Chapter 5

Trends in the Evaluation of Lipid Peroxidation Processes

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1. Introduction

Oxidative stress occurs as a result of imbalance between the antioxidant and prooxidant systems acting at certain points in metabolic processes, in favor of the last. The oxidative stress, defined by H Sies following extensive research performed between 1981 and 1993, is the outcome of intense generation of reactive oxygen species (ROS), which are not counteracted by endogenous antioxidant molecules [Sies, 1985]. Current knowledge links many types of pathologies to oxidative damage; among them, most cited are atherosclerosis, diabetes mellitus, neurodegenerative disorders, cancers, rheumatic diseases, autoimmune disorders, etc. Figure 1, sometimes referred to as "oxidative stress wheel", presents the most important diseases in which oxidative stress is involved resulting in biochemical lessions

Free radicals are chemical species containing unpaired electrons, which can increase the reactivity of atoms or molecules. Free radicals are highly reactive and unstable, due to their impaired electrons; they can react locally, accepting or donating electrons, in order to become more stable. The reaction between a radical and a non-radical compound generally leads to the propagation of the radical chain reaction, and to an increasing generation of new free radicals. During biochemical processes that normally take place in living cells, many types of free radicals are generated: oxygen-, sulfur-, bromide- and chloride- centered species [Halliwell & Gutteridge, 2007]. The most common reported cellular free radicals are singlet oxygen ($^1\Sigma g^+O_2$), hydroxyl (OH·), superoxide ($O_2^{-}\cdot$) and nitric monoxide (NO·). Also, some other molecules like hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) (which are not free radicals from the chemical point of view, having all-paired electrons) are reported to generate free radicals in living organisms through various chemical reactions [Halliwell, 2006].

In this context, it is extremely important to evaluate the extent and rate of the lipid peroxidation process using different methods and experimental models, ranging from

quantitative assay of lipoperoxides end products to the evaluation of changes in certain metabolic processes under the influence of pro-oxidative or antioxidative known substances. The present article aims at reviewing different techniques, methods and experimental models for the evaluation of lipid peroxidation that can be used in clinical research and in basic biochemical research as well. Simple, rapid, cost effective, and more elaborated, expensive methods are critically evaluated, presenting the advantages and limitations of each one. A special emphasis is given to fluorescent methods, which our team is frequently using to evaluate the lipid peroxidation processes.

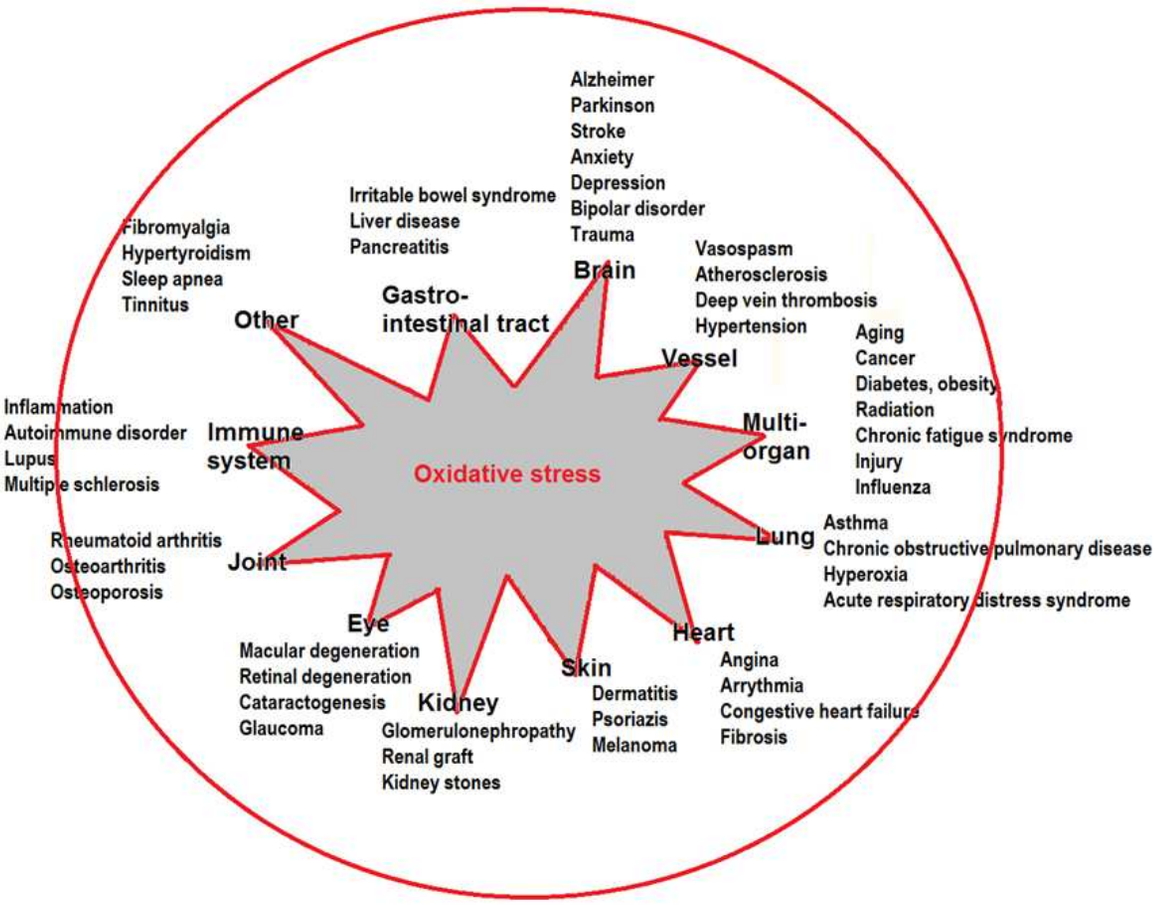

Figure 1. Implication of oxidative stress in pathology ("oxidative stress wheel")

2. Oxidative stress, ROS and implication in metabolic procesess

2.1. Oxygen centered reactive species (ROS)

Reactive oxigen species is a generic term that includes both oxygen radicals and certain non-radicals that are oxidizing agents and/or are easily converted into radicals, such as H_2O_2, ozone (O_3), singlet oxygen (Δg^1O_2), peroxynitrite, hypochlorous acid (HOCl), etc. [Halliwell, 2006].

ROS are generated as a result of oxygen action on nutrients or on physiological components in living organisms.

The sources for oxidative stress are either endogenous (abnormal mithocondria and peroxisomes function, lipoxygenase, NADPH-oxidase, cytochrome P450 activity), endogenous antioxidant systems dysfunction (low amount of non-enzymatic antioxidants such as gluthatione, vitamins A, C and E, reduced enzimatic activity) or exogenous agents (ultraviolet or ionizing radiation, toxins, chemotherapy, bacteria, etc.) (Figure 2).

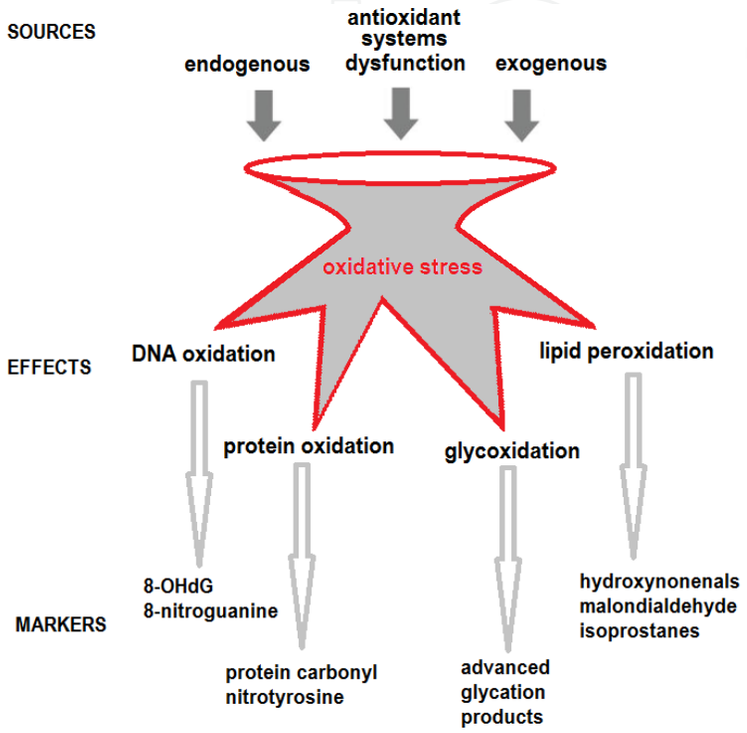

Figure 2. Sources, effects and main markers of oxidative stress

ROS can induce many damaging cellular processes, such as DNA oxidative lesions, loss of membrane integrity due to lipid peroxidation, protein and functional carbohydrate structural changes, etc. All these structural and functional changes have direct clinical consequences, leading to the acceleration of the general aging process, but also to some pathological phenomena, associated with the increase of the capillary permeability, impairment of the blood cell function, etc. ROS lesions are frequently associated with aging [Dröge & Schipper, 2007; Griffiths et al., 2011], atheroclerosis [Hulsmans & Holvoet, 2010], cardio-vascular disease [Dikalov & Nazarewicz, 2012; Puddu et al., 2009], type I or type II diabetes mellitus [Cai et al., 2004], autoimmune disorders, neurodegenerative disorders such as Parkinson [Yoritaka et al., 1996] or Alzheimer's disease [Sayre et al., 1997; Takeda et al., 2000], inflammatory diseases such as reumatoid arthritis [Griffiths et al., 2011] or different types of cancers [Lenaz, 2012; Li et al., 2009; Manda et al., 2009].

In order to counteract the damaging action of the physiologically generated ROS, the living organisms developed efficient antioxidant systems [Christofidou-Solomidou & Muzykantov 2006; Halliwell, 2006; Sies, 1997; Veskoukis et al., 2012]. Endogenous antioxidants in the human body act through different types of mechanisms:

- reducing the ROS generation – through chelating the metal ions (ferum, copper, etc.) by specific or non-specific proteins (ferritine, transferrine, albumin), so that these ions can no longer participate to redox reactions [Aruoma et al., 1989; Elroy-Stein et al., 1986; Freinbichler, 2011; Halliwell & Gutteridge, 1984; Velayutham, 2011]
- stopping the ROS formation chain reaction – generally via antioxidants with small molecular mass, such as reduced glutathion (GSH), vitamins E and C, uric acid, etc. [Gordon, 2012; Halliwell, 2006]
- scavenging ROS with antioxidant enzymes such as superoxyde dismutase (SOD), catalase (CAT), glutathion-peroxidase (GPx), glutathion-reductatse (GR), etc. [Halliwell, 2006; Sies, 1997]
- reparing lesions caused by ROS via specific enzymes such as endonucleases, peroxidases, lipases, etc. [Sies, 1997].

All these antioxidant systems act differently, depending on their structure and properties, their hydrophilic or lipophilic character, and also depending on their localization (intracellular or extracellular, in cell or organelles membrane, in the cytoplasm, etc.). All the aforementioned systems act sinergically and form a network which protects living cells from the destructive action of ROS (Figure 3).

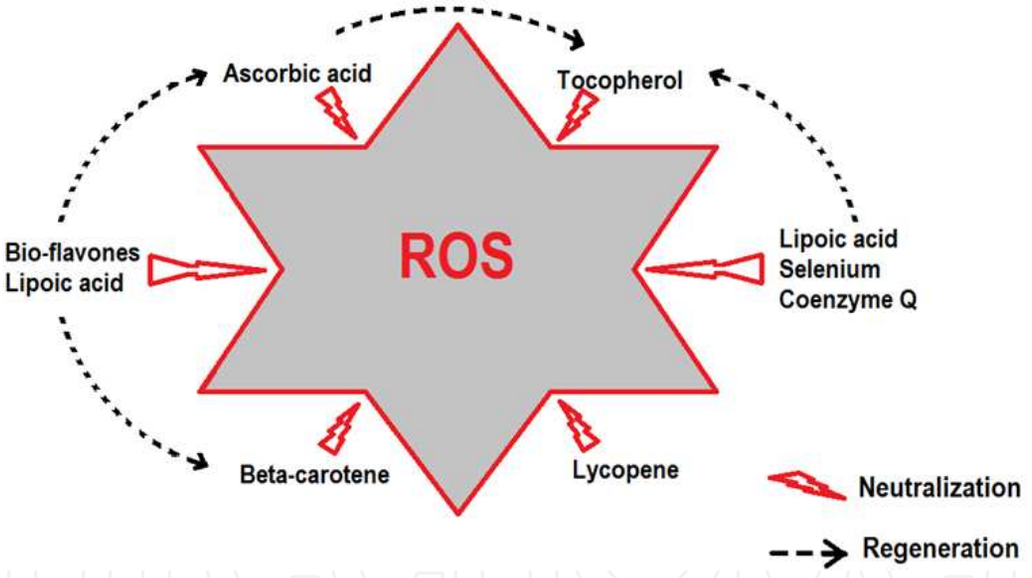

Figure 3. ROS neutralization by several biomolecules

2.2. Nitrogen centered radical species (RNS)

After the discovery of the physiological role of endogenously produced nitric oxide (NO), the capacity of this bio-molecule to react with other cellular components (such as proteins and lipids), specific nitrosative chemical changes have emerged as a key signaling mechanism in cell physiology. Several studies reported the involvement of excess generation of NO and its adducts in the etiology of multiple disease states, including insulin resistance and diabetes, atherosclerosis or Alzheimer's disease [Duplain et al., 2008; Parastatidis et al., 2007; Uehara, 2007; Yasukawa T et al., 2005; White et al., 2010].

The nitrosative modifications of proteins take two main forms: either S-nitrosylation of cysteine thiols or nitration of tyrosine residues. Both chemical processes may arise from protein interactions with NO or with secondary intermediates of NO, otherwise termed reactive nitrogen species (RNS) [White et al., 2010]. One of the very important members of the RNS group is represented by peroxynitrite ($ONOO^-$), produced from the reaction of NO with the superoxide anion (O^{2-}), which is considered as one of the major cellular nitrating agents [Hogg, 2002; White et al., 2010]. Other nitrating agents are the nitrosonium cation (derived from the action of myeloperoxidase), produced from the reaction of nitrite with hydrogen peroxide and nitroso-peroxocarbonate, which results from the reaction of carbon dioxide with peroxynitrite. Lipid peroxyl radicals have been recently shown to promote tyrosine nitration by inducing tyrosine oxidation and also by reacting with NO_2^- to produce $\cdot NO_2$ [Bartesaghi et al., 2010; Denicola et al., 1996; Lang et al., 2000].

2.3. Oxidative stress and lipid peroxidation

Among the targets of ROS and RNS, lipids are basically the most vulnerable, as their peroxidation products can result in further propagation of free radical reactions [Halliwell & Chirico, 1992]. The brain is a high oxygen-consuming organ and the nervous cell has also the greatest lipid-to-protein ratio; besides, the brain has a relatively week protection systems against ROS generation, therefore it is particularly vulnerable to oxidative stress. The age-related increase in oxidative brain damage results in intense generation of lipid peroxidation products, protein oxidation, oxidative modifications in nuclear and mitochondrial DNA [Grimsrud et al., 2008].

Polyunsaturated fatty acids (PUFAs) and their metabolites have many physiological roles such as energy generation, direct involvement in cellular and sub-cellular membrane structure and function, implication in cell signaling processes and in the regulation of gene expression as well. They constitute the main target of ROS action in the lipid peroxidation reactions.

The general process of lipid peroxidation consists of three stages: initiation, propagation, and termination [Auroma et al., 1989; Leopold & Loscalzo, 2009]. Many species can be responsible for the initiation of the chain reaction the radicals: hydroxyl, alkoxyl, peroxyl, superoxide or peroxynitrite. As a consequence, a free radical atracts a proton from a carbon of a fatty acyl side chain leaving the remaining carbon radical accessible to molecular oxygen to form a lipid peroxyl radical. This is also highly reactive and the chain reaction is propagated further. As a result, PUFA molecules are transformed into conjugated dienes, peroxy radicals and hydroperoxides, which will undergo a cleavage mainly to aldehydes. More than 20 lipoperoxidation end-products were identified [Niki, 2009]; among the components of PUFAs oxidative degradation products, the most frequently mentioned were acrolein, malondialdehyde (MDA), 4-hydroxyalkenals and isoprostanes [Esterbauer et al., 1991; Leopold & Loscalzo, 2009].

RNS also play a major role in lipid biology, by two major pathways: targeting some enzymes (COX-2 and cytochrome P-450) and thus influencing bioactive lipid synthesis and interacting with unsaturated fatty acids (such as oleate, linoleate and arachidonate) and generating novel

nitro-fatty acids. Nitrated lipids, such as nitroalkenes, may undergo aqueous decay and release independent of thiols, isomerize to a nitrite ester with N-O bond cleavage, or generate an enol group and ·NO [Leopold & Loscalzo, 2009]. The nitro-fatty acids have distinct bioactivities from their precursor lipids [Baker et al., 2009; Freeman et al., 2008; Kim et al., 2005; Lee et al., 2008; White et al., 2010]. Studies identified a high number of nitro-fatty acid species and proved an elevated formation of RNS in hydrophobic environments, such as the lipid bilayer, suggesting that lipids might constitute candidates for nitrosative signal transduction [Jain et al., 2008; Moller et al., 2005; Moller et al., 2007; Thomas et al., 2001].

Nitroalkenes may also participate in reactions with cysteine and histidine residues in proteins and with the thiolate anion of glutathione (GSH) to initiate reversible modification(s) of proteins. Thiyl radicals may also initiate lipid peroxidation by extraction of a hydrogen atom from bis-allylic methylene groups of fatty acids generating pentadienyl radicals. These radicals, in turn, may react with oxygen to generate peroxyl radicals [Leopold & Loscalzo, 2009].

Lipid hydroperoxides (LOOHs) are intermediates of PUFAs lipid peroxidation and can be also found as minor constituents of cell membranes; these compounds are also final products of prostaglandin and leukotriene biosynthesis, and can be decomposed by transition metals to form alkoxyl and lipoperoxyl radicals. Furthermore, biomolecules such as proteins or amino-lipids, can be covalently modified by lipid decomposition products (i.e. by forming Schiff bases with aldehydes or/and by activating membrane-bound enzymes). In consequence, lipid peroxidation may alter the arrangement of proteins in bilayers and thereby interfere with their physiological role in the membrane function.

2.4. Pathological involvement of the lipid peroxidation process

Many lipid peroxidation products, either full chain or chain-shortened, have been reported to be harmful or to have pro-infl ammatory effects [Birukov 2006; Niki 2009; Salomon 2005].

Lipid peroxidation increases the permeability of cellular membranes, resulting in cell death.

The lipid peroxidation process located at the cell membrane level may lead to loss of integrity and viability, and also to altered cell signaling and finally to tissue dysfunction; the oxidation of plasma lipoproteins is probably a major contributor to the formation of lipid peroxidation products and is widely thought to be involved in atherosclerosis. Malondialdehyde and 4-hydroxy-2-nonenal (HNE), the well known products of lipid peroxidation, react with a variety of biomolecules, such as proteins, lipids and nucleic acids, and they are thought to contribute to the pathogenesis of human chronic diseases [Breusing et al., 2010].

A clear example of the pathological role of PUFAs peroxidation is the evolution of atherosclerotic lesions to cardio-vascular disease. Until 1970, it was considered that dyslipidemia was the main factor initiating the atherosclerotic lesions. Later on, researchers emphasized the involvement of inflammatory processes, growth factors, smooth muscle cells proliferation, as well as viruses, bacteria or tumor phenomenon in the atherosclerosis, besides lipids and lipoproteins.

RNS interaction with different types of proteins is directly involved in physio-pathological processes. Several studies proved that key enzymes involved in glycolysis, β-oxidation, the tricarboxylic acid cycle and electron transport chain are targets of tyrosine nitration or *S*-nitrosylation. Modifications by nitrosylation that reduce the activity or function of important tricarboxylic acid cycle and electron transport proteins have the potential to slow substrate oxidation and probably lead to the build up of metabolic intermediates (particularly lipids) that could impair signaling pathways to reduce insulin action. Also, key insulin-signaling intermediaries are *S*-nitrosylated, and this could constitute a potential mechanism of insulin resistance [Chouchani et al., 2010].

3. Evaluation of the end products of lipid peroxidation

Malonidialdehyde (MDA) is one of the most cited lipoperoxidation product originating from PUFAs. Several generation mechanisms have been proposed for MDA. Pryor & Stanley (1975) considered them as bicyclic endoperoxydes coming up from nonvolatiles MDA precursors, similar to prostaglandins. This mechanism was confirmed in 1983 by Frankel & Nef. Two other mechanisms were postulated by Esterbauer (1991) and consist in the successive generation of peroxydes and β-cleavage of the lipid chain or as a reaction of acroleine radical with a hydroxyl moiety (exemplification for arachidonic acid in Figure 4, adapted from Esterbauer, 1991). Hecker & Ulrich (1989) consider that MDA can be generated *in* vivo by means of enzymatic processes linked to prostaglandins.

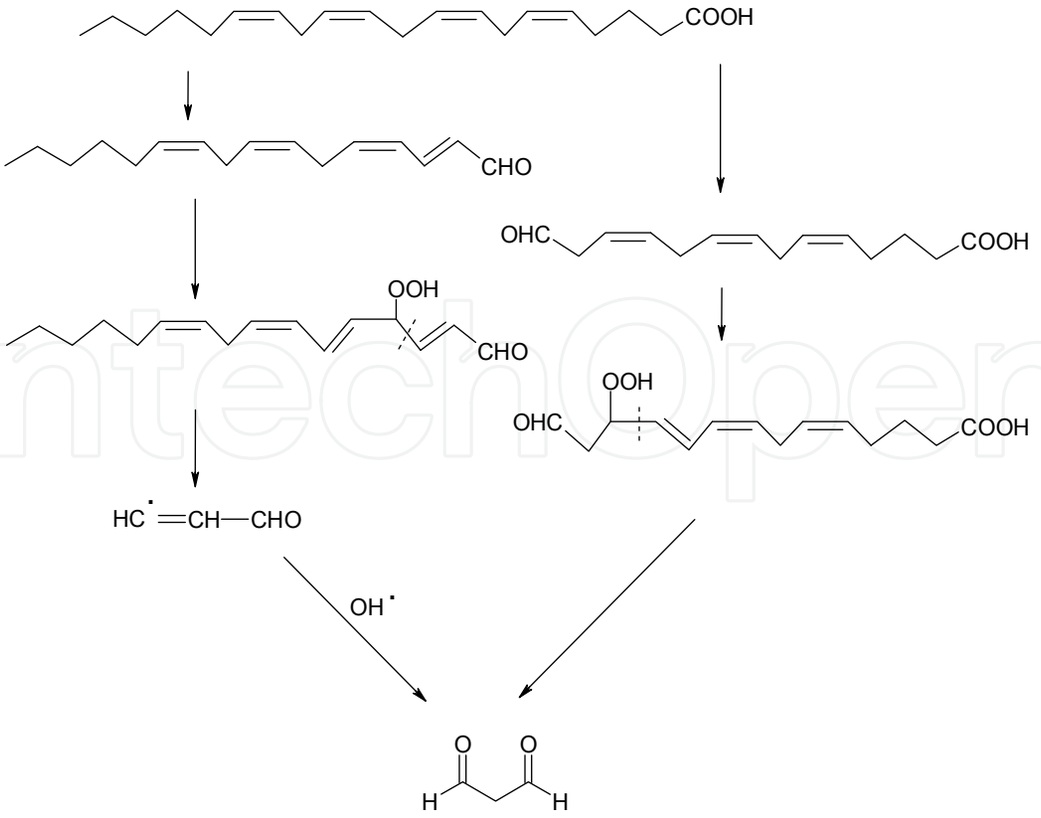

Figure 4. Generation of MDA as proposed by Esterbauer (1991)

Because the evaluation of the end products of lipid peroxidation at the tissue level is considered of maximum importance in both clinical and toxicological research, several methods to assay MDA have been proposed [Del Rio, 2005]. Since 1948, the most used method to assay lipoperoxidation end products is based on the studies of Bernheim et al., consisting in MDA condensation with the thiobarbituric acid (TBA) leading to a red complex which can be quantified by visible absorption spectrophotometry (in the range 500-600 nm, depending on the procedure used) or fluorescence spectroscopy (Figure 5). As TBA reacts also with several other aldehydes commonly present in the biological sample, the agents reacting with TBA are frequently denoted as thiobarbituric acid reacting species (TBARS). It must be said that TBARs and MDA are a rather imprecise measure of the lipid peroxidation process, since many substances that are present in human biological fluids can also react with TBA.

Moreover the reaction conditions (heating) can lead to the degradation of other molecules in the sample, increasing the amount of MDA that is available for the reaction with TBA. Therefore this assay usually gives an overestimation of free radical damage [Cherubini et al., 2005].

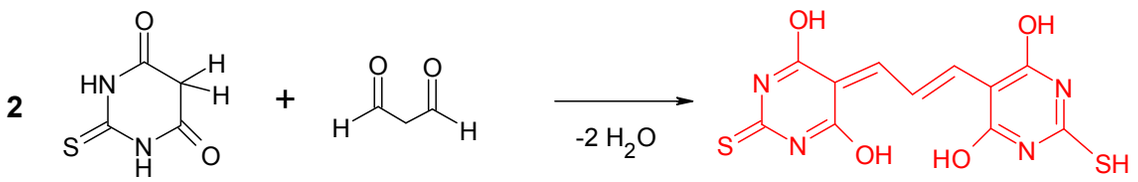

Figure 5. Mechanism of reaction for TBARS quantification

The authors generally avoid interferences by using different methods: Kwiecieñ et al., 2002, used BHT (butylated hydroxytoluene) to prevent further oxidation of the sample; deproteinisation was performed with trichloracetic acid [Cassini et al., 1986], forced the lipoperoxidation and stopped the reaction with SDS and acetic acid [Gautam et al., 2010; Ohkawa et al., 1979], added EDTA and refered the results to standards prepared from tetramethoxypropane [Houglum et al., 1990] or used the standard addition method [Sprinteroiu et al., 2010].

The specificity of the measurement is improved by HPLC to separate the MDA-TBA adduct from interfering chromogens [Agarwal & Chase, 2002; Del Rio et al., 2003; Lykkersfeldt, 2001; Templar et al., 1999].

The principles of TBARS assay is so popular, that a few companies even developed kits for clinical research to assay MDA spectrophotometrically from biological samples.

Apart from the above mentioned method, other methods were applied for the quantitative assay of lipoperoxidation end-products: direct HPLC [Karatas et al., 2002], capillary electrophoresis [Wilson et al., 1997], RP-HPLC, derivatisation with 2,4-diphenylhydrazine [Sim et al., 2003], pre-column derivatisation with diaminonaphtalene at acidic pH (for protein bound MDA) or alkaline pH (for non protein-bound MDA), followed by HPLC-UV

analysis [Stegens, 2001], GC-MS analysis following derivatisation with phenylhydrazine [Cighetti et al., 2002], GC-ECD-MS after derivatisation with 2,4,6-trichlorophenylhydrazine [Stalikas & Konidari, 2001].

4. Evaluation of the antioxidant status

For the evaluation of the antioxidant status in biological samples several markers (either enzymatic or non-enzymatic) can be used. Generally, the results obtained from the evaluation of antioxidant status markers should be correlated with certain peroxidative parameters, in order to be able to draw conclusions from the experiments.

Among the enzymatic markers of the antioxidant defense mechanism of the biological samples, literature cites some specific enzymes, such as catalase, superoxide-dismutase, glutathione-reductase, glutathione-peroxidase, etc. Each of these enzymes can be assayed using specific kits.

Commonly used are also non-enzyimatic markers, such as reduced glutathione, some vitamins (ascorbic acid, tocopherols, carotenoides).

A series of commercial kits for measuring the antioxidant status in biological samples (blood, serum, but also food products) are also available - the so-called *total antioxidant status* kits. The assays can be colorimetric (one example is the kit using as a chromogen 2,2'-azino-di-[3-ethylbenzthiazole sulfonate], which reacts with methmyoglobin and hydrogen peroxide to give a coloured cation), chemiluminometric (using the reaction of luminol with hydrogen peroxyde), or physico-chemical (potentiometric).

These methods allow the evaluation of the total antioxidant capacity in the biological samples, thus accounting both for enzymatic and for non-enzymatic bio-molecules.

Our group developed a method enabling a distinct evaluation for the biological samples antioxidant capacity as resulting exclusively from redox hydrophilic biomolecules. The method is based on potentiometric evaluation of the status of oxidant and reducing species in samples of human serum. We used a micro Pt/AgCl combination redox electrode, with an internal reference, and a Tistand 727 Potentiometer (Metrohm AG, Switzerland). The baseline apparent redox potential of the human serum (ARP0) was measured; than a mild prooxidant chemical system (quinhydrone) was added to the biological samples. After incubating at 25°C for 1h respectively 3 hours, two final apparent redox potentials were recorded (ARPf). Quinhydrone mimics the prooxidant conditions developing *in vivo* and consumes the reducing species leading to an increase of the apparent redox potential in time. This dynamic recording of the data allowed the calculation of a difference between the final value of the ARP (ARPf) and the initial one (ARP0), thus defining the redox stability index (RSI). This parameter illustrates the serum sample capacity to counteract the prooxidant agent. The lower the RSI, the higher the activity of the hydrosoluble antioxidant protective systems in the serum [Margina et al., 2009].

5. Monitoring the induced-peroxidation process

J. Goldstein, M. Brown, and D, Steinberg emphasized more than a decade ago, that low density lipoproteins can be chemically modified, loosing their ability to be recognized by the classical LDL receptors [Brown & Jessup, 1999; Steinberg, 2009]. The oxidation of LDL does not take place in the blood stream, but inside the intimae, after lipoprotein complexes crossing through the endothelium. Therefore, in the oxidation process intracellular as well as extra cellular components are involved. This change of the LDL is realized mainly by oxidation with the free radicals that appear in large amounts in hypertension, diabetes mellitus, as a consequence of smoking, or in viral and bacterial infections, but LDL may be also modified by glycation (in type II diabetes mellitus), by association with proteoglycans or by incorporation in immune complexes [Ross, 1999].

Oxidised LDL particles (LDLox) have been proved to have different proatherogenic effects that can be predominantly attributed to their lipid components. Mainly, the uptake of LDLox by macrophages is enhanced through the scavenger receptor. Products generated from the decomposition of peroxidized lipids, such as aldehydes, modify the apolipoprotein B-100 (apoB-100) structure to a more electronegative form able to interact with the macrophage scavenger receptor. The modified LDL particles are taken up by scavenger receptors on the macrophages instead of the classical LDL receptors. This process is not regulated by feed back inhibition and allows the excessive build-up of cholesterol inside the cells, leading to their transformation into foam cells that are involved in the initiation and progression of atherosclerotic lesion [Parthasarathy et al., 1999; Shamir, 1996].

Alpha-tocopherol is an antioxidant from the LDL structure, and is the first one degraded during the radical attack on these lipoproteins. When this antioxidant protection is exhausted, PUFA are changed into lipid hydroperoxides. There is a great variability between subjects regarding the amount of PUFA and of antioxidants from LDL, which explains the variability concerning the susceptibility to oxidation of LDL particles. There are also a lot of other factors that influence *in vitro* evaluation of LDLox: some endogenous compounds, diet, some medicines, and probably genetic factors. Therefore, the assay of the *in vitro* LDL susceptibility to lipid peroxidation constitutes an important marker in the evaluation of atherogenic models/patients [Parthasarathy et al., 1999; Shamir, 1996].

The susceptibility of lipoprotein particles to lipid peroxidation can be assessed, after the isolation of LDL, either by treatment with copper salts, with mixtures of ferric compounds and ascorbic acid, or other prooxidant systems.

In order to evaluate this susceptibility to oxidation Esterbauer et al. proposed in 1989 a method based on the variation of absorbance of conjugated dienes in time at 234 nm. These dienes are relatively stable products resulted by a rearrangement of the double bonds from the PUFA molecules after the radical hydrogen abstraction. The increase in absorption at 234 nm is due to the formation of conjugated dienes during the peroxidation of polyunsaturated fatty acids. Absorbance at 234 nm shows an initial slower increase (lag phase) as antioxidants are destroyed and then increases more rapidly (propagation phase) reaching a

plateau phase at which the absorbance is maximal as the rate of formation of dienes approaches their rate of decomposition [Esterbauer et al., 1990].

Another method for the assay of lipoprotein susceptibility to lipid peroxidation is based on the ability of lipid hydroperoxides to convert iodide (I^-) to iodine (I_2), which will than react with the iodide excess and form I_3^- that absorbs at 365 nm. There is a direct stoechiometric relationship between the amount of organic peroxides that resulted from the reaction and the concentration of I_3^- [Steinberg D., 1990, Esterbauer H. 1993]

One of the symplest methods for the assay of the susceptibility of LDL particles to lipid peroxidation is based on the selective precipitation of serum LDL with heparin at isoelectrical point (pH=5.4), method which is cheaper and quicker than the one based on ultracentrifugation. Personal results proved that the susceptibility of LDL to lipid peroxidation is correlated with the fasting plasma glucose level as well as with the lipid level [Margina et al., 2004].

The same type of assay can be used in order to evaluate the susceptibility to induced peroxidation for other kinds of biological samples (red blood cells, sub-cellular fractions such as mitochondria, or even tissue homogenates).

Previously published results [Margina D et al., 2011] proved that, for patients diagnosed with central obesity (BMI>30Kg/m2), adipose tissue susceptibility to lipid peroxidation correlated significantly with the total cholesterol (TC) level and with the LDL level. The susceptibility of adipose tissue to lipid peroxidation was assessed on white adipose tissue harvested from the abdominal area, homogenated in NaOH 0.015M, followed by TBARS evaluation. This parameter reflects the tendency to accumulation of free radicals in the adipose tissue of obese patients. In the same study, we pointed out that patients with impaired lipid profile (TC>220 mg/dl, LDL>150 mg/dl) had a significantly higher susceptibility of the adipose tissue to lipid peroxidation ($p=0.036$), associated with the decrease of the adiponectin level (Figure 6), compared to obese patients with physiologic lipid profile (TC<220mg/dl, LDL<150mg/dl).

Literature data also mention the assay of circulating LDLox, using different ELISA methods; one of these methods uses antibodies against a conformational epitope in the apolipoprotein B-100 (apoB-100) moiety of LDL that is generated as a consequence of substitution of at least 60 lysine residues of apoB-100 with aldehydes. This number of substituted lysines corresponds to the minimal number required for scavenger-mediated uptake of ox-LDL. The substituting aldehydes can be produced by peroxidation of lipids of LDL, leading to the generation of ox-LDL [Holvoet et al., 2006]. Another method might be the electrophoretic separation of LDL and LDLox particles from serum samples; studies proved that the electrophoretic mobility of oxidized LDL particles is increased compared to that of standard LDL [Lougheed et al., 1996].

Besides biochemical determination, noninvasive, real-time monitoring of lipid peroxidation using fluorescent probes has also been developed. The assays can be performed either on living cells (for example using cis-parinaric acid, fluoresceinated phosphoethanolamine, undecylamine-fluorescein, diphenyl-1-pyrenylphosphine – DPPP or other fluorescent

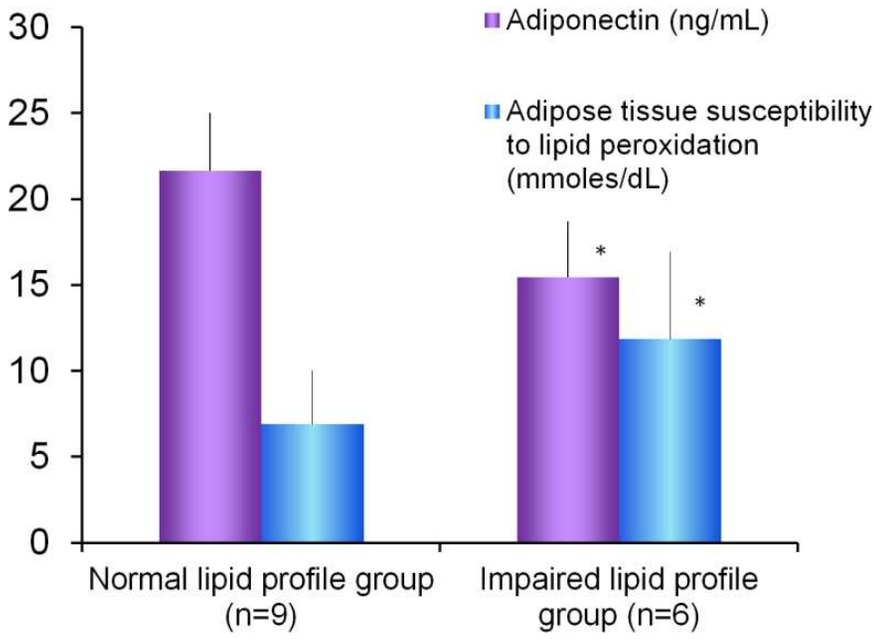

Figure 6. Obese patients with impaired plasma lipid profile (TC>220mg/dl, LDL>150mg/dl) are characterized by significantly different levels for the susceptibility of adipose tissue to induced lipid peroxidation as well as adiponectin level, compared to obese patients with normal plasma lipid profile (TC<220mg/dl, LDL<150mg/dl); * $p<0.05$ for cardio-vascular group compared to normal lipid profile group)

markers) or non-living samples (liposomes, tissue homogenates, plasma, serum, etc). In the cases of experiments that are performed on living cells, common limitation of use of fluorescent probes is that the probes often are cytotoxic or affect physiological activities of the cell [Drummen et al., 2004; Margina et al., 2012, Takahashi M. et al., 2001]. DPPP stoichiometrically reduces biologically generated hydroperoxides (such as fatty acid hydroperoxides, and triacylglycerol hydroperoxides) to their corresponding alcohols, and is transformed consequently into its oxide. DPPP is essentially non-fluorescent until oxidized to a phosphine-oxide by peroxides. Due to its solubility in lipids, DPPP intercalates into the membrane leaflets and reacts with lipid hydroperoxides, thus allowing the evaluation of peroxide formation in the membranes of live cells [Kawai et al.,, 2007]. Due to these chemical properties, the probe can be used in order to evaluate the extent of lipid peroxidation of biological materials such as cell membranes [Akasaka et al., 1993; Ohshima et al., 1996; Takahashi et al., 2001]. Because DPPP molecules are incorporated into the cell membranes, hydroperoxides located in the membrane are supposed to preferably react with DPPP.

Diphenyl-1-pyrenylphosphine (DPPP) is a synthetic compound with high reactivity against hydroperoxides, which has been used as a sensitive fluorescent probe for hydroperoxide analysis for HPLC methods. H_2O_2, which is least lipid-soluble, does not induce the peroxidation reaction of DPPP located in cell membranes. Although H_2O_2 is highly permeable to the membrane, it may not stay within the membrane long enough to react with DPPP effectively. Experimental lipid peroxidation can be induced by 10μM cumene hydroperoxide (CuOOH) which generates an effective reaction with DPPP in the membrane [Gomes et al., 2005; Takahashi et al., 2001].

We proved using DPPP in cell models (U937 human macrophage cell line as well as Jurkat lymphocytic cells) that the increase of certain polyphenol concentration (quercetin or epigallocatechin gallate) induces a decrease of the CuOOH induced lipid peroxidation of cell membranes. Fluorescence signals for the cells labeled with 5 μM DPPP are presented in Figure 7 [Margina et al, 2012].

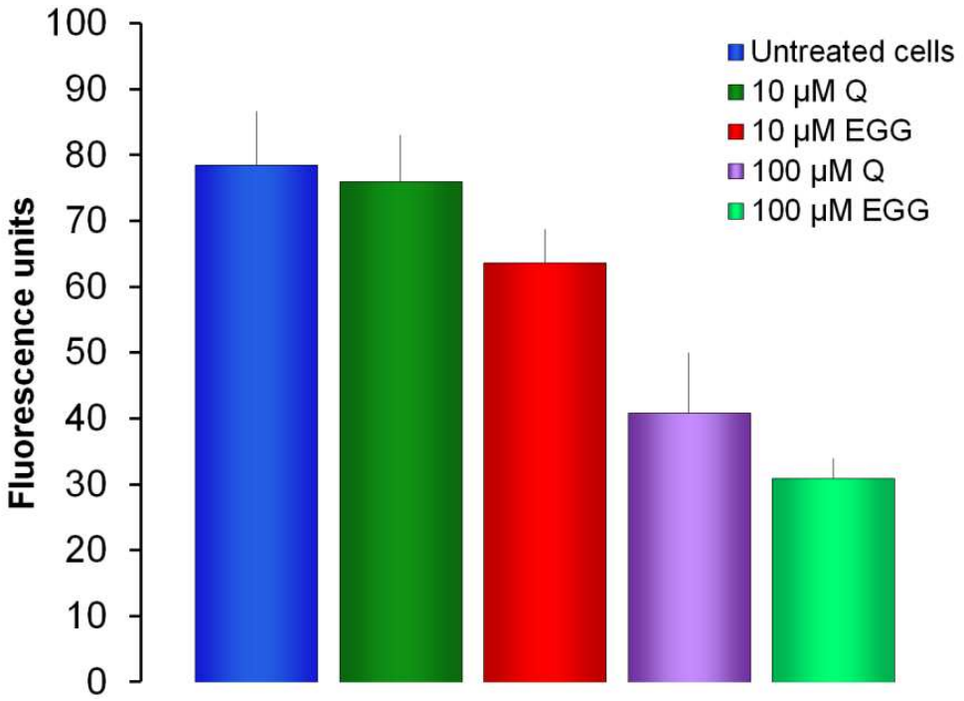

Figure 7. DPPP-oxide generation from Jurkat cell membrane before and after 20 minutes exposure to 10μM and 100 μM quercetin (Q) and epigallocatechin gallate (EGG) respectively; the fluorescence intensity was registered after 5 minutes of incubation of DPPP-labeled cells with CuOOH (for the induction of the lipid peroxidation)

6. Conclusions

Oxidative stress is among the most claimed causes of disease, as by its very definition indicates an abnormal biochemical function of the body. Among the targets of the oxidative stress, lipids are favorites, susceptible to structural changes that can decisively influence their normal function, and also generating hydroxyperoxides that further propagate the lipoperoxidation process.

Lipid peroxidation has been intensively studied in connection with normal and pathological metabolic processes; one of the main purposes was the understanding of toxicity triggered by lipoperoxidation end-products. That is why direct or indirect quantification of these products (TBARS, MDA, hydroxynonenals, prostaglandins, DNA and protein-adducts of the former, etc.) remains of interest for traditional and nowadays methods. The process of lipoperoxidation is often monitored in dynamics, even on living cells, using various techniques.

It is also of a great interest to seek for efficient antioxidants to prevent the excess lipoperoxidation, therefore one component of such kind of studies consist in finding out

efficient markers for the oxidative stress and the antioxidant status. This can be fulfilled if a right and comprehensive understanding of the lipoperoxidation process is achieved. But this is still a faraway target.

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