



## Lipid Peroxidation and Atherosclerosis: the Importance of Selected Patient Group Analysis

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It is widely accepted that lipid peroxidation plays a central role in the development of cardiovascular diseases, and that low density lipoprotein oxidation is considered to be the hallmark of early atherosclerosis [1,2]. Oxidized LDL is atherogenic – it causes arterial cell death, accumulation of inflammatory cells in the arterial wall, and stimulation of growth factors and cytokine release. In addition, Ox-LDL contributes to platelet aggregation, smooth muscle cell proliferation and thrombotic and inflammatory processes. Increased susceptibility of LDL to oxidation was shown in patients with hypercholesterolemia, hypertension, diabetes mellitus, chronic renal failure, and in smokers [3]. Upon specific treatment with statins, angiotensin-converting inhibitors,  $\beta$ -carotene or selenium respectively, LDL oxidizability in these patients returned to normal levels [3]. Patients on hemodialysis do not have a significantly increased LDL oxidation rate [4,5].

In the present issue of *IMAJ*, Boaz et al. [6] present a study on copper ion-induced lipid oxidation kinetics in unfractionated serum from hemodialysis patients. Selecting males with a history of myocardial infarction, and comparing them to a matched group of dialysis patients (age, diabetes mellitus and smoking status) with no cardiovascular disease revealed a significant increased oxidative stress in the dialysis patients with CVD vs. those with no CVD.  $T_{max}$ , the oxidation kinetic parameter defined as the time at which the rate of absorbing lipid peroxidation products accumulation was maximal, was significantly shorter in dialyzed patients with a history of MI than in those without CVD. Similarly, serum levels of thiobarbituric acid reactive substances (measured as malondialdehyde equivalents) were increased in dialysis patients with MI, in comparison to dialysis patients with no CVD.  $T_{max}$  and MDA were negatively correlated to one another. Unlike  $T_{max}$  values,  $OD_{max}$  or  $V_{max}$  (absorbance and rate of conjugated dienes formation) was not significantly different between the two hemodialysis patient groups, probably because these measurements are not good predictors of oxidative stress *in vivo*.

Oxidation of LDL is a free radical-driven lipid peroxidation process. Oxidative stress is defined as a disturbance in the balance

between the production of reactive oxygen and/or nitrogen species and the antioxidant defense. Oxidative stress includes ROS/RNS endogenous sources (mitochondria, peroxisomes, inflammatory cells) and exogenous sources (radiation, ozone, xenobiotics). On the other hand, the defense against oxidative stress includes enzymatic systems (superoxide dismutase, catalase, glutathione peroxidase, paraoxonase) and non-enzymatic systems (vitamin E, vitamin C, glutathione, flavonoids). In biological systems, ROS/RNS include: superoxide anion, hydrogen peroxide, hydroxyl radical, nitrogen oxide, peroxynitrite, and hypochlorous acid, which are all formed under normal metabolism, taking part in the signaling cascade, and involved in cellular functions such as proliferation, inflammation and adhesion processes.

Oxidative stress can be assessed [7] by indirect measurements like conjugated dienes formation and the accumulation of oxidized degradation products of polyunsaturated fatty acids. The kinetic analysis of serum lipid peroxidation allows for a dynamic quantification of conjugated dienes formed as a result of the conversion of the C=C double bond in polyunsaturated fatty acids into the conjugated double bond (-C=C=C-), which is characterized by a strong ultraviolet absorption at 245 nm.

During lipoprotein oxidation, peroxides are formed, with a subsequent formation of peroxy radicals, followed by a decomposition phase to yield aldehydes such as hexanal, MDA and 4-hydroxynonenal. The assay is based on the detection of a stable product, which is formed between aldehydes and thiobarbituric acid in the aqueous phase. Oxidation is also determined as end-products of lipid peroxidation, like isoprostanes, linoleic acid and cholesteryl linoleate hydroxide/hydroperoxides, oxysterols, protein oxidation products and DNA oxidation products [7].

Some of the detection methods are non-specific, measuring products that are not necessarily a result of oxidative stress, and their application is questionable (e.g., conjugated dienes, peroxide values, thiobarbituric acid reactive substances). Some of the measures are confounded by diet [7,8]. Other markers are indeed produced *in vivo* (oxysterols, isoprostans, ketoproteins), but their accumulation under increased oxidative stress is not certain, and they do not provide information on the types and sources of the stress [7,8]. For the above reasons, until a reliable method for *in vivo*

ROS/RNS = reactive oxygen and/or nitrogen species

LDL = low density lipoprotein  
Ox-LDL = oxidized LDL  
CVD = cardiovascular disease  
MI = myocardial infarction  
MDA = malondialdehyde

lipid peroxidation becomes available, the need for more than one type of measurement is required. Indeed, Boaz et al. [6] now demonstrate that both Tmax and MDA analyses show increased serum lipids peroxidation in dialysis patients with CVD vs. those without.

As lipid peroxidation is thought to play a major role in atherogenesis, it is expected that antioxidants would attenuate the progression of atherosclerosis. Indeed, in some observational and secondary prevention trials, vitamin E alone or in combination with  $\beta$ -carotene attenuated cardiovascular disease [9]. In hemodialysis patients, whose CVD death rate is 5–20 times higher than that of the general population, Boaz and colleagues [10] demonstrated the favorable effect of vitamin E against heart diseases. That study showed the beneficial effects of high dose (800 IU/day) oral vitamin E therapy in the prevention of secondary CVD events in hemodialysis patients with a history of CVD. Comparing dialysis patients with and without CVD is an important approach to explain excess cardiac morbidity and mortality in such patients. It might be that vitamin E was beneficial only in those dialyzed patients with increased oxidative stress. Similarly, subgrouping of hypercholesterolemic patients with low serum high density lipoprotein levels, or with low serum paraoxonase activity, as well as diabetic patients homozygous for the haptoglobin 2 allele, may enable discrimination related to oxidative stress. Oxidative stress is not determined only by the balance between arterial cells oxidases/oxygenases and cellular antioxidants, or only by serum oxidants/antioxidants ratio. Human serum PON1, an esterase that is bound to HDL, was shown to hydrolyze specific lipid peroxides in lipoproteins, macrophages, and in human atherosclerotic coronary and carotid lesions [11,12]. Serum PON1 activity is inversely related to atherosclerosis development. In hemodialysis patients, as well as in patients with hypercholesterolemia, hypertension or diabetes, analysis of serum PON1 activity may provide an additional sensitive tool to discriminate between those patients with and without CVD and, hence, may further assist in the decision as to who should be given antioxidant treatment. Several primary prevention trials showed no beneficial effect of vitamin E and, in some cases, even adverse effects [9,13]. This may be related to the ineffectiveness of the antioxidants vitamin E and  $\beta$ -carotene used in those specific trials.

Some flavonoids are much more potent antioxidants than vitamin E or carotenoids [14]. The antioxidative capacity of an antioxidant is determined by several characteristics, such as biological absorption, concentration, rate constants of radical reactions, location in the aqueous or the lipid domains (or in both phases), mobility in hydrophobic domains, lifetime, rate of regeneration, recycling activity, and metal scavenging activity (chelating, binding).

Flavonoids can reduce LDL lipid peroxidation by scavenging ROS/RNS, by chelation of transition metal ions and also by sparing LDL-associated antioxidants. They can also reduce macrophage oxidative stress by inhibition of cellular oxygenases (such as NADPH oxidase, lipoxygenase) and/or stimulation of cellular

antioxidants (such as the glutathione system). Thus, plant flavonoids, as potent natural antioxidants that protect against lipid peroxidation in arterial cells and lipoproteins, can significantly attenuate the development of atherosclerosis [14,15].

Compounds called "antioxidants" differ in their ability to react with different types of free radicals, therefore a combination of antioxidants can provide a wider range of free radical scavenging than an individual antioxidant.

A most important issue in relation to antioxidant anti-atherogenicity is that antioxidant treatment might be beneficial only in subjects under oxidative stress. Indeed, in their current study Boaz et al. [6] demonstrated that increased oxidative stress in hemodialysis patients was limited to the group with CVD, and that only this group, but not the non-CVD dialysis patients, could probably benefit from antioxidant therapy.

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PON1 = paraoxonase

HDL = high density lipoprotein