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Cardiovascular Event Risk

High-Density Lipoprotein and Paraoxonase*

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Low High-Density Lipoprotein Cholesterol (HDL-C)

Low HDL-C has been established as a cardiovascular risk factor at least since the Framingham study observation of a significant inverse relationship between HDL-C and coronary heart disease (CHD) in 1977 (1). Since then, multiple prospective epidemiological studies have demonstrated this inverse relationship (1–9). The mechanism of this increased risk has been attributed to multiple factors including impaired reverse cholesterol transport, adenosine triphosphatase–binding cassette transporter, and reduced oxidative protection.

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This inverse relationship between low HDL-C and increased risk has also been attributed to a specific high-density lipoprotein (HDL) subclass, described by various laboratory techniques as HDL2, HDL2b, alpha1 HDL, and LpAI (10). Differences in HDL subclass distributions in blood were first described by Gofman et al. (11,12) in 1954 using analytic ultracentrifugation (11,12). They noted that the concentrations of the more buoyant particles (HDL2) were 50% higher in women than in men. Later, in 1966, they reported that baseline HDL mass concentrations were 32% lower for HDL2 and 8% lower for HDL3 (the less buoyant particles) in patients who had CHD develop during 10 years of follow-up compared with patients who did not (13). The HDL subclasses appear to have a differential effect on lipoprotein oxidative protection (14).

Oxidation, Paraoxonase, and CHD Risk

Modification, or oxidation, of apoproteins may contribute to atherosclerosis in humans. Oxidation of apoprotein B has been shown to result in a modified low-density lipoprotein (m-LDL) particle that is taken up rapidly by a scavenger receptor on tissue macrophages, resulting in atherogenic foam cell formation, inhibition of macrophage egress from tissue, and damage to the endothelial border that results in atherosclerosis in animal models (15). Incubation of LDL with cultured endothelial cells results in a modified low-density lipoprotein (m-LDL) that is taken up by macrophages 3 to 10 times more rapidly than native LDL, resulting in atherogenic foam cell formation (16,17). This m-LDL undergoes many structural changes, most of which depend on peroxidation of polyunsaturated fatty acids in the LDL lipids that can be inhibited by vitamin E (18,19). In plasma, aldehydes (namely, malondialdehyde or 4-hydroxynonenal) are generated by peroxidation of polyunsaturated fatty acids, which are part of LDL phospholipids. These aldehydes may alter lysine residues of apoprotein B and result in m-LDL (19,20). Once the LDL contains fatty acid lipid peroxides, a propagation follows that amplifies the number of free radicals and leads to extensive fragmentation of the fatty acid chains (21).

Paraoxonase (PON) is an enzyme initially of interest in the field of toxicology because it is an "A" esterase and hydrolyzes organophosphate compounds used as insecticides and nerve gases (22). PON is associated with HDL particles, and in sheep, most of the PON activity is associated with the apolipoproteinAI-only particle (23). Thus, part of the protective effect of some, but perhaps not all, HDL particles may be the association of PON and its putative role in decreasing lipid peroxide accumulation on LDL particles (24). The HDL from transgenic mice lacking PON-1 fails to protect LDL against oxidative modification. Thus, PON-1 may be a determinant of resistance to the development of atherosclerosis by protecting lipoproteins against oxidative modification, perhaps by hydrolyzing phospholipid and cholesteryl-ester hydroperoxides.

PON and HDL Link

Both PON-1 and PON-3 reside on HDL particles (25). All 3 are associated with oxidative protection. The PON-1 activity is associated with HDL2 and may be a contributing factor to the cardioprotection attributed to elevated HDL2 levels (26).

PON Polymorphisms and Atherosclerosis

The PON family is made up of 3 related genes termed PON-1, PON-2, and PON-3 that reside on chromosome 7q21.3 (27). The PON-1 is bound to HDL and appears to hydrolyze inflammatory phospholipids in both LDL and HDL particles, and plays a role in physiologic anti-

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inflammatory activity (28,29). There are 3 common PON-1 polymorphisms: L55M, Q192R, and the promoter polymorphism T(−107)C (30).

PON polymorphisms have been associated with CHD risk in some, but not all, investigations. Serrato and Marian (31) described an association between HUMPONA and CHD in 1995. Subsequently, a plethora of investigations and associations have been reported (32). In the Nurses' Health and Health Professionals Follow-up Study, the PON-1 polymorphisms Q192R and L55M were not associated with increased CHD risk (33).

The lack of clarity in the association of PON-1 polymorphisms and CHD risk may, in part, be due to the physiologic role of PON, which may be to play a minor role in the early pathogenesis of CHD but a more powerful role in the interaction with lipid and glucose metabolism and the later macrovascular aspects of atherosclerosis (34). The likelihood of severe stenosis has been reported to be greater in CHD patients with hyperglycemia and serum PON-1 activity (35). Differences in genotype distribution may be related to severity of CHD in patients with established CHD (36). Smoking may be a contributing factor, because the Northwick Park Heart Study II reported that the L55M and Q192R genotype did not differ between cases and control subjects but CHD risk associated with smoking was significantly modified by the L55M genotype (37). Ethnic differences may also play a role, as it has been reported that in a Turkish patient population PON-1 L55M was associated with CHD but not PON-1 Q192R (38). The PON-1 Gln192Arg SNP has been associated with a significantly increased risk of stroke (39).

Some uncertainty exists in the relationship between PON polymorphisms and PON concentration or activity. In case-control studies, serum PON-1 concentration and activity were found to be decreased in CHD independent of the PON-1 polymorphism, and in diabetes mellitus, serum PON-1 specific activity decrease is also independent of the PON-1 genetic polymorphism (40).

Comments on the Current Article

Within the context of this lack of clarity in regard to PON polymorphisms and CHD risk, the article by Regieli et al. (41) in this issue of the *Journal* addresses a clinically important issue, which is the importance of HDL and reverse cholesterol transport and the physiologic and genetic attributes that may confer risk reduction benefit. The REGRESS (REgression GRowth Evaluation Statin Study) study population is well described and has been utilized to address other medically relevant questions. The role of oxidative protection attributed to PON residing on HDL particles has been explored by other investigators. What is new is the relationship of PON-1 genetic variants and prospective long-term clinical outcome in well-characterized male Caucasian CHD patients. Their data indicate a significant increase in CHD death in the 14% of the population with the MM L55M genotype. Furthermore, there was an allele-dose effect in that the 10-year risk of CHD death was 4.6% in L55 homozygotes, 7.1% in heterozygotes, and 10.9% in 55M homozygotes. Importantly, this relationship was unchanged when corrected for HDL-C, LDL cholesterol, triglycerides, and current smoking status, suggesting that the PON-1 genotype may be an independent risk factor for CHD death. Some conflict may exist with other case-control studies but is adequately addressed by Regieli et al. (41). This study contributes new knowledge regarding the risk of CHD death in male CHD patients and the relationship of common PON polymorphisms.

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