

Oxidative Stress

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Definitions

Oxidative stress is the condition that occurs when the steady-state balance of pro-oxidants to antioxidants is shifted in the direction of the former, creating the potential for organic damage. Pro-oxidants are by definition *free radicals*, atoms or clusters of atoms with a single unpaired electron. Physiologic concentrations of pro-oxidants are determined both by internal and external factors. Pro-oxidant reactive oxygen species (ROS), for example, are normal products of aerobic metabolism. However, under pathological conditions ROS production can increase, surpassing the body's detoxification capacity and thus contribute to molecular-level organic pathology. External sources of free radicals include exposures to environmental toxins such as ionizing radiation, ozone and nitrous oxide, cigarette smoke (including passive inhalation) and heavy metals, as well as dietary intake of excess alcohol, unsaturated fat, and other chemicals and compounds present in food and water.

Antioxidants are chemical compounds that can bind to free radicals and thus prevent them from damaging healthy cells. Antioxidants can be divided into enzymatic and non-enzymatic subtypes. Several antioxidant enzymes are produced by the body, with the three major classes being catalase, the glutathione (GSH) peroxidases, and the superoxide dismutases (SODs). Non-enzymatic antioxidants include the innate compound glutathione as well as antioxidant vitamins obtained through the diet, such as α -tocopherol (vitamin E), ascorbic acid (vitamin C), and β -carotene.

Measurement

Because free radicals are unstable, and difficult to measure, traditional indices of oxidative stress include downstream markers of oxidative damage to macromolecules such as lipids, proteins and DNA. Oxidative stress is also indirectly assessed by estimating capacity for antioxidant defense in serum, plasma, or other body fluids. Such measures include assessment of enzymatic antioxidant activity, individual quantitative assessment of circulating non-enzymatic antioxidant levels, and estimation of total antioxidant status (ability of antioxidants in the blood to neutralize a pro-oxidant compound *in vitro*). Tables 1 and 2 below list a number of commonly used measures of oxidative damage and antioxidant defense, their availability in biological samples, and frequently used assays. These tables provide neither exhaustive catalogues of all available oxidative stress measures, nor recommendations for which measures to employ. Rather, they provide an overview of the types of measures commonly used in research.

Table 1. Biomarkers of oxidative damage

BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
Lipid Peroxidation		
F ₂ -isoprostanes	Plasma, urine	GC/MS, HPLC-MS/MS
Oxidized low-density lipoprotein (oxLDL)	Plasma, serum	ELISA
Malondialdehyde (MDA)	Plasma, serum, saliva, urine, exhaled breath condensate	Colorimetry, spectrophotometry, HPLC + fluorescence, GC/MS
Protein Oxidation		
Protein carbonyls	Plasma, serum	ELISA
DNA Oxidation		
8-hydroxy-2-deoxyguanosine (8-	Plasma, serum, urine	HPLC-EC, HPLC-MS/MS*, GC/MS,

BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
OHdG)		Comet assay*

Table 2. Antioxidant measures

BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
Non-enzymatic Antioxidants		
<i>Lipid-soluble</i>		
α -tocopherol (vitamin E)	Plasma, serum	HPLC
Carotenoids	Plasma, serum	GC/MS, reversed-phase HPLC, HPLC-MS
α -carotene		
β -carotene		
lycopene		
Retinol (vitamin A)	Plasma, serum	GC/MS, reversed-phase HPLC, HPLC-MS
Retinol binding protein (RBP)	Plasma, serum	Radial immunodiffusion
<i>Water-soluble</i>		
Ascorbic acid (vitamin C)	Plasma, serum	HPLC-EC, spectrophotometry, fluorimetry
Glutathione (GSH)	Plasma, serum	Colorimetry, fluorimetry, HPLC
Uric acid	Plasma, serum, urine	Colorimetry, fluorimetry, HPLC
Total antioxidant status (TAS)	Plasma, serum	TRAP, ORAC, TEAC, FRAP
Enzymatic Antioxidants		
Catalase activity	Plasma, serum, RBCs	Colorimetry, ELISA, spectrophotometry
Glutathione peroxidase (GPx)	Plasma, serum, RBCs	Colorimetry, ELISA, spectrophotometry
Superoxide dismutase (SOD)	Plasma, serum, RBCs, CSF	Colorimetry, ELISA, spectrophotometry

CSF= cerebrospinal fluid; ELISA = enzyme-linked immunosorbant assay; FRAP = ferric reducing ability of plasma; GC/MS = gas chromatography/mass spectrometry; HPLC = high performance liquid chromatography; HPLC-EC = high performance liquid chromatography with electrochemical detection; HPLC-MS/MS = high performance liquid chromatography/mass spectroscopy; ORAC = oxygen radical absorbance capacity; RBC = red blood cell; TEAC = trolox equivalent antioxidant capacity; TRAP = total radical trapping antioxidant parameter

Physiological Mechanisms

The single unpaired electron characteristic of free radicals contributes to the instability and high reactivity of these chemical species. Interaction of free radicals with other compounds results in a chain reaction of oxidation and reduction wherein uncharged molecules consecutively lose and gain electrons. Changes in electron configuration ultimately can lead to cellular damage. Oxidation of DNA molecules, for example, can result in mutation, and oxidation of lipid molecules can result in decreased structural fluidity of these compounds thus resulting in loss of integrity of cellular membranes.

Relevant Research

Findings from experimental animal research have demonstrated that exposure to acute psychosocial stress might promote transient increases in oxidative damage. For example, exposure of rats to acute immobilization stress has been demonstrated to increase markers of lipid peroxidation in plasma (Liu, Wang, & Mori, 1994), and in myocardial and hepatic tissue (Davydov & Shvets, 2001; Zaidi, Al-Qirim & Banu, 2005).

The results of acute stress and oxidative damage research are complemented by those from both human and animal research examining the association of repeated, chronic, or sub-chronic stress on levels of oxidative damage. A series of rodent studies conducted by Sahin and Gumuslu (Gumuslu, Sarikcioglu, Sahin, Yargicoglu & Agar, 2002; Sahin & Gumuslu, 2004; 2007a; 2007b) revealed that daily exposure to cold stress and/or immobilization stress over a period of two weeks was associated with elevated levels of oxidized proteins and lipids in peripheral tissues. Similarly, evidence from observational studies in humans supports an association of both chronic and brief naturalistic stress with increased oxidative damage. Epel and colleagues found that more years of giving care to an ill child and greater perceived stress each were correlated with increased levels of oxidized lipids (Epel et al., 2004). When compared to a lower stress time period, blood samples taken from students during academic examination week had increased DNA damage, increased sensitivity of lipids to oxidation, and decreased free radical trapping ability, suggesting an increase in oxidative stress (Sivonova et al., 2004).

Recommended Reading

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