exhibits other important biological functions, such as inhibition of uptake of apoptotic cells by macrophages *in vitro* [17,22], but may promote complement-mediated enhanced clearance of apoptotic cells *in vivo*, as has been demonstrated for IgM antibodies [23]. Interestingly, pneumococcal vaccination of cholesterol-fed LDLR^{-/-} mice increased the T15/E06 titers and, most strikingly, reduced the progression of atherosclerosis [24,25]. Overall, these data suggest that PC-based OxPL represents a danger-associated molecular pattern and that effector molecules, such as IgM natural antibodies (e.g., antibody E06/T15), scavenger receptors (e.g., CD36 and SR-B1) and C-reactive protein of the innate immune system evolved to bind and potentially neutralize them [26].

In the next section we provide a detailed summary of the OxPL/apoB assay in various applications in CVD. Other assays to measure the presence of OSEs on lipoprotein particles exist, and the interested reader is referred to recent articles on this topic that review the advantages and limitations of each assay [27–29].

OxPL/apoB methodology

Using E06, we have developed a chemiluminescent ELISA to detect OxPL on human apoB-100 (OxPL/apoB)-containing lipoprotein particles in plasma. This well-validated assay has been previously described in detail [27,28,30,31]. The assay is performed by plating overnight murine monoclonal antibody MB47 (50 μ l at 5 μ g/ml) [29,32], which under the conditions used, captures a saturating amount of apoB-100 on all apoB-containing lipoproteins from plasma. After washing, the plates are coated with 1% bovine serum albumin in tris-(hydroxymethyl) aminomethane-buffered saline. Plasma (50 μ l at 1:50 dilution) is added and allowed to incubate for 75 min. This initial step is designed so that each well captures a constant, saturating amount of apoB-100 from plasma and therefore normalizes the OxPL measure to an equal amount of apoB in the well for each patient. Thus, by definition, the OxPL/apoB measurement is independent of apoB and LDL cholesterol levels. Biotinylated E06 (1 μ g/ml) is added and allowed to incubate for 1 h. Alkaline phosphatase neutravidin (1:40,000 dilution) is added for 1 h. Lumi-Phos[®] 530 (Lumigen, Inc., Southfield, MI, USA; 25 μ l) is added for 75 min to detect OxPL per unit of apoB captured (e.g., OxPL in relative light units [RLU]/apoB). Since an equal amount of

apoB-100 is captured in each well from each subject, the denominator is the same in all wells (e.g., 1) and, thus, the actual read out is the amount of OxPL as detected by E06. This is detected by chemiluminescent technique and reported in RLU in 100 ms (Figure 3). The OxPL/apoB assay is highly specific to the number of OxPL epitopes on individual apoB-100 particles, but does not measure the total amount of OxPL in plasma.

In an early version of this assay prior to 2006, parallel plates were used to document that the wells captured equal amounts of apoB by first capturing apoB with MB47 and then adding the biotinylated murine monoclonal antibody MB24, which detects a separate apoB epitope, to quantitate the amount of captured apoB. The numerator represented OxPL on apoB detected by E06 (in RLU) and the denominator represented the amount of apoB (in RLU) detected on a parallel plate (i.e., $E06 \text{ in RLU} / \text{apoB in RLU} = \text{OxPL:apoB ratio}$, without units). Subsequently, we demonstrated that the correlation between OxPL/apoB RLU vs OxPL/apoB ratio was $r = 0.99$ in more than 1500 samples [28]. Therefore, this additional step is not performed routinely in current human studies and the data are presented as OxPL/apoB in RLU, measured as the E06 binding only. In the near future, we will report the values as moles of oxidized PC, as we have generated a standard curve using a small peptide with a known amount of OxPL to equate the amount of OxPL detected by E06 as moles PC. For the data reported thus far, one can compare relative changes or values within assays, but not absolute values among different studies. To minimize variability we have generally performed all the samples in one batch with high and low OxPL standards on each plate. Our coefficient of variability has been 5–10%, which should improve further with the standard curve.

Table 1 displays the various studies performed with this assay prior to 2006. The OxPL/apoB assay is reported as OxPL/apoB ratio prior to 2006 and as OxPL/apoB RLU following 2006.

Relationship of OxPL/apoB & Lp(a): Lp(a) as a preferential carrier of E06-detectable OxPL

Several early clinical studies documented, unexpectedly, that OxPL/apoB correlated strongly with Lp(a) [27,33], and not with LDL as expected [31]. Lp(a), which is secreted from the liver, is an independent, causal, genetic risk factor for cardiovascular death and myocardial infarction, and this risk is continuous and linear with increasing Lp(a) levels [34–41]. A physiological role of Lp(a) and the underlying mechanisms through which it contributes to CVD are still unknown. However, we have shown that Lp(a) preferentially binds OxPL, compared with other lipoproteins [42,43], and have proposed that a unique physiological role of Lp(a) may be to bind and transport proinflammatory OxPL in plasma. This would suggest that a sufficient and low level of Lp(a) is beneficial. Indeed, a J-shaped curve relates Lp(a) levels to CVD [44,45], suggesting that a small amount of Lp(a) (2–7 mg/dl) is associated with reduced CVD risk, but that higher levels are associated with increased risk (>25 mg/dl). The OxPL content may also explain this pathophysiological role [31]. When present at high plasma concentrations, Lp(a) would be more atherogenic than native LDL, as it binds with increased affinity to arterial intimal proteoglycans [46] resulting in increased intimal concentration of LDL along with associated proinflammatory OxPL. This hypothesis is now supported by several levels of evidence, including the correlation between OxPL/apoB and Lp(a) in multiple clinical studies [30,44,47]; the presence of OxPL on apo(a) and Lp(a), as detected by a variety of biochemical, immuno-precipitation and ultracentrifugation experiments, which demonstrate that approximately 85% of E06 reactivity (i.e., OxPL) coimmunoprecipitated with Lp(a) [42,43]; *in vitro* transfer studies demonstrating that OxPL from OxLDL are preferentially transferred to Lp(a) rather than LDL in a time-/temperature-dependent fashion [42]; extraction of purified human Lp(a) with organic solvents followed by liquid chromatography tandem mass spectrometry studies showing that 30–70% of OxPL, both E06-detectable and E06-nondetectable, are extractable;

lack of evidence of oxidation of Lp(a) itself (e.g., the lack of malondialdehyde epitopes) [42]; large clinical studies showing CVD event prediction by elevated baseline levels of OxPL/apoB, particularly those with small isoforms [28,42,44,47–49]; and accentuation of CVD risk and event prediction by OxPL/apoB with either lipoprotein-associated phospholipase A₂ (Lp-PLA₂) or secretory-PLA₂ (sPLA₂), suggesting an additive effect of substrate (OxPL) and enzyme activity of phospholipases [44,47].

Oxidized phospholipid/apoB levels were measured in 3481 subjects (1831 black, 1047 white and 603 Hispanic) in the Dallas Heart Study, where it was demonstrated that they were highest in black people followed by white and Hispanic people ($p < 0.001$ for each comparison) (Figure 4). OxPL/apoB levels did not correlate significantly with cardiovascular risk factors, age or gender. However, OxPL/apoB levels strongly correlated with Lp(a) ($r = 0.85$, $p < 0.001$), with the correlation showing a ‘reverse L’ shape when values were log-transformed (Figure 5). In this relationship, there was no correlation between OxPL/apoB and Lp(a) at Lp(a) levels < 30 nmol/l (~ 10 mg/dl), but a very strong correlation above this threshold. Within racial groups, the highest r -values were highest in black individuals, then white and the Hispanic individuals. The OxPL-Lp(a) correlation was highly dependent on underlying apo(a) isoform size, with strong correlations in subjects with small apo(a) isoforms (number of kringle type-IV repeats) that became progressively weaker or absent with larger apo(a) isoforms. Interestingly, there was a negative association between the size of the major apo(a) isoform and OxPL/apoB ($r = -0.50$, $p < 0.001$) (Table 2), irrespective of racial group. The relationship between OxPL/apoB and Lp(a) remained significant ($r = 0.67$, $p < 0.001$) after adjusting for apo(a) isoform size. In summary, this suggests that the OxPL/apoB levels reflect the most atherogenic Lp(a) particles, irrespective of race, and may allow clinical selection of risk profiles above and beyond measuring Lp(a) levels, as recently shown in the European Prospective Investigation of Cancer (EPIC)-Norfolk study [47]. Although basal Lp(a) levels are mainly genetically determined, modest changes above this pre-set baseline do occur in a number of situations, such as in acute phase responses [34], low fat diet [50,51] and statin therapy [52]. As Lp(a) is a lipoprotein carrier of OxPL, it is likely that the OxPL/apoB levels are also genetically determined to some extent, but in certain situations these values do change from baseline. Studies in twins are underway to examine the strength of the heritability of this relationship.

Relationship of OxPL/apoB & CVD

Association with acute coronary syndromes & percutaneous coronary intervention—Acute coronary syndromes are associated with increased oxidative stress [53–55]. In two studies, it was demonstrated that acute increases occur in OxPL/apoB in patients following acute coronary syndrome [27] or during uncomplicated percutaneous coronary intervention [33], suggesting generation and/or release of oxidized lipids into the circulation from atherosclerotic lesions. In a prospective study in patients with acute coronary syndrome [27], it was demonstrated that OxPL/apoB levels rise rapidly by approximately 54% after an acute myocardial infarction and then tend to decrease toward baseline levels over the next 7 months (Figure 6a). By contrast, no significant changes were noted in patients with stable coronary artery disease (CAD), patients with normal coronary angiograms and a control group of healthy subjects followed for the same period of time. Further supporting this finding, a follow-up study demonstrated that OxPL/apoB and Lp(a) levels also acutely increased (by 36%; $p < 0.0001$) (Figure 6b) and 64%; $p < 0.0001$, respectively) immediately following percutaneous coronary intervention and then returned to baseline by 6 h [33]. Immunoprecipitation experiments showed that approximately 50% of OxPLs were present on Lp(a) immediately after percutaneous coronary intervention, whereas the rest were present on non-Lp(a) apoB-100 particles. However, by 6 h more than

90% of OxPL were again present on Lp(a). This supports the role of Lp(a) as a preferential binder and transporter of OxPL [42].

Association with CAD—It was demonstrated in 504 patients undergoing clinically indicated coronary angiography that elevated levels of OxPL/apoB were strongly and independently correlated with the presence and extent of angiographically documented CAD, defined by >50% diameter stenosis and measured as one-, two- or three-vessel CAD, particularly in patients 60 years of age or younger [48]. In patients <60 years old, the highest quartile of OxPL/apoB was associated with an odds ratio (OR) for CAD of 3.12 ($p = 0.004$), compared with the lowest quartile. This relationship was markedly accentuated in the setting of hyper-lipidemia (OR: 16.8) (Table 3). Importantly, in patients <60 years old, OxPL/apoB remained an independent predictor of CAD, even with Lp(a) in the model (Figure 7). In the entire cohort, OxPL/apoB levels were independently associated with obstructive CAD for all clinical and lipid measures except for Lp(a), suggesting a common biologic influence on CAD risk. These observations support the hypothesis that although much of the risk attributable to Lp(a) can be explained by its binding of OxPL, additional risk associated with OxPL may be present in younger patients, perhaps through proinflammatory pathways independent of Lp(a). Furthermore, in this specific population selected for clinically indicated coronary angiography, the most atherogenic Lp(a) particles may be better reflected by OxPL/apoB, as suggested by the significantly higher OR for CAD. It is likely that smaller Lp(a) particles have the most OxPL on them, and this leads to some dissociation of OxPL/apoB and Lp(a) levels *per se*. In fact, in all studies published to date OxPL/apoB was equivalent or superior to risk compared with Lp(a) in risk prediction.

Relationship to peripheral artery disease—In the Bruneck study, a large prospective population-based survey of 40–79-year-old men and women initiated in 1990 where serial plasma levels of OxPL/apoB were measured in 765 and 671 subjects in 1995 and 2000, OxPL/apoB levels predicted the presence of symptomatic CVD and were significantly associated with the presence, extent and development (1995–2000) of carotid and femoral atherosclerosis (Figure 8) [28]. The association of OxPL/apoB and Lp(a) was strongest in subjects with small apo(a) isoforms and the highest Lp(a) concentration, suggesting that OxPL/apoB levels may be influenced by the number of K-IV2 repeats (Figure 9).

Prediction of cardiovascular events—The Bruneck study [28,44] was the first prospective epidemiological study in an unselected population derived from the general community to demonstrate the prognostic utility of OxPL/apoB levels in predicting future death, myocardial infarction, stroke, and transient ischemic attack and revascularization. OxPL/apoB levels predicted the development of cardiovascular events over a 10-year prospective follow-up period. For example, subjects in the highest tertile of OxPL/apoB had a significantly higher risk of cardiovascular events than those in the lowest tertile (hazard ratio: 2.4, 95% CI: 1.3–4.3, $p = 0.004$) (Figure 10) following multivariable adjustment for traditional risk factors, high-sensitivity C-reactive protein and Lp-PLA₂ activity. These findings were confirmed and extended in the prospective case-controlled EPIC-Norfolk study, consisting of 763 cases and 1397 matched controls composed of 45–79-year-old apparently healthy men and women followed for 6 years [47]. This study showed the highest tertiles of OxPL/apoB were associated with a significantly higher risk of CAD events (OR: 1.67; $p < 0.001$) compared with the lowest tertiles (Table 4), after adjusting for age, smoking, diabetes, LDL and high-density lipoprotein cholesterol, and systolic blood pressure.

Furthermore, OxPL/apoB levels provided additional predictive value to the Framingham Risk Score (FRS). By measuring OxPL/apoB in each tertile of FRS, the predicted risk can be either increased or decreased depending on the tertiles of OxPL/apoB. This would allow

fine tuning of risk prediction and more accurate assessment of treatment options. For example, in the Bruneck Study, the graded increase in CVD risk across OxPL/apoB tertile groups was evident in the low-, moderate- and high-risk groups as defined by the FRS (Figure 11a) [44]. This result was validated in the EPIC-Norfolk study (Figure 11b) [47]. Finally, OxPL/apoB values are independent of metabolic syndrome parameters [47].

Receiver-operator characteristic c-index values—To assess the predictive value of the utility of these biomarkers above the FRS, receiver-operator characteristic unconditional c-indexes were generated [47]. The c-index discriminates between individuals at different risk levels and measures the probability that a randomly chosen individual who experienced an event has a higher risk score than a randomly chosen individual who did not experience an event during the same, specific follow-up interval. The c-index is a standard measure of the effect of a new marker in risk prediction and helps to quantify its predictive discrimination, but does have limitations [56].

The c-index for the FRS was 0.584 (95% CI: 0.558–0.609), a relatively low value that reflects the fact that age and sex were already accounted for as part of the matching design. Adding individual biomarkers to the FRS shows that the c-index increased from 0.584 (95% CI: 0.558–0.609) to 0.618 (95% CI: 0.593–0.642), in progressing order of myeloperoxidase mass, Lp-PLA₂ activity, OxPL/apoB, high-sensitivity C-reactive protein, Lp(a), sPLA₂ mass and sPLA₂ activity. Adding biomarkers to FRS until all biomarkers were present in the model progressively increased the c-index from 0.584 (95% CI: 0.558–0.609) to 0.651 (95% CI: 0.627–0.675) (Table 5).

Relationship of OxPL/apoB to Lp(a), Lp-PLA₂ and sPLA₂—In prior studies, we have demonstrated that OxPL/apoB reflect the biological activity of small apo(a) isoforms associated with high Lp(a) levels [44,49]. Furthermore, the OxPL/apoB assay represents OxPL bound by Lp(a) (~85–90%) and non-Lp(a) apoB (10–15%), on average [42]. This relationship is not constant and depends on the underlying LPA genetics, where small isoforms are associated with high OxPL/apoB and high correlations with Lp(a) [49,57], but large isoforms are associated with low Lp(a) levels (i.e., <25 mg/dl) and weak-to-absent correlations [49]. To assess whether adding Lp(a) levels to OxPL/apoB enhances the predictive value for CVD events, we performed a 3 × 3 tertile analysis evaluating relationship to CAD events in EPIC-Norfolk. This demonstrated that the relationship of OxPL/apoB and Lp(a) to fatal and nonfatal CAD was accentuated in the highest tertiles of both biomarkers (OR: 1.77, 95% CI: 1.31–2.37), suggesting that they can provide independent and additive information for risk prediction (Figure 12) [47].

Lipoprotein-associated phospholipase A₂ and sPLA₂ are enzymes that react with OxPLs and cleave the oxidized fatty acid side chain at the sn-2 position of OxPL to generate lyso-phosphatidylcholine and an oxidized free fatty acid. Both of these biomarkers have been associated with the prediction of cardiovascular events when elevated and are targets of ongoing therapeutic trials to inhibit their activities [50]. Since OxPL and phospholipases may share similar pathophysiology, we evaluated whether the combination of these biomarkers provides enhanced predictive value. In the Bruneck Study, the strength of the association between OxPL/apoB and CVD risk significantly increased with increasing Lp-PLA₂ activity (p = 0.018 for interaction) (Figure 13a). Similarly, the OR of CAD events associated with the highest tertiles of OxPL/apoB was significantly potentiated (approximately doubled) by the highest tertiles of sPLA₂ activity and mass (Figure 13b). In clinical risk prediction, using combinations of these biomarkers may allow stronger predictive value for ascertaining CVD risk. Although these phospholipases are clearly risk predictors, their role as causal risk factors has not been proven yet [58]. In fact, Phase III

clinical trials are currently underway to assess whether inhibition of their mass and/or activity will lead to improvement in cardiovascular outcomes [51,52].

Change in OxPL/apoB & therapeutic interventions—The OxPL/apoB assay was originally developed as an indicator of minimally oxidized LDL in plasma that might reflect the overall content of circulating OxLDL. We initially postulated that OxPL/apoB levels would increase during hypercholesterolemia and/or atherosclerosis progression and decrease during atherosclerosis regression. Counterintuitively, we have found the opposite, in both animals [59] and humans [33,58,60–65], suggesting that increases in OxPL/apoB in plasma may reflect reduction of the OxPL content of arterial lesions. For example, in New Zealand white rabbits and cynomolgus monkey models of atherosclerosis, which do not have Lp(a) or their Lp(a) does not bind OxPL and/or has E06 immunoreactivity, respectively, OxPL/apoB levels increased 50–100% in plasma in the setting of lesion regression, concomitant with reduced immunostaining of OxPL in atherosclerotic lesions (Figure 14) [59]. Note that the OxPL staining in the middle panel of Figure 14 has disappeared from the luminal surface, while the OxPL/apoB level goes up in plasma, suggesting a flux of OxPL from the vessel wall to the circulation during dietary induced regression. This suggests that efflux of OxPL occurs preferentially early during atherosclerosis regression from arterial lesions into plasma, even more extensively than depletion of apoB-100 or physical plaque regression. These data suggest that changes in the OxPL/apoB ratio may reflect early atherosclerosis regression. Similarly, in human studies with low fat diets [60,62], aged garlic supplementation [58,63] and statin therapy (Table 1) [33,60,61,65], significant increases in OxPL/apoB have been noted shortly after the initiation of the intervention. Although the etiology of these changes is not fully defined, the data are consistent across studies and are associated with treatments considered to be of clinical benefit. For example, in the Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering (MIRACL) trial, an increase in OxPL/apoB and Lp(a) was observed at 16 weeks after initiation of atorvastatin therapy, consistent with a decreased reoccurrence of clinical events, while no such change was observed in the placebo arm. Similarly, an increase in OxPL/apoB, and Lp(a) in response to aged garlic supplements was associated with less progression of coronary artery calcium and improvement in vascular function [58,63]. Based on the animal data, one may postulate a flux of OxPL from sites of vessel injury or inflammation to the circulation, where they are bound to apoB lipoproteins. In humans, the OxPL preferentially bind to Lp(a), but we have also noted an increase in the Lp(a) levels as well, suggesting that perhaps there is some signaling mechanism leading to increased Lp(a) levels in these settings. These findings are consistent with our suggestion noted above that a ‘physiological’ role of Lp(a) may be to bind and transport cellular sources of OxPL, such as from apoptotic cells or during normal cellular metabolism where OxPL may be generated. Whether these increases in OxPL/apoB found in the settings of low fat diets, statin therapy, or other potentially beneficial interventions are biomarkers of enhanced efflux from the artery of OxPL, and hence a surrogate of regression, remains to be established.

The apparent paradox is that OxPL/apoB remain strongly independent predictors of CVD in prospective studies, yet here we find they are increased, at least in the short term, in settings of effective therapy. We suggest that a resolution of this paradox may be that with continued therapy, the initial efflux of OxPL from artery into plasma will return to baseline, and the elevated OxPL/apoB will revert to basal levels, or even lower, consistent with reduced risk caused by the intervention. These ideas are currently being tested by extended analyses of adequately powered prospective intervention trials. If confirmed, an early rise in OxPL/apoB might then be used as a useful surrogate biomarker of a beneficial intervention. Such biomarkers are urgently needed.

Conclusion & future perspective

Oxidized phospholipids are strongly implicated in several aspects of CVD. The OxPL/apoB assay provides diagnostic information by strongly reflecting the presence and progression of CVD and prognostic information for predicting future cardiovascular events. It independently complements established risk factors in risk prediction, is independent of the metabolic syndrome and optimizes the predictive value of the FRS. Importantly, from a pathophysiological perspective, OxPL/apoB appears to most closely reflect the biological activity of the most atherogenic Lp(a) particles that are associated with both high Lp(a) levels and small apo(a) isoforms. Since apo(a) isoforms are laborious and expensive to measure, OxPL/apoB levels may accurately reflect the CVD risk of these atherogenic lipoproteins. With Lp(a) now being established as an independent, genetic risk factor for CVD, it may become a target of therapy in future studies. In fact, the antisense oligonucleotide mipomersen and a more specific antisense oligonucleotide targeted to KIV-2 repeats of apo(a) have recently been shown to reduce plasma levels of Lp(a)/apo(a), and their associated OxPL, by 75–86% in Lp(a)-transgenic mice [66,67], setting the stage for future clinical development.

Currently, the OxPL/apoB assay is a research tool, but the large clinical database already established suggests that with standardization of the methodology, it will be a useful biomarker that can be used clinically for risk stratification. Future research will focus on the early (and possibly late) changes in OxPL/apoB that occur with therapeutic interventions and whether therapeutic decisions can be guided by assessing these changes in response to the intervention. Finally, since OxPL are implicated in a variety of disorders that have oxidative stress as a key component, such as Alzheimer's disease, multiple sclerosis, nonalcoholic steatohepatitis, rheumatologic disease such as lupus and rheumatoid arthritis, infectious disease and cancer, it would be of interest to assess whether OxPL/apoB and related oxidative biomarkers are associated with and predict noncardiovascular outcomes in patients with these disorders.

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Executive summary

- Oxidation of lipoproteins, especially low-density lipoprotein (LDL), is a necessary mechanism for foam cell generation as well as initiation and destabilization of atherosclerotic lesions.
- A variety of oxidation-specific epitopes, such as oxidized phospholipids (OxPL) and malondialdehyde-lysine epitopes, are generated during oxidation.
- EO6 is a murine monoclonal antibody that binds to the phosphocholine head group of oxidized but not native phospholipids.
- The OxPL/apoB-lipoprotein(a) (Lp(a)) correlation is highly dependent on apo(a) isoform size, with strong correlations with small apo(a) isoforms and weak or absent with larger apo(a) isoforms.
- OxPL/apoB levels correlate with Lp(a) and not with LDL, which could propose a unique physiological role of Lp(a) to bind and transport pro-inflammatory OxPL in plasma. OxPL/apoB reflects the most atherogenic Lp(a) particles associated with small isoforms and high Lp(a) levels.

Relationship of OxPL/apoB & cardiovascular disease

- OxPL/apoB increases acutely in patients following acute coronary syndromes or during uncomplicated percutaneous coronary intervention.
- OxPL/apoB levels are independently correlated with the presence and extent of angiographically documented coronary artery disease and the presence and progression of carotid and femoral atherosclerosis.
- Elevated baseline levels of OxPL/apoB independently predict the development of cardiovascular events over a 10-year prospective follow-up in a population derived from general community and provide additional predictive value to the Framingham Risk Score, are independent of the metabolic syndrome and increase the c-index for predicting events in a model with Framingham Risk Score, traditional risk factors, and other oxidative and inflammatory biomarkers, including high-sensitivity C-reactive protein.
- The strength of the association between OxPL/apoB and cardiovascular disease risk significantly increases with increasing Lp-PLA₂ activity, secretory phospholipase A₂ activity and mass.
- In both animals and humans, efflux of OxPL from arterial lesions into plasma was observed to occur preferentially early during atherosclerosis regression (low fat diets, statin therapy and aged garlic supplements).

Conclusion

- OxPL/apoB levels provide diagnostic and prognostic information in strongly reflecting the presence and progression of cardiovascular disease and predicting future cardiovascular events, and may be a useful assay in research and clinical applications.

Figure 1. Oxidized lipid moieties induce lipoprotein accumulation in macrophages

Macrophage lipoprotein uptake mechanisms can be separated into: macropinocytosis, when actin polymerization and extensive membrane ruffling result in the ruffles closing into large endosomes and capture of large volumes of extracellular material, including all classes of native and OxLDL present in the vicinity of the cell; and micropinocytosis, when ligand–receptor binding leads to membrane invagination and nearly stoichiometric internalization of the ligand or the lipoprotein carrying this ligand. mmLDL and polyoxygenated OxCEs induce Syk recruitment to TLR-4, Syk and TLR-4 phosphorylation and subsequent ERK1/2-dependent activation of small GTPases Rac, cdc42 and Rho and phosphorylation of paxillin, leading to actin reorganization and membrane ruffling. Resulting macropinocytosis promotes foam cell formation [74]. Binding of OxLDL or OxPL to CD36 initiates Lyn-dependent phosphorylation of JNK, which is essential for CD36-mediated OxLDL uptake, although the mechanism linking JNK with the membrane dynamics is unclear [75]. The TLR-4- and CD36-mediated uptake mechanisms are only examples; there are numerous other pattern recognition receptors involved in oxidation-specific epitope-stimulated lipoprotein internalization by macrophages.

mmLDL: Minimally modified low-density lipoprotein; nLDL: Native low-density lipoprotein; OxCE: Oxidized cholesterol ester; OxLDL: Oxidized low-density lipoprotein; OxPL: Oxidized phospholipid; POVPC: 1-palmitoyl-2-oxovaleroyl-sn-glycero-3-phosphorylcholine; TLR: Toll-like receptor.

Adapted with permission from [26].

Figure 2. Pattern recognition of oxidation-specific danger-associated molecular patterns and microbial pathogen-associated molecular patterns

Using the example of the PC epitope, in this illustration, we demonstrate our hypothesis of the emergence and positive selection of multiple pattern recognition receptors (PRRs) that recognize common epitopes, shared by modified self- and microbial pathogens. According to this hypothesis, oxidation of plasma membrane phospholipids in apoptotic cells alters the conformation of the PC head group, yielding an exposed epitope, accessible to recognition by macrophage scavenger receptors, natural antibodies and pentraxins, such as C-reactive protein. These PRRs were selected to clear apoptotic cells from developing or regenerating tissues. Recognition by the same receptors of the PC epitope of capsular polysaccharide in Gram-positive bacteria (e.g., *Streptococcus pneumoniae*) strengthened positive selection of these PRRs and probably helped to select additional strong proinflammatory components to PRR-dependent responses. (Note that the PC on the bacteria is not part of a phospholipid.) Finally, oxidized lipoproteins, prevalent in experimental animals and humans as a result of enhanced oxidative stress, dyslipidemia and impact of environmental factors, bear OxPLs with the PC epitope exposed in an analogous manner to that of apoptotic cells. This leads to OxLDL recognition by PRRs and initiation of innate immune responses. The balance between proinflammatory responses of cellular PRRs and atheroprotective roles of natural antibodies plays an important role in the development of atherosclerosis. There are likely many more oxidation-specific epitopes that represent such danger-associated molecular patterns and corresponding PRRs that represent respective innate responses. C-PS: Cell wall polysaccharide; OxPL: Oxidized phospholipid; PC: Phosphocholine. Adapted with permission from [26].

Figure 3. The oxidized phospholipid/apoB assay

Microtiter well plates are coated with the murine antibody MB47 and plasma added to bind apoB-100 particles. OxPL on apoB-100 are then detected with biotinylated murine monoclonal antibody E06.

LDL: Low-density lipoprotein; Lp(a): Lipoprotein(a); OxPL: Oxidized phospholipid; VLDL: Very low-density lipoprotein.

Figure 4. Levels of oxidized phospholipid/apoB and lipoprotein(a) categorized by racial group

Boxes indicate medians, 25th and 75th percentile, whiskers indicate 10th and 90th percentile. Differences among racial groups are all significant ($p < 0.001$).

BF: Black females; BM: Black males; HF: Hispanic females; HM: Hispanic males; Lp(a): Lipoprotein(a); OxPL: Oxidized phospholipid; RLU: Relative light units; WF: White females; WM: White males.

Adapted with permission from [49].

Figure 5. Correlation between oxidized phospholipid/apoB and Lipoprotein(a) in the Dallas Heart Study

(A) Relationship plotted on a geometric scale. (B) Relationship plotted on a logarithmic scale. (C) Relationship in the entire cohort according to apo(a) isoform sizes.

Lp(a): Lipoprotein(a); OxPL: Oxidized phospholipid; RLU: Relative light units.

Adapted with permission from [49].

Figure 6. Change in oxidized phospholipid/apoB following acute coronary syndromes and uncomplicated percutaneous coronary intervention

(A) Percent change from baseline in OxPL/apoB measured by antibody E06 in patients with ACS. The p-values at the 30-, 120- and 210-day labels represent differences between groups at each time point. Changes in LDL cholesterol are given for comparison. (B) Absolute changes in RLU in oxidized phospholipid/apoB after PCI. $p < 0.001$ compared with other time points.

ACS: Acute coronary syndrome; LDL: Low-density lipoprotein; OxPL: Oxidized phospholipid; PCI: Percutaneous coronary intervention; RLU: Relative light units. Adapted with permission from [27,33].

Figure 7. Odds ratios for obstructive coronary artery disease associated with selected risk factors among patients 60 years of age or younger from the multivariable analysis

Risk factors are shown in descending order of significance. In this analysis, Lp(a) was forced into the model with the OxPL:apoB-100 ratio.

CAD: Coronary artery disease; CRP: C-reactive protein; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; Lp(a): Lipoprotein(a); OxPL:apoB-100 ratio: Ratio of oxidized phospholipid content per particle of apoB-100.

Adapted with permission from [48].

Figure 8. Multivariate analysis showing the association of oxidized phospholipids/apoB-100 particle tertile groups with the presence and progression of carotid and femoral artery atherosclerosis and with cardiovascular disease

* $p < 0.05$ for the comparison between the first tertile group (reference category) and the third tertile group. The p-values presented in the figures are the overall p-values for the three tertiles (test for trend).

Adapted with permission from [28].

Figure 9. 3D plot of oxidized phospholipid/apoB levels according to lipoprotein(a) mass and apo(a) phenotypes expressed as the number of kringle IV type 2 repeats

The OxPL/apoB levels presented are geometric means (taken as the anti-log of the mean of log-transformed OxPL/apoB values).

Lp(a): Lipoprotein(a); OxPL: Oxidized phospholipid.

Adapted with permission from [28].

Figure 10. Cumulative hazard curves of incident cardiovascular disease from 1995 to 2005 for tertiles of oxidized phospholipid/apoB in the Bruneck study

OxPL: Oxidized phospholipid.

Adapted with permission from [44].

Figure 11. Oxidized phospholipid/apoB ratio within each Framingham Risk Score group
(A) Relationship between tertile groups and cardiovascular disease risk (tertile 1: <0.0379 relative light units (RLU), tertile 2: 0.0379–0.0878 RLU and tertile 3: >0.0878 RLU). **(B)** Relationship between tertile groups and future coronary artery disease risk (tertile 1: <1150 RLU, tertile 2: 1151–2249 RLU and tertile 3: >2249 RLU). Framingham Risk Score was calculated as low risk (<10% risk of events over 10 years), moderate risk (10–20%) and high risk (>20%).
 * $p < 0.05$ and ** $p < 0.001$ for comparison of each tertile of the respective biomarkers with the lowest tertile in the low Framingham Risk Score category of each biomarker.
 EPIC: European Prospective Investigation of Cancer; Lp(a): Lipoprotein(a); OxPL: Oxidized phospholipid.
(A) Adapted with permission from [44].

(B) Adapted with permission from [47].

Figure 12. Odds ratios for fatal and nonfatal coronary artery disease based on tertiles of oxidized phospholipid/apoB and lipoprotein

(a). The tertile cutoffs for OxPL/apoB are tertile 1: <1150 relative light units (RLU), tertile 2: 1151–2249 RLU and tertile 3: >2249 RLU, and for Lp(a) they are tertile 1: <7.25 mg/dl, tertile 2: 7.25–11.69 mg/dl and tertile 3: >11.69 mg/dl.

CAD: Coronary artery disease; Lp(a): Lipoprotein(a); OR: Odds ratio; OxPL: Oxidized phospholipid.

Adapted with permission from [47].

Figure 13. Relationship of oxidized phospholipid/apoB to lipoprotein-associated phospholipase A₂ and soluble phospholipase A₂ activity

(A) Relationship between OxPL/apoB tertile groups and CVD risk according to tertiles of Lp-PLA₂ activity. (B) ORs for CAD based on tertiles of OxPL/apoB and sPLA₂ activity.

The tertile cutoffs for OxPL/apoB are tertile 1: <1150 relative light units (RLU), tertile 2: 1151–2249 RLU and tertile 3: >2249 RLU, and for sPLA₂ activity levels, tertile 1: <4.05 nmol/min/ml, tertile 2: 4.05–4.83 nmol/min/ml and tertile 3: >4.83 nmol/min/ml.

CAD: Coronary artery disease; CVD: Cardiovascular disease; EPIC: European Prospective Investigation of Cancer; HR: Hazard ratio; Lp-PLA₂: Lipoprotein-associated phospholipase

A₂ activity; OR: Odds ratio; OxPL: Oxidized phospholipid; sPLA₂: Soluble phospholipase A₂.

(A) Adapted with permission from [44].

(B) Adapted with permission from [47].

Figure 14. Oxidized phospholipid:apoB ratio and immunohistochemistry in a New Zealand white rabbit study

(A) OxPL:apoB ratio in the New Zealand white rabbit study in the baseline ($n = 15$), low cholesterol ($n = 10$) and high cholesterol ($n = 5$) groups. (B) Immunohistochemistry of New Zealand white aortas with antibody E06 staining (brown color pattern) for OxPL in the baseline, low cholesterol and high cholesterol diet groups. The arrow represents lack of OxPL at the luminal surface in a representative rabbit with pre-existing atherosclerosis that was subsequently switched to a low-cholesterol diet.

OxPL: Oxidized phospholipid.

Adapted with permission from [59].

Table 1
Clinical studies examining the role of oxidized phospholipids/apoB in cardiovascular disease.

Study	Year	Study name	Patient population	Number of patients/samples	Outcome: change in OxPL/apoB levels	Ref.
Wu <i>et al.</i>	1999	–	Borderline hypertension	146/146	Increased in borderline hypertension and may reflect early vascular changes	[68]
Penny <i>et al.</i>	2001	UCSD regression study	Hypercholesterolemic patients undergoing quantitative angiography before/after lipid-lowering therapy	29/54	Related significantly to the severity of endothelial dysfunction and was the single most powerful independent risk factor	[69]
Tsimikas <i>et al.</i>	2003	ACS	Acute coronary syndromes Acute MI, unstable angina, stable CAD	66/272	Increased after acute MI	[27]
Tsimikas <i>et al.</i>	2004	Toronto PCI	Patients with stable angina pectoris undergoing PCI	141/1269	Increased immediately after PCI and return to baseline after 6 h	[33]
Segev <i>et al.</i>	2005				No relationship to restenosis	[70]
Tsimikas <i>et al.</i>	2004	MIRACL	Impact of atorvastatin in ACS	2341/4682	Increased 9.6% with atorvastatin 80 mg/day	[71]
Fraley <i>et al.</i>	2009				Baseline levels varied according to specific CVD risk factors and were largely independent of inflammatory biomarkers	[72]
Silaste <i>et al.</i>	2004	–	Healthy young women	37/74	Increased 19–27% with low-fat, high-vegetable diet	[62]
Tsimikas <i>et al.</i>	2005	Mayo	Coronary angiography	504/504	Strong and graded association with presence and extent of CAD	[48]
Tsimikas <i>et al.</i>	2006	Bruneck	Random sample of population (40–79 year old males and females)	765/1436	Predict presence and progression of carotid and femoral atherosclerosis	[28]
Kiechl <i>et al.</i>	2007				Predict 10-year CVD event rates independently of traditional risk factors, hsCRP and FRS	[44]
Rodenburg <i>et al.</i>	2006	–	Children with familial hypercholesterolemia and unaffected siblings	256/512	Increase 29% with Step II AHA diet Increase 49% with pravastatin 40 mg/day	[61]
Bossola <i>et al.</i>	2007	–	End-stage renal failure patients undergoing chronic hemodialysis	52/104	Reduced in end-stage renal failure patients following hemodialysis	[73]
Ky <i>et al.</i>	2008	PROXI	Hypercholesterolemic patients were randomized to different types and doses of statin	120/240	Increased 26% with pravastatin 40 mg/day and 20% with atorvastatin 80 mg/day	[60]
Choi <i>et al.</i>	2008	REVERSAL	Patients with CAD who underwent coronary IVUS and were assigned to statin therapy	214/428	Increased 48% with atorvastatin 80 mg/day and 39% with pravastatin 40 mg/day	[65]
Tsimikas <i>et al.</i>	2009	Dallas Heart Study	Multietnic, probability-based sample of the Dallas County population	3481/3481	Varied according to race/ethnicity, independent of cardiovascular risk factors and were inversely associated with apo(a) isoform size	[49]
Tsimikas <i>et al.</i>	2010	EPIC-Norfolk Study	45–79-years-old healthy males and females followed for 6 years	2160/2160	The highest tertiles are associated with higher risk of CAD events	[47]

Study	Year	Study name	Patient population	Number of patients/samples	Outcome: change in OxPL/apoB levels	Ref.
Budoff <i>et al.</i>	2009	Garlic study	Asymptomatic patients with CAD treated with aged garlic extract plus supplement followed with coronary artery calcium scan	60/120	Increase with aged garlic extract predicts lack of coronary artery calcium progression	[63]
Ahmadi <i>et al.</i>	2010				Increase with aged garlic extract correlates with improvement in vascular function	[58]
Arai <i>et al.</i>	2010	I4399M SNP	Carriers and noncarriers of I4399M single nucleotide LPA polymorphism	174/174	Elevated in carriers compared with noncarriers, while patients with small apo(a) isoforms had the highest OxPL/apoB levels	[57]
Faghihnia <i>et al.</i>	2010	CHORI	Healthy subjects consuming a high-fat low-carbohydrate diet and a low-fat high-carbohydrate diet	63/126	OxPL/apoB and OxPL/apo(a) are increased by a low-fat high-carbohydrate diet	[64]
Total: 21	—	—	—	10609/15782	—	—

ACS: Acute coronary syndrome; AHA: American Heart Association; CAD: Coronary artery disease; CHORI: Children's Hospital Oakland Research Institute; CVD: Cardiovascular disease; EPIC: European Prospective Investigation of Cancer; FRS: Framingham Risk Score; hsCRP: High-sensitivity C-reactive protein; IVUS: Intravascular ultrasound; MI: Myocardial infarction; MIRACL: Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering; OxPL: Oxidized phospholipid; PCI: Percutaneous coronary intervention; PROXI: Pravastatin and Atorvastatin on Markers of Oxidative Stress in Hypercholesterolemic Humans; REVERSAL: Reversal of Atherosclerosis with Aggressive Lipid Lowering; SNP: Single nucleotide polymorphism; UCSD: University of California at San Diego.

Spearman correlation (r-values) between lipoprotein(a) and oxidized phospholipid/apoB by race and sex.

Table 2

Correlation	All	BF	BM	WF	WM	HF	HM
<i>Correlation between Lp(a) and OxPL/apoB by race and sex</i>							
Lp(a) vs OxPL/apoB	0.84*	0.87*	0.87*	0.72*	0.68*	0.69*	0.53*
<i>Correlation between major apo(a) allele and OxPL/apoB by race and sex</i>							
apo(a) vs OxPL/apoB	-0.50*	-0.47*	-0.48*	-0.46*	-0.46*	-0.50*	-0.32*
<i>Correlation between Lp(a) and OxPL/apoB by race and sex stratified by number of apo(a) isoforms in the major allele</i>							
12-20	0.85*	0.81*	0.84*	0.84*	0.85*	0.85*	0.80*
21-26	0.88*	0.86*	0.85*	0.74*	0.62*	0.80*	0.69*
27-41	0.47*	0.67*	0.71*	0.16**	0.13**	0.38*	0.25*

* p < 0.001.
** p < 0.05.
BF: Black females; BM: Black males; HF: Hispanic females; HM: Hispanic males; Lp(a): Lipoprotein(a); OxPL: Oxidized phospholipid; WF: White females; WM: White males.
Data taken from [49].

Table 3

Odds ratios for obstructive coronary artery disease according to quartiles of the ratio of oxidized phospholipids to apoB-100 and levels of lipoprotein(a) in patients with and without hypercholesterolemia.

Patient group	Oxidized phospholipid:apoB-100 ratio			
	No hypercholesterolemia		Hypercholesterolemia	
	Number with CAD (%)	OR (95% CI)	Number with CAD (%)	OR (95% CI)
All patients				
Quartile I	29	1.00	67	4.93 (2.31–10.5)
Quartile II	44	1.92 (0.91–4.06)	56	3.10 (1.54–6.25)
Quartile III	38	1.47 (0.68–3.19)	64	4.36 (2.16–8.79)
Quartile IV	39	1.54 (0.70–3.40)	77	8.13 (3.88–17.1)
Total	150	–	264	–
Age ≤60 years				
Quartile I	14	1.00	61	9.33 (2.64–33.0)
Quartile II	27	2.21 (0.61–7.97)	37	3.53 (1.03–12.0)
Quartile III	28	2.33 (0.64–8.45)	57	8.00 (2.51–25.5)
Quartile IV	43	4.59 (1.39–15.1)	74	16.8 (5.11–55.2)
Total	112	–	229	–
Age >60 years				
Quartile I	45	1.00	71	3.00 (1.10–8.18)
Quartile II	61	1.85 (0.67–5.15)	68	2.57 (1.01–6.54)
Quartile III	48	1.11 (0.39–3.14)	71	2.90 (1.11–7.58)
Quartile IV	31	0.55 (0.15–1.92)	80	4.95 (1.76–13.9)
Total	185	–	290	–

For the oxidized phospholipid:apo B-100 ratio, quartiles I–IV correspond to the following values: <0.047, 0.047–0.089, 0.089–0.294 and >0.294, respectively. For lipoprotein(a), quartiles I–IV correspond to the following values: <8.8, 8.8–21.1, 21.1–39.7 and >39.7 mg per deciliter, respectively. The p-values indicate whether any two of the eight groups (defined by quartile and hypercholesterolemia status) have significantly different proportions of subjects with CAD.

CAD: Coronary artery disease; OR: Odds ratio.

Data taken from [48].

Table 4

Odds ratio (95% CI) of coronary artery disease by tertiles of oxidized phospholipid/apoB.

OxPL/apoB	Tertile 1 <1150 RLU; OR	Tertile 2 1150–2249 RLU; OR (95% CI)	Tertile 3 >2249 RLU; OR (95% CI)	p-value trend linearity
Entire cohort				
Unadjusted	1.00	1.21 (0.97–1.52)	1.61 (1.29–2.01)	<0.001
Adjusted model 1	1.00	1.32 (1.04–1.68)	1.67 (1.32–2.12)	<0.001
Adjusted model 2	1.00	1.27 (1.01–1.60)	1.66 (1.32–2.09)	<0.001
Men				
Unadjusted	1.00	1.31 (0.99–1.75)	1.59 (1.20–2.11)	0.001
Adjusted model 1	1.00	1.43 (1.05–1.94)	1.65 (1.21–2.24)	0.001
Adjusted model 2	1.00	1.42 (1.06–1.92)	1.70 (1.26–2.29)	<0.001
Women				
Unadjusted	1.00	0.96 (0.67–1.39)	1.60 (1.12–2.28)	0.009
Adjusted model 1	1.00	1.05 (0.70–1.56)	1.67 (1.14–2.44)	0.007
Adjusted model 2	1.00	0.97 (0.67–1.41)	1.58 (1.10–2.26)	0.01

Model 1 matched for sex, age and enrollment time, and adjusted for diabetes, smoking, systolic blood pressure, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. Model 2 matched for sex, age and enrollment time, and adjusted for Framingham Risk Score.

OR: Odds ratio; OxPL: Oxidized phospholipid; RLU: Relative light units.

Data taken from [47].

Table 5

Statistical values for area under receiver operating characteristic curves.

Biomarkers	c-index (95% CI)
FRS	0.584 (0.558–0.609)
FRS, MPO	0.586 (0.561–0.612)
FRS, Lp-PLA ₂	0.587 (0.562–0.613)
FRS, OxPL/apoB	0.597 (0.572–0.623)
FRS, hsCRP	0.605 (0.580–0.630)
FRS, Lp(a)	0.607 (0.582–0.632)
FRS, sPLA ₂ mass	0.609 (0.584–0.634)
FRS, sPLA ₂ activity	0.618 (0.593–0.642)
FRS, MPO, Lp-PLA ₂ ,	0.590 (0.565–0.616)
FRS, MPO, Lp-PLA ₂ , OxPL/apoB	0.603 (0.578–0.628)
FRS, MPO, Lp-PLA ₂ , OxPL/apoB, hsCRP	0.614 (0.590–0.639)
FRS, MPO, Lp-PLA ₂ , OxPL/apoB, hsCRP, Lp(a)	0.625 (0.600–0.649)
FRS, MPO, Lp-PLA ₂ , OxPL/apoB, hsCRP, Lp(a), sPLA ₂ mass	0.635 (0.610–0.659)
FRS, MPO, Lp-PLA ₂ , OxPL/apoB, hsCRP, Lp(a), sPLA ₂ mass, sPLA ₂ activity	0.651 (0.627–0.675)

FRS: Framingham Risk Score; hsCRP: High-sensitivity C-reactive protein; Lp(a): Lipoprotein(a); Lp-PLA₂: Lipoprotein-associated phospholipase A₂ activity; MPO: Myeloperoxidase; OxPL: Oxidized phospholipid; sPLA₂: Soluble phospholipase A₂.

Data taken from [47].