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Oxidation-Specific Biomarkers and Risk of Peripheral Artery Disease

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Objectives The goal of this study was to examine the prospective association between oxidation-specific biomarkers, primarily oxidized phospholipids (OxPL) on apolipoprotein B-100–containing lipoproteins (OxPL/apoB) and lipoprotein (a) [Lp(a)], and risk of peripheral artery disease (PAD). We examined, as secondary analyses, indirect measures of oxidized lipoproteins, including autoantibodies to malondialdehyde-modified low-density lipoprotein (MDA-LDL) and apolipoprotein B-100 immune complexes (ApoB-IC).

Background Biomarkers to predict the development of PAD are lacking. OxPL circulate in plasma, are transported by Lp(a), and deposit in the vascular wall and induce local inflammation.

Methods The study population included 2 parallel nested case-control studies of 143 men within the Health Professionals Follow-up Study (1994 to 2008) and 144 women within the Nurses' Health Study (1990 to 2010) with incident confirmed cases of clinically significant PAD, matched 1:3 to control subjects.

Results Levels of OxPL/apoB were positively associated with risk of PAD in men and women: pooled relative risk: 1.37, 95% confidence interval: 1.19 to 1.58 for each 1-SD increase after adjusting age, smoking, fasting status, month of blood draw, lipids, body mass index, and other cardiovascular disease risk factors. Lp(a) was similarly associated with risk of PAD (pooled adjusted relative risk: 1.36; 95% confidence interval: 1.18 to 1.57 for each 1-SD increase). Autoantibodies to MDA-LDL and ApoB-IC were not consistently associated with risk of PAD.

Conclusions OxPL/apoB were positively associated with risk of PAD in men and women. The major lipoprotein carrier of OxPL, Lp(a), was also associated with risk of PAD, reinforcing the key role of OxPL in the pathophysiology of atherosclerosis mediated by Lp(a). (J Am Coll Cardiol 2013;61:2169–79) © 2013 by the American College of Cardiology Foundation

Approximately 10 million U.S. adults have peripheral artery disease (PAD), including 23% of those 70 years of age or older (1,2). Peripheral artery disease is associated with substantial morbidity, cost (3), and functional decline (4) and might require limb amputation in extreme cases. Despite its high prevalence and associated morbidity, risk factors for PAD are less well-studied than those for coronary and cerebrovascular disease.

Evidence from cellular and animal experiments suggests that oxidative stress plays a key, modifiable role in the etiology of atherosclerosis (5,6). However, there is a lack of appropriate epidemiological markers to measure oxidation:

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Abbreviations and Acronyms

ABI = ankle-brachial index
ApoB = apolipoprotein B-100–containing lipoproteins
ApoB-IC = apoB-immune complexes
BMI = body mass index
BSA = bovine serum albumin
CI = confidence interval
CVD = cardiovascular disease
Ig = immunoglobulin
LDL = low-density lipoprotein
Lp(a) = lipoprotein (a)
MDA = malondialdehyde
MI = myocardial infarction
OxPL = oxidized phospholipids
PAD = peripheral artery disease
PC = phosphocholine
RLU = relative light units
RR = incidence rate ratio/ relative risk

many biomarkers lack the necessary combination of reliability, accuracy, cost-effectiveness, and ease of measurement; and few have been examined specifically with respect to PAD. Oxidized phospholipids (OxPL), a marker of lipid oxidation transported by lipoprotein (a) [Lp(a)] in plasma (7,8), might provide insight into the role of oxidative stress in atherosclerosis. Pro-inflammatory OxPL on Lp(a) and other apoB-100–containing lipoproteins upregulate proinflammatory genes and proinflammatory responses of several arterial wall cells and initiate a localized inflammatory cascade (9,10).

These oxidation-specific epitopes—such as OxPL and malondialdehyde (MDA) epitopes—represent danger-associated molecular patterns that are detrimental to the host; are present on apoptotic cells, oxidized low-density lipoprotein (LDL), and lipids; and often share molecular identity/mimicry with epitopes on pathogens (11). In response to such danger-associated molecular patterns, macrophage scavenger receptors, immune effector proteins such as autoantibodies to malondialdehyde-modified low-density lipoprotein (MDA-LDL), complement factor H (12), and C-reactive protein have been selected and expanded to bind and neutralize their pro-inflammatory effects.

Oxidized phospholipids on apolipoprotein B-100–containing lipoproteins (OxPL/apoB) have been associated with carotid and femoral atherosclerosis measured by ultrasound (13), myocardial infarction (MI), stroke, revascularization, and total mortality in selected, largely clinical populations (7). Immunoglobulin (Ig) M autoantibodies to MDA-LDL have also been inversely associated with ultrasound-detected carotid and femoral atherosclerosis (14,15); and in another small cross-sectional study, autoantibody titers against oxidized LDL were increased in patients with early onset PAD (16). However, no adequately powered prospective cohort studies have examined the association between oxidation-specific biomarkers and risk of incident clinically manifested PAD in healthy populations. The goal of this study was to examine the prospective association between oxidation-specific biomarkers, primarily oxidized phospholipids (OxPL) on apolipoprotein B-100–containing lipoproteins (OxPL/apoB) and lipoprotein (a) [Lp(a)], and risk of peripheral artery disease (PAD). We examined, as secondary analyses, indirect measures of oxidized lipoproteins, including autoantibodies to malondialdehyde-modified low-density lipoprotein (MDA-LDL) and apolipoprotein B-100 immune complexes (ApoB-IC).

Methods

Study population. The HPFS (Health Professionals Follow-up Study) is a prospective cohort study of 51,529 male dentists, optometrists, pharmacists, podiatrists, osteopathic physicians, and veterinarians 40 to 75 years of age that began in 1986. The NHS study (Nurses' Health Study) is a prospective cohort study of 121,700 female nurses 30 to 55 years of age that began in 1976. From individuals in these 2 studies, 18,224 men provided blood specimens in 1994, and 32,826 women provided a blood sample in 1989. We excluded individuals who had a history of cardiovascular disease (CVD)—including MI; surgical/percutaneous revascularization of the coronary, carotid, or peripheral beds; confirmed PAD; stroke; and transient ischemic attack.

The case-control analytic datasets include 143 incident cases and 429 control subjects in the HPFS study and 144 incident cases and 432 control subjects in the NHS study. Cases were matched 1:3 to control subjects on age, race (NHS study only), month of blood draw (within 3 months), fasting status, and smoking history (never/former/current). We selected control subjects at random, conditional on the matching factors, from participants free of PAD at the time the case occurred (risk set sampling). Because older age categories had fewer participants, we relaxed the age match range year-by-year if necessary to a maximum of within 3 years. The Harvard School of Public Health Human Subjects Committee approved both studies.

Assessment of OxPL, Lp(a), autoantibodies, and immune complexes. Blood samples were shipped overnight with a cold pack to the central laboratory, centrifuged on arrival, aliquotted, and stored in liquid nitrogen at −130°C to −196°C. The HPFS specimens were anticoagulated with ethylenediaminetetraacetic acid, and NHS specimens were anticoagulated with heparin, and 95% of HPFS bloods and 97% of NHS bloods were received within 24 h. A validated plasma assay was used to measure OxPL/apoB, with the murine monoclonal antibody E06 that recognizes the phosphocholine (PC) group on oxidized but not on native phospholipids (reviewed in detail in Taleb et al. [7] and references therein). E06 similarly recognizes the PC covalently bound to bovine serum albumin (BSA), as in PC-BSA. A 1:50 dilution of plasma in phosphate-buffered saline is added to microtiter wells coated with monoclonal antibody MB47, which binds a saturating amount of apoB-100 to each well. Finally, biotinylated E06 is used to determine OxPL/apoB in relative light units (RLU). Within-person 5-year reproducibility of frozen samples is high (r = 0.78) (13), and pilot-tests showed that OxPL/apoB levels are stable over 24 h on ice (intra-class correlation coefficient = 0.96).