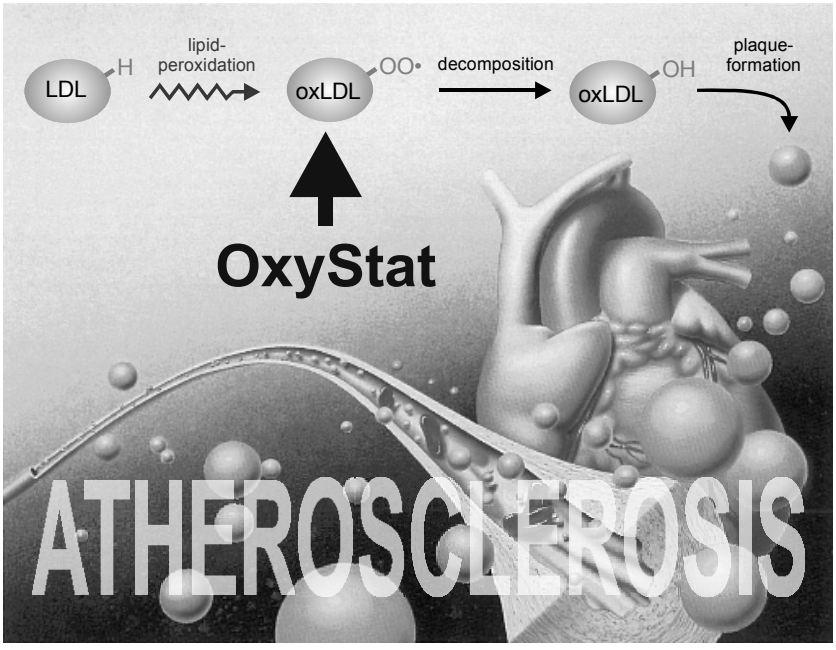
OxyStat

1. Oxidative stress - the war within

Oxidative stress could be defined as a disturbance in the pro-oxidant / antioxidant balance in favour of the pro-oxidant state. In this situation the organism is under increased exposure to reactive oxygen species (ROS) which participate in free radical-induced alterations of cellular components through exponential chain radical-carrying mechanisms.

ROS such as hydrogen peroxide H_2O_2, the superoxide anion O_2^- and the hydroxyl radical •OH can damage a wide variety of cellular components causing lipid peroxidation, protein oxidation and genetic damage through oxidation of DNA. Such damage has been implicated in various disease processes including cancer, cardiovascular disease, arthritis and aging. In addition, lipid peroxidation is the cause of food rancidity in which fats and oils oxidize with characteristic changes in taste, color and odor.
Cells are exposed to these ROS during the course of normal aerobic metabolism and through exposure to light, ionizing radiation, metal ions as well as by the action of various redox cycling drugs. To protect against the deleterious effects of oxidative damage, most organisms contain defense systems including enzymes such as catalase and superoxide dismutase and antioxidants including vitamins E and C and glutathione.

The following scheme shows the theoretical model of the reaction cascades in an organism exposed to oxidative stress:



January, 03

Free radicals are continuously produced during metabolism. Some cells generate free radicals deliberately to perform pathophysiological functions, and free radicals are formed in other cells during leaks in the electron transfer chain.
We have an intricate natural anti-free radical or anti-oxidant system that controls free radical compounds and limits the amount of free radical damage to proteins, lipids, and DNA.

When the production of free radicals exceeds the body's natural antioxidant defense mechanisms, oxidative stress can occur causing damage to key biomolecules. Since this damage occurs throughout an individual's life, it is now argued that oxidative damage is a major contributor to aging and to the degenerative diseases associated with aging such as cancer, cardiovascular disease, immune-system decline, Alzheimer's disease, and Parkinson's disease.

The free radical mechanisms of the human body might be viewed in analogy to an army that has the potential for great good, but must be kept well disciplined and well fed. When exhausted by repeated attacks and poorly fed, this army has the potential for vast damage.

2. Free radicals and lipid peroxidation

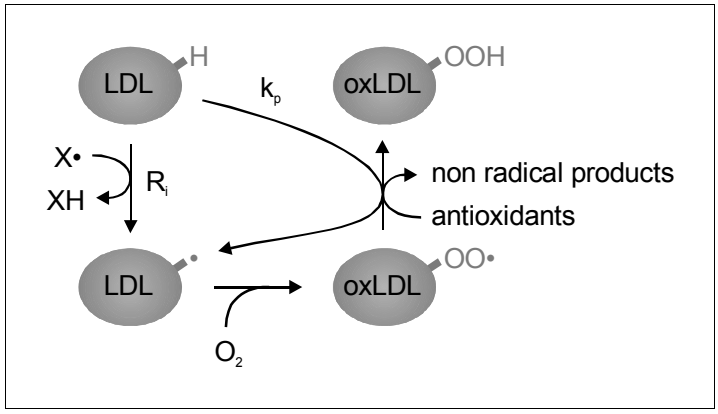
The LDL oxidation possesses the general characteristics of lipid peroxidation reactions.

In most instances, three consecutive time phases can clearly be detected:

lag-phase	radical capture by antioxidants	duration: 0.5 - 2 h
propagation-phase	lipid peroxidation	duration: 1 - 2 h
decomposition-phase	decomposition of lipid hydroperoxides	duration: 15 h

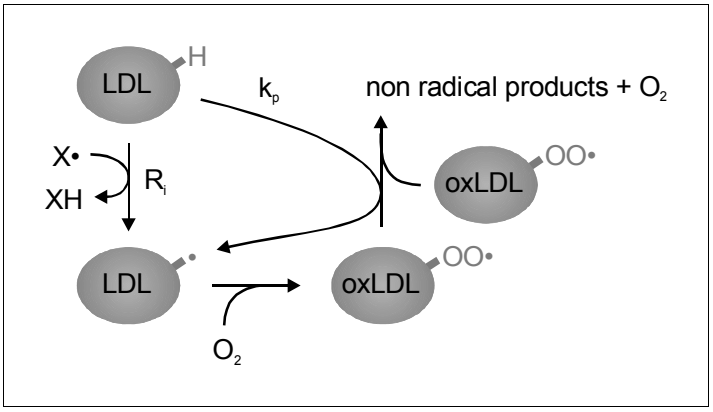
The Biomedica OxyStat assay measures the total peroxide concentration, which is formed in the propagation-phase of the LDL oxidation process.

Lipid peroxidation begins when an initiating radical X• abstracts a hydrogen atom from one of the polyunsaturated fatty acids (PUFAs) contained in the LDL lipids [11]:



lipid peroxidation with antioxidants

The rate of initiation R_i is essential for the whole peroxidation process. Once formed, the carbon centered lipid radical LDL• reacts very quickly with molecular oxygen yielding a lipid peroxyl radical LDL-OO• which then abstracts with a certain rate constant k_p (p = propagation) a hydrogen atom from an adjacent LDL yielding a lipid hydroperoxide and a new LDL• radical.
It is this latter reaction, termed propagation, that causes the lipid peroxidation chain.
The antioxidants in LDL, especially β-carotin, vitamin E and ubiquinol-10 compete with chain propagation by scavenging LDL-OO• radicals.
When LDL has lost most of the antioxidant compounds, the propagation phase commences and the lipids in LDL are rapidly oxidized to lipid hydroperoxides:



lipid peroxidation without antioxidants

At this stage the only competing reaction is the termination by radical-radical combination: LDL-OO• + LDL-OO• → non radical products + O_2.
This mechanism leads to keto- and hydroxy-fatty acids containing conjugated double bonds as end products.

The propagation phase is finally followed by the decomposition phase, where the lipid hydroperoxides break down to a wide range of products including aldehydes, hydrocarbon gases, epoxides, alcohols and others.
Such a three phase sequence has been demonstrated for oxidation of LDL initiated by macrophages and copper ions and is probably common to all processes of LDL oxidation regardless of the method of initiation.

Free radicals can be generated in a wide variety of normal physiological functions. In some cases they are protective in nature but they can be harmful if not processed rapidly. Free radicals and lipid peroxides have been found to be elevated in patients with rheumatoid arthritis and systemic lupus erythematosus, as well as patients with glomerular disorders [12,13]. They play an important role in regulating hypertension through the degradation of prostacyclin and nitric oxide [14].

Free radicals have been implicated in the etiology of diabetes and potentially in some of its long term complications through the destruction of pancreatic beta cells [15]. Notably, patients with Type I diabetes and angiopathy were found to have substantially higher lipid peroxides than controls, leading to the suggestion that this might be involved in the development of atherosclerosis.

Reactive oxygen species have also been implicated in the development of tissue damage in ulcerative colitis, in breast cancer risk where lipid peroxides were found to be highest in women with mammographic dysplasia, and in a variety of liver diseases [16-18].

All these processes have the potential to impact one another through the free radicals that they generate.

Lipid peroxides result from hydroxyl radical-attack on polyunsaturated fatty acids (PUFAs). Elevated levels of lipid peroxides are thus strongly suggestive of hydroxyl radical activity and reflect oxidative damage.
The production of toxic radicals and metabolites is thought to be the main cause of much systemic damage.
It has been suggested that hydroxyl radical attack upon membrane bound essential fatty acids (EFAs), leading to a loss of highly unsaturated EFAs, may have a direct relationship to EFA deficiencies, free radical damage and the aging process [19].

Summary:

The main steps of lipid peroxidation are:

1. Stress induces formation of radicals, which generate reactive oxygen species (ROS) like O_2 or H_2O_2.
2. These ROS attack polyunsaturated fatty acids (PUFAs) located in LDL.
3. Antioxydants (e.g. β-carotin, vitamin E) capture the ROS in LDL.
4. As soon as the antioxidants are used up, biological peroxides are formed.
   The Biomedica OxyStat assay directly measures these peroxides, and thus enables a direct correlation of biological peroxidation in a sample.
5. Biological peroxides are further decomposed into numerous substances, like aldehydes, hydrocarbon gases, epoxides, alcohols and so on.
6. After this decomposition phase, autoantibodies directed against oxidized LDL are produced in vivo, which are therefore an indicator for long-term exposure to oxidative stress.
   The Biomedica oLAB ELISA enables the measurement of these autoantibodies.

3. Clinical therapeutics for oxidative stress

In the 1970s, Pauling discussed the use of therapeutic doses of vitamin C to prevent and treat viral infections. Cathcart reported in numerous clinical instances therapeutic benefit of using "bowel tolerance" doses of vitamin C (the oral dose which will initiate diarrhea) for the treatment of many virus-related disorders [(20)]. Although his theories have not been proven through detailed mechanistic studies, many clinicians have reported anecdotally that their patients have benefited from this therapeutic approach.

There is emerging recognition that individual antioxidants may not have as wide-ranging clinical benefits as the intake of balanced antioxidants which incorporate all the dietary redox-active substances people have consumed for millennia. This is due in part to the involvement by different antioxidants in the process of regenerating each other.

Specific antioxidants include ascorbate, carotenoids and tocopherols, but also extend into other phytonutrients such as phenols, flavonoids, and quinoids. There is growing acceptance among scientific and medical communities that enhanced antioxidant intake in the diet and specific application of antioxidants in certain states of oxidative stress may provide both preventive and therapeutic advantage [(21)].

4. Interpretive Guidelines

In case of high lipid peroxide concentrations measurement results: (> 400 µmol/l using the OxyStat assay), a possible diagnosis could be: Increased cellular lipid peroxidation.

Possible causes: Excess exposure to xenobiotics or gut-derived toxins. Upregulated cytochrome P450 activity.

Other possible sources of formation of free radicals: Inflammation, infection, intestinal dysbiosis, trauma, radiation, ischemia, inadequate nutritional antioxidant reserves.

Actions to be considered: Identification and reduction of exposure to toxic substances or other sources of free radicals. Check for gut-derived toxins. Consider elemental analysis to rule out heavy metal toxicity or nutrient insufficiencies. Consider increasing intake of antioxidants, especially fat-soluble nutrients: tocopherols, ascorbic acid, carotenoids, coenzyme Q10, melatonin, lipoic acid, glutathione, N-acetylcysteine. Consider herbal antioxidants: milk thistle, catechin, ginkgo biloba, licorice, hawthorne, reishi, anthocyanidins, curcumin. Consider nutritional cell membrane support: phosphatidyl choline, taurine, essential fatty acids. Reduce partially hydrogenated fats.

5. Implications

1. Esterbauer H. et al., Anti-oxidants in disease mechanisms and therapy (Sies H., ed.) (1997) 38: 425-456
2. Montine et al., American Journal of Pathology (1999) 155: 863-868
3. Roozendaal et al., Clin. Exp. Immunol. (1999) 116: 206-213
4. Mattacks et al., Cytokine (1999) 11: 334-346
5. Smolle K.H. et al., Abstracts of the 7th Vienna Shock Forum (1999)
6. Trinker M. et al., Abstracts of the Int. Congress of Vascular Medicine (1998)
7. Roob G. et al., Abstracts of the 31st Annual Meeting of the American Society of Nephrology (1998)
8. Bahadori et al., Clinical Biochemistry (1998) 31: 557-608
9. Girotti A.W., Journal of Lipid Research (1998) 39: 1529-1541
10. Wendel A., Free Radical Biology & Medicine (1987) 3: 355-358
11. Esterbauer H. et al., Role of natural antioxidants in inhibiting Cu^{2+} mediated oxidation of LDL, in: Free Radicals, Lipoprotein Oxidation and Atherosclerosis (1995) 11-26
12. Surya Prabha P et al., Reactive oxygen species, lipid peroxides and essential fatty acids in patients with rheumatoid arthritis and systemic lupus erythematosus Prostaglandins, Leukotrienes and EFAs. (1991) 43: 251-255
13. Das UN. et al., Oxy-radicals, lipid peroxides and essential fatty acids in patients with glomerular disorders. Prostaglandins, Leukotrienes and EFAs (1993) 49: 603-607
14. Surya Prabha P. et al., Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. Prostaglandins, Leukotrienes and EFAs (1990) 41: 27-33
15. Oberley LW., Free radicals and diabetes, Free Radical Biology & Med (1988) 5: 113-124
16. Sedghi S. et al., Elevated breath ethane levels in active ulerative colitis: evidence for excessive lipid peroxidation. Am J Gastroenterol (1994) 89 (12): 2217-2221
17. Boyd NF. et al., The possible role of lipid peroxidtion in breast cancer risk. Free Radical Biology & Med (1991) 10: 185-190
18. Britton RS. et al., Role of free radicals in liver diseases and hepatic fibrosis. Hepato-Gastroenterol (1994) 41: 343-348
19. Horrobin DF., Is the main problem in free radical damage caused by radiation, oxygen and other toxins the loss of membrane essential fatty acids rather than the accumulation of toxic materials? Med Hypoth (1991) 35: 23-26
20. Cathcart RF 3d; Vitamin C: the nontoxic, nonrate-limited, antioxidant free radical scavenger. Med Hypoth (1985) 18: 61-77
21. Marantz PR., Beta carotene, vitamin E, and lung cancer. N Eng J Med (1994) 331: 611