The hypothesis that oxidized LDL (ox-LDL) is necessary, if not obligatory, in the development of atherosclerotic lesions was formulated 20 years ago with the seminal observation that uptake of native LDL by macrophages did not result in foam cell formation. In contrast, uptake of ox-LDL via scavenger receptors resulted in the unregulated accumulation of lipid. Since then, multiple studies in experimental animal models have provided firm evidence of an important role of ox-LDL in atherogenesis. However, the role of ox-LDL in the clinical arena has not yet been established. The report in this issue of Circulation by Ehara et al, as well as several other studies, lends support to the idea that the oxidation of LDL is also relevant in humans and continues to move ox-LDL research from the bench to the bedside.

There is substantial evidence that ox-LDL is present in vivo within atherosclerotic but not normal blood vessels. LDL that is extracted from human and animal atherosclerotic lesions has all of the physical, chemical, immunological, and biological properties of ox-LDL. Monoclonal antibodies to epitopes of ox-LDL immunostain animal and human atherosclerotic lesions. Products of lipid peroxidation, such as oxidized cholesterol, fatty acids, phospholipids, and isoprostanes, are found in atherosclerotic lesions and plasma. Autoantibodies to epitopes of ox-LDL, reflecting the fact that ox-LDL is very immunogenic, are found in lesions and plasma of animals and patients with various manifestations of atherosclerosis. In addition, radiolabeled oxidation-specific antibodies image ox-LDL in the artery of live animals, and plasma autoantibody titers of ox-LDL correlate with both the accumulation and depletion of arterial ox-LDL. Finally, a large number of animal studies have shown that antioxidants reduce the progression of atherosclerosis in animal models.

When it is present in vivo, ox-LDL has a wide range of atherogenic properties, from early lesion formation to plaque rupture. These processes include inducing the expression of adhesion molecules on endothelial cells, monocyte chemotaxis and adhesion, cytotoxicity, upregulating inflammatory genes and growth factors, endothelial dysfunction, platelet aggregation and thrombus formation, and destabilizing plaques through several mechanisms, including increased expression of metalloproteinases.

Concurrent with the experimental work on ox-LDL, pathological studies have shown that a majority of acute coronary syndromes (ACS) result from ruptured or disrupted coronary plaques. Several elegant studies have demonstrated that the development of human coronary atherosclerosis involves vessel remodeling, inflammation, and disruption of thin, weakened fibrous caps overlying a large pool of extracellular lipid. Because of the difficulties in obtaining access to freshly disrupted plaques in patients and the lack of techniques to image these lesions easily, a deficiency of knowledge exists regarding the contents of these lipid pools. However, several post mortem studies have shown that oxidized cholesterol, cholesterol esters, phospholipids, and their breakdown products are present within this lipid gruel in substantial quantities. Presumably, this oxidized lipid material is derived, in part, from necrotic foam-cells rich in ox-LDL that have released their contents into the extracellular space.

It is generally believed that “fully oxidized LDL” does not exist in the circulation. Blood is rich in a variety of antioxidants. Even if ox-LDL entered the circulation in minute quantities, it would be rapidly cleared by the reticuloendothelial system, particularly in the liver. In addition, both animals and patients with atherosclerosis have preexisting circulating autoantibodies to ox-LDL that could rapidly remove any ox-LDL present. In contrast, circulating minimally modified LDL (MM-LDL), in which oxidative modification has not been sufficient to cause changes recognized by scavenger receptors, was clearly described by Avagaro and Sevanian. Subsequently, several groups reported evidence for the presence of oxidation-specific epitopes on plasma LDL, presumably such MM-LDL.

In their report, Ehara et al also measured levels of oxidation-specific epitopes on plasma LDL (eg, MM-LDL) in several subsets of patients with different manifestations of coronary artery disease (CAD), using a sandwich ELISA with the murine monoclonal antibody DLH3. This antibody recognizes oxidized phosphatidylcholine, including adducts with proteins that are not apoB. They observed that plasma levels of ox-LDL were significantly elevated in the patients with CAD compared with a control group. In addition, they showed that the ox-LDL levels correlated with the severity of the clinical presentation, ie, the patients with acute myocardial infarction (AMI) had the highest levels, followed by those with unstable angina and then those with stable angina. Impressively, their work was complemented by immunocy-
tochemical staining with DLH3 for epitopes of ox-LDL in coronary atherectomy specimens (from a different cohort of patients), which confirmed the presence of this epitope of ox-LDL.

Although the average measurement of ox-LDL discriminated patients from controls, the measurements would have distinguished only 7 of the 45 AMI patients from the 45 patients with unstable angina. These data suggest that at their present stage of development, such measurements of epitopes of ox-LDL may not yet be sufficiently robust to be used alone to discriminate the risk of acute coronary events in individual patients. However, combining these measurements of ox-LDL epitopes with other lipid and inflammatory markers may ultimately lead to additional insights into risk and diagnosis of plaque instability.

Ehara et al. estimated that the ox-LDL in the AMI patients was only 0.25% of the total LDL. Several other studies have also attempted to quantify circulating ox-LDL, and they have measured levels from 0.001% in normal patients (using DLH3) to ~5% in patients with AMI (using different monoclonal antibodies to malondialdehyde [MDA]-LDL [eg, LDL particles containing the oxidation-epitope MDA-lysine] and copper-oxidized LDL). However, there are several key caveats concerning the accuracy of these measurements. These include the extent of modification and reproducibility of the reference ox-LDL preparations that are used as standards for these assays, the fact that only one epitope is measured in these assays whereas potentially many epitopes may exist on any one individual LDL, and the fact that different populations of ox-LDL may exist. The only way to be certain of the amount of a particular epitope that is quantitated is to ensure that the reference standard has the same extent of oxidation on LDL as the measured LDL. The quantitation of ox-LDL will be improved when the epitopes are better delineated and the reference standard can be a pure preparation of the epitope. In addition, a practical limitation of the method used by Ehara et al. is the need to isolate the LDL by density ultracentrifugation and to remove other apoB-containing lipoproteins which, although more precise in evaluating LDL-specific associated oxidation, is impractical for performing this outside of an investigational arena.

The work from the laboratory of Holvoet et al. who used immunological techniques to measure circulating levels of ox-LDL and MDA-LDL, also supports the fact that LDL containing oxidation-specific epitopes seems to differentiate normal patients from patients with CAD, transplant atherosclerosis, and ACS. They also recently showed that plasma MDA-LDL levels, when measured in the emergency room and combined with troponin I measurements, have very high sensitivity and specificity for detecting ACS. We, too, recently observed in a 7-month prospective study that patients with ACS had up to a 60% increase in autoantibody titers to model-epitopes of ox-LDL, such as MDA-LDL, copper-oxidized LDL, and 1-palmitoyl-2-(5-oxgualoryl)-3-phosphorylcholine (POVPC), an oxidized phospholipid epitope, as well as an increase in circulating LDL-immune complexes, which peaked over the first month. In addition, circulating levels of an oxidation-specific epitope of ox-LDL, defined by EO6, a natural murine monoclonal autoantibody that likely binds to an oxidized phospholipid epitope similar to DLH3, was increased by up to 35% in the patients with AMI in the first month after the event.

The above data clearly demonstrate that relatively small amounts of LDL containing different oxidation-specific epitopes can be measured in the bloodstream and may reflect different manifestations of CAD. At present, the etiology of the oxidation-specific epitopes present on plasma LDL is unknown. The source of such ox-LDL could be the direct release of modified LDL from ruptured or permeable plaques, ischemic injury due to damaged cell membranes (either acutely or after reperfusion), or even remote nonatherosclerotic inflammatory sources. Finally, the transfer of such oxidation-specific epitopes from any of these sources to a lipoprotein acceptor, such as LDL, could occur. It is likely that, at least in ACS, all of these mechanisms may contribute.

In more stable patients, it is likely that elevated levels of ox-LDL reflect the turnover of ox-LDL in newly formed or progressing lesions not just in the coronary arteries, but also in the systemic arteries, which have a substantially greater amount of atherosclerotic burden and ox-LDL. For example, noninvasive imaging of hypercholesterolemic rabbit arteries shows that the aorta contains a substantially greater amount of oxidation-specific epitopes than the relatively small coronary arteries. It is also well appreciated that in an atherosclerotic milieu, LDL is oxidized more easily. For example, injecting native LDL into rats, or humans with atherosclerosis, results in rapid accumulation of ox-LDL within the artery wall, which can be prevented by pretreatment with probucol or vitamin E, respectively. Improved methods are needed to evaluate both the rate of oxidation and the presence of ox-LDL within the vessel wall.

Another major question that arises is which epitopes of ox-LDL are better markers of the atherosclerotic process. ox-LDL is a generic term for many different modifications of LDL. For example, MM-LDL primarily represents the oxidation of polyunsaturated fatty acids on phospholipids and cholesterol esters, but the LDL particle is still recognized by the LDL receptor. Fully oxidized LDL represents the depletion of polyunsaturated fatty acids and antioxidants within the LDL core and significant derivatization, cross-linking, and fracturing of the apoB of LDL so that the LDL particle is now recognized by scavenger receptors. Ehara et al. are likely measuring an oxidized phospholipid epitope of MM-LDL. At this point, it is not clear which epitopes of ox-LDL (eg, oxidized phospholipids, MDA-lysines such as those on MDA-LDL, or others) will be more relevant to certain clinical syndromes. What is needed to answer this question are large prospective trials with clinical endpoints. A comprehensive panel of different indices of ox-LDL should be measured, including autoantibody measurements to different epitopes of ox-LDL, immune complexes of LDL, and different epitopes of ox-LDL itself to provide a better assessment of the role of ox-LDL in ACS. In addition, the clinical utility of these measurements should be compared with other markers of enhanced lipid peroxidation, such as plasma or urinary isoprostanes, and with other inflammatory markers of plaque activity, such as C-reactive protein or interleukins. The current data on circulating levels of
various markers of ox-LDL do not yet provide enough information for us to determine if they will be of clinical relevance, either in the acute setting or as novel risk factors. Finally, although the studies to date have related indices of ox-LDL to clinical events, levels of ox-LDL may also directly relate to coronary vasomotion, as recently reported by Penny et al.11

Because ox-LDL can be thought of as integral in the final pathway of the many risk factors leading to foam cell formation, it is likely that assays for oxidation-specific epitopes in LDL will have substantial predictive qualities when assessed in larger studies. More studies like that of Ehara et al2 are needed, together with the other measures noted above. If large studies show that such ox-LDL measurements do have predictive value, then their measurement along with the lipid profile and markers of inflammation may improve our ability to provide a more accurate atherosclerotic risk analysis, particularly of acute clinical events.

References

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Measuring Circulating Oxidized Low-Density Lipoprotein to Evaluate Coronary Risk
Sotirios Tsimikas and Joseph L. Witztum

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