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Oxidative stress detection: what for?
Part I

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Abstract. – In this paper we review the diseases, such as cancer, arteriosclerosis, arthritis, neurodegenerative disorders, whose emerging pathogenetic evidence by Free Radicals has been shown either from experimental or from clinical point of view.

In the last 10 years the growing evidence of a co-causative responsibility of oxidative stress in some chronic and acute illnesses highlighted the need to improve the diagnostic potential as well as the algorythm of an effective treatment plan.

An exhaustive basic description of the Free Radicals action mechanism in different parenchymas to produce damage and clinical symptoms is the very preliminary background to any further investigation as to the specific diseases-oriented diagnostic tests as well as to a rationale therapy.

Key Words:

Free radicals, Reactive oxygen species, Lipid peroxide, Polyunsaturated fatty acids, Antioxidants.

Introduction

The role of free radicals in human pathology is quite a puzzling issue and the benefits of oxidative stress drug therapy in terms of disease prevention or therapy are still debated.

On the other hand new inputs are coming from the market about the diagnostic methods and instruments, with special attention to the point of care phylosophy, making easier cheaper and quicker the epidemiological and clinical investigations.

In the market perspectives, the Reactive Oxygen Species (ROS) level detection in plasma in the next years, is supposed to be competitive with the Erythrocyte Sedimentation Rate test (ESR), being the latter an aspecific marker, bystander of inflammation or other degenerative or proliferative diseases, enclosed the cancer and the former a sensitive monitor of a dangerous unsteady background of pathogenetic processes and illnesses: ESR reflects in fact plasma proteins and fibrinogen increased turnover and concentration, ROS an imbalance of oxidative stressors often not adequately controlled by physiological antioxidant activity.

In this short review we'll summarize the up to date of the studies exploring the physiopathology of free radicals and the role of diagnostic strategies targeted to proper therapy.

Free Radicals

A free radical can be defined as a chemical species possessing an unpaired electron. It can also be considered as a fragment of a molecule. As such, free radicals can be formed in 3 ways:

1. By the homolytic cleavage of a covalent bond of a normal molecule, with each fragment retaining one of the paired electrons ($X{:}Y \rightarrow X^{\bullet} + Y^{\bullet}$);
2. By the loss of a single electron from a normal molecule ($X{:}Y \rightarrow X{:}^{-} + Y^{+}$);
3. By the addition of a single electron to a normal molecule ($A + e^{-} \rightarrow A^{-\bullet}$).

The latter, electron transfer, is a far more common process in biological systems than is homolytic fission, which generally requires high energy input from either high temperatures, UV light or ionising radiation. Heterolytic fission, in which the electrons of the covalent bond are retained by only one of the fragments of the parent molecule does not result in free radicals but in

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ions, which are charged or electrically neutral. The unpaired electron and the radical nature of a species are conventionally indicated by writing it with a heavy superscript dot.

It can be a source of confusion that the electrons in one of the most relevant molecules in free radical biochemistry, oxygen, are distributed in such a way that two of the electrons are "unpaired". Thus, oxygen is sometimes considered a di-radical. While the di-radical nature of oxygen does enable it to react readily with many other free radicals, generally, it reacts relatively slowly with non-radical species. When considering its reactions in the context of free radical biochemistry, it is usually easiest to simply consider it as a normal molecule that can readily add to free radicals or accept a single electron from them, while not itself being a free radical.

The most relevant free radicals in biological systems are radical derivatives of oxygen. Reduction of oxygen by the transfer to it of a single electron will generate the superoxide free radical anion ("superoxide"):

$$O_2^{\bullet} + e^- \rightarrow O_2^{-\bullet}$$

A two-electron reduction of oxygen would yield hydrogen peroxide:

$$O_2^{\bullet} + 2e^- + 2H^+ \rightarrow H_2O_2$$

Hydrogen peroxide is often produced in biological systems *via* the generation of superoxide: two superoxide molecules can react together to form hydrogen peroxide and oxygen:

$$2O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$$

Because the free radical reactants generate non-radical products this is known as a dismutation reaction. It can take place spontaneously (even if rather slowly) or can be catalysed by the enzyme superoxide dismutase. Hydrogen peroxide is not a free radical but falls into the category of "reactive oxygen species" (ROS) that includes not only oxygen free radicals but also non-radical oxygen derivatives that are involved in oxygen radical production.

Hydrogen peroxide is a relevant molecule in free radical biochemistry because it can rather easily break down, particularly in the presence of transition metal ions, to generate the most reactive and damaging of the oxygen free radicals, the hydroxyl radical (· OH):

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$

This reaction is often referred to as the iron-catalysed Haber-Weiss reaction. The non-catalysed Haber-Weiss reaction is the reaction of superoxide directly with hydrogen peroxide:

$$O_2^{-\bullet} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2^{\bullet}$$

The spontaneous reaction is less likely in biological systems due to low steady-state concentrations of the reactants. The iron (or copper) catalysed reaction can still be considered to be dependent on superoxide as both the source of the hydrogen peroxide and as the reductant of the transition metal ion:

$$O_2^{-\bullet} + Fe^{3+} \rightarrow Fe^{2+} + O_2^{-\bullet}$$
$$O_2^{-\bullet} + Cu^{2+} \rightarrow Cu^{+} + O_2$$

Ferrous (Fe^{2+}) iron and cuprous (Cu^{+}) copper are much more reactive with hydrogen peroxide than their oxidised counterparts, ferric (Fe^{3+}) and cupric (Cu^{2+}), respectively.

The autoxidation of reduced transition metals can also produce superoxide:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-\bullet}; \; Cu^{+} + O_2 \rightarrow Cu^{2+} + O_2^{-\bullet}$$

Thus, the reactions of the transition metal ions with oxygen can be considered reversible redox reactions and are extremely relevant in the promotion of free radical reactions. The "key players" in the biochemistry of oxygen free radicals are oxygen itself, superoxide, hydrogen peroxide, transition metal ions and the hydroxyl radical, the first four of which conspire by a variety of reactions to produce the last[1].

Superoxide, although a free radical, is not a particularly damaging species: it is a mostly reductive in nature and its main significance is probably as a source of hydrogen hydroperoxide and as a reductant of transition metal ions. Its reaction with $NO^{\cdot}$, which is believed to be the identity of Endothelium Derived Relaxing Factor, may also prove to be physiologically relevant[2]. At low pH values superoxide will protonate to form the perhydroxyl radical ($HO_2^{\bullet}$), a more reactive, oxidising species but a physiological pH less than 1% will be in the protonated form.

Hydrogen peroxide is an oxidising agent but not especially reactive and its main significance lies in it being a source of hydroxyl radicals in the presence of reactive transition metal ions. In

the absence of metal catalysts, superoxide and hydrogen peroxide are readily removed and are virtually harmless.

The **hydroxyl radical** is an extremely reactive oxidising radical that will react with most biomolecules at diffusion-controlled rates. Is therefore will not diffuse a significant distance within a cell before reacting and has an extremely short half-life but is able to cause great damage within a small radius of its site of production.

Singlet oxygen is another non-radical, reactive oxygen species often related to oxygen free radicals; it can lead and be produced by free radical reactions. Oxygen free radicals are not the only relevant free radicals in biochemistry, even if they are often the first species formed. Other free radicals of importance are the wide range of carbon-centred radicals ($R^{\bullet}$) that arise form the attack of an oxidising radical (e.g. $OH^{\bullet}$) on a biomolecule (RH) such as carbohydrate, lipid, nucleic acid or protein. These react very slowly with oxygen to form the corresponding peroxyl radicals ($ROO^{\bullet}$). In turn, these peroxyl radicals can participate in reactions that produce alkoxyl radicals ($RO^{\bullet}$). Sulphur atoms can also be the centre for free radicals (thiyl radicals, $RS^{\bullet}$) generated, for example, in the oxidation of glutathione.

Production of Free Radicals in Cells

With the exception of unusual circumstances such as the influence of ionising radiation, free radicals are generally generated in cells by electron transfer reactions. These can be mediated by the action of enzymes or non-enzymatically, often through the redox chemistry of transition metal ions.

Free radical production in animal cells can either be accidental or deliberate. Free radicals are produced deliberately by animal cells in some special conditions because they can be useful entities if constrained and targetted. Some enzymes utilise a free radical at their active site in the process of catalysis; for example ribonucleotide reductase[3,4]. In these situations the free radical is not really "free" at all and its reactivity is targetted towards a specific reactions. Activated phagocytes also deliberately produce superoxide as part of their bactericidal role[5]. Although the free radicals are generated only at the interface of the phagocyte plasma membrane and bacterium, some leakage of superoxide, hydrogen peroxide and other reactive oxygen species is inevitable.

Under normal conditions, the major source of free radicals in cells is electron "leakage" from electron transport chains, such as those in mitochondria and in endoplasmic reticulum, to molecular oxygen, producing superoxide. Other enzymes can also generate superoxide or hydrogen peroxide, such as the range of flavin oxidases situated in peroxisomes. Another source of superoxide in animal cells is the autoxidation of some compounds including ascorbic acid (vitamin C), thiols (e.g. glutathione, cysteine), adrenaline and flavin co-enzymes. These autoxidation reactions can be greatly enhanced by the involvement of transition metal ions. This accidental production of free radicals is kept to a minimum by the high efficiency of enzyme-mediated electron transfer and by keeping metal ions tighly sequestered; these are fundamental means of preventive antioxidant defence. Such precautions cannot be completely efficient and animals have evolved enzymic and non-enzymic antioxidant defences to deal with the inevitable low-level generation of free radicals during normal metabolic activity.

Free radical generation in cells can be greatly increased by some toxic foreign compounds. The typical example is carbon tetrachloride, which was the first such compound to be shown to exert its toxicity through a free radical mechanism, being metabolised to the trichloromethyl free radical by the action of cytochrome P-450 in the liver[6,7]. The production of reactive free radicals overwhelms the antioxidant defences in the liver and results in the oxidative destruction of cellular membranes and serious tissue damage. Other examples of toxic compounds exerting their toxicity *via* the generation of free radicals are "redox-cycling" compounds that readily accept an electron to form a free radical and then transfer it to oxygen, producing superoxide and thence hydrogen peroxide. The efforts of GSH-peroxidase to remove the continously-produced hydrogen peroxide result in the depletion of GSH (glutathione) and allows oxidative damage to the cell[8,9]. It is possible to postulate the involvement of free radical mechanism in the toxicity of many compounds but caution must be exercised. In many conditions, the free radical generation may be secondary to the initial toxic mechanism, a consequence rather than the cause of cell damage.

Damaging Reactions of Free Radicals

All of the most important classes of bio-molecules may be attacked by free radicals but lipids are probably the most sensitive. Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs), which are readily attacked by oxidising radicals. The oxidative destruction of PUFAs, kown as lipid peroxidation, is particularly damaging because it proceeds as a self-perpetuating chain-reaction[10]. The general process of lipid peroxidation can be envisaged as in the scheme below, where LH is the target PUFA and R· the initiating, oxidising radical. Oxidation of PUFA produces a fatty acid radical (L·) that rapidly adds oxygen to form a fatty acid peroxyl radical (LOO·). The peroxyl radicals are the carriers of the chain-reaction, they can oxidise further PUFA molecules and initiate new chains, generating lipid hydroperoxides (LOOH) that can break down to yet more radical species and to a wide range of compounds, notably aldehydes[11,12]:

$$LH + R^{\bullet} \rightarrow L^{\bullet} + RH$$
$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$
$$LOO^{\bullet} + L^{\bullet}H \rightarrow LOOH + L^{-\bullet}$$
$$LOOH \rightarrow LO^{\bullet}, LOO^{\bullet}, \text{aldehydes}$$

The breakdown of lipid hydroperoxides often involves transition metal ion catalysis, in reactions analogous to that with hydrogen peroxide yielding lipid peroxyl and lipid alkoxyl radicals. Aldehydes are always formed when lipid hydroperoxides break down and many of them are biologically active, particularly a class known as the hydroxyalkenals, whose best known member is 4-hydroxynonenal[13,14]. These molecules can diffuse from the original site of attack and spread the damage to other parts of the cell. In summary, lipid peroxidation is of particular significance as a damaging reaction consequent to free radical generation in cells because:

- It is a very likely occurence, given the availability and sensitivity of PUFA in membranes;
- It is a very destructive chain-reaction that can directly damage the membrane's structure and indirectly damage other cell components by the production of reactive aldehydes;
- Lipid peroxidation has been implicated in a wide range of tissue injures and diseases. It has been established to be casually involved in such tissue injuries as carbon tetrachloride hepatotoxicity[15] and may be involved in the pathogenesis of atherosclerosis[16].

Proteins and nucleic acids appear less sensitive than PUFAs to free radical attack in that there seems less possibility of rapidly-progressing, destructive chain reactions being initiated. Random attack of radicals on proteins is unlikely to be very damaging unless very extensive. Free radical damage to proteins is only likely to be important to the viability of the cell if the damage is allowed to accumulate, which in most cells is not likely, or if the damage is somehow focused on specific sites of particular proteins. One way that damage may be focused on specific sites of particular proteins is if the protein binds a transition metal ion at a particular site eg the binding of copper by a histidine residue. In this case, the reaction of the transition metal with hydrogen peroxide can produce hydroxyl radical that will react at or near the metal-binding site; this concept is known as "site-specific" damage[17,18]. A wide range of residue modifications can occur such as the formation of peroxides[19] and carbonyls[20], the latter of which may be a useful measure of oxidative damage to proteins.

DNA is readily attacked by oxidising radicals if they are formed in its vicinity as has been clearly demonstrated by radiation biologists. It must therefore be considered a susceptible and relevant target. As with proteins there appears little possibility of rapid chain-reactions occurring and again it is important that it must be either "site-specific" such that damage is focused and of high intensity, leading to strand breaks, or must elude the repair systems before replication occurs, leading to mutations. The detection of oxidised nucleobases in human urine has been taken as evidence for a continual oxidative attack on DNA[21,22]. Even with a very high level of efficiency of repair, sufficient damage may accumulate over a lifetime to lead to mutations and thence cancer.

Defences Against Free Radicals

Because some free radical generation in animal cells is inevitable and because they can be vey damaging, defences against the severe actions of free radicals have evolved. These are known as antioxidant defences and the two main categories are those whose role is to prevent the production of free radicals and those that intercept any that are produced[23]. They exist in both the aqueous and membrane compartments of cells and can be enzymes or non-enzymes.

The preventive defences include efficiency of electron transfer and sequestration of transition metal ions. Iron, for example, is held tightly bound to special proteins such as transferrin and ferritin[24]. Some iron, however, is postulated to exist in a more reactive low-molecular weight pool and, moreover, the production of free radicals may really release free transition metal ions[25]. Another form of preventive antioxidant defence is the removal of peroxides that react with transition metal ions to generate reactive free radicals. This includes both hydrogen peroxide and also the lipid hydroperoxides that are generated during lipid peroxidation. Catalase and glutathione peroxidase are enzymes whose role is to safely decompose peroxides. The former is mainly located in peroxisomes and acts upon hydrogen peroxide; the latter is found in the cytosol of most cells and is active towards both hydrogen peroxide and, if first cleaved from membrane phospholipids by a phospholipase, fatty acid hydroperoxides[26]:

$$2H_2O_2 \rightarrow \text{catalase} \rightarrow 2H_2O + O_2$$

$$ROOH + 2GSH \rightarrow \text{GSH peroxidase} \rightarrow ROH + GSSG$$

Other defences exist to intercept, or "scavenge", free radicals. One of this is the only known enzyme whose substrate is a free radical, superoxide dismutase. Most free radical scavengers are not enzymes, however. In cell membranes, the best characterised and perhaps the most relevant is α-tocopherol, the major member of the vitamin E family[27-29]. This compound is known as a "chain-breaking antioxidant" because it functions to intercept lipid peroxyl radicals (LOO·) and so terminate lipid peroxidation chain reactions:

$$LOO^{\bullet} + \alpha\text{-tocopherol-OH} \rightarrow LOOH + \alpha\text{-tocopherol-O}^{\bullet}$$

The resultant tocopheroxyl radical is relatively stable and, in physiological conditions, insufficiently reactive to initiate lipid peroxidation itself; an essential criterion of a good antioxidant. Other lipid-soluble chain-breaking antioxidants, such as ubiquinol[30], are as yet insufficiently characterised to establish their physiological relevance.

In the aqueous phase other molecules act as free radical scavengers. Ascorbic acid is a relevant antioxidant both within cells and in the plasma[31]. It has been shown to produce α-tocopherol from the tocopheroxyl radical *in vitro*[32].

Uric acid in plasma and glutathione in cell cytosol also possess strong radical scavenging properties. It is readily apparent that cells have evolved an array of antioxidant defences designed to severely limit damage caused by free radicals wherever and whenever it might occur. This condition is perhaps the most persuasive argument that exists for the significance of free radicals as a threat to the viability of cells and organisms.

Many biological molecules will react with oxidising free radicals and it might be thought that they are all antioxidants, but to qualify as such they must not be converted to a reactive radical themselves and the proposed target that they are supposed to be defending should also be identified[33]. Many putative biological antioxidants are really just innocent bystanders caught up in the action.

A third group of natural antioxidant defence are repair processes, which remove damaged bio-molecules before they can accumulate and before their presence results in altered cell metabolism or viability. Oxidatively damaged nucleic acids are repaired by specific enzymes, oxidised proteins are removed by proteolytic systems and oxidised membrane lipids acted upon by lipases, peroxidases and acyl transferases.

Finally, there is a great deal of effort being expended currently on finding effective antioxidant drugs for treatment or prevention of free radical-mediated tissue damage. Such compounds include metal-chelating agents and radical scavengers. Very few have as yet been proven to be useful. A notable exception is Probucol, used clinically as a lipid-lowering drug and since found to be an effective antioxidant that may help protect against atherosclerosis by preventing oxidation of low density lipoprotein[34].

One of the major problems related to efforts to antioxidant intervention regimes is that reactive free radicals cannot easily be specifically targetted and scavenged in biological systems. The hydroxyl radical, for example, will react readily with practically any compound, so the concept of a "specific ·OH scavenger" in a biological system is nonsense. To compete with the cell components for reaction with the ·OH radical scavenger would have to be present at concentrations much higher than the biomolecules, which is not feasible.

Free Radicals in Human Disease

With the increasing acceptance of free radicals as commonplace and relevant biochemical intermediates, they have been implicated in a very great number of human diseases. Free radicals have lifetimes measured in microseconds and are extremely difficult to measure *per se*, not least in the clinical condition. The researcher must generally rely on the measurement of products of free radical reactions, referred to as their "footprints" and there are often transitory in nature. The technique available for studying free radicals and their reactions is improving all the time but it must be applied with scientific rigour to elucidate the true role of free radicals in human diseases, if the field is not to be brought into disrepute. Sensitive and specific techniques should be applied to prove the presence of free radicals at the site of injury. It is essential that the role of free radicals in the causation of disorders and their generation as a consequence of disorders be clearly distinguished. To do this it is necessary to understand the time course of the free radical generation and injury. Preventing free radical generation, possibly by the enhancement of the natural antioxidant defences, should decrease both the detectable free radicals and the severity of the injury.

Ageing

The ageing process is very complex: it depends on genetic disposal, but on life style and environmental conditions at the same time.

Harman suggested, fifty years ago, that the accumulation of oxidants could explain the alteration of physical and cognitive functions of ageing. Oxygen metabolism leads to reactive species, including free radicals, which tend to oxidize the surrounding molecules such as DNA, proteins and lipids. Oxidative stress is an adaptive process which is triggered upon oxidant accumulation and which comprises the induction of protective and survival functions. Experimental evidence suggests that the ageing organism is in a state of oxidative stress, which supports the free radical theory. The free radical theory is not consistent with programmed senescence theories involving the cell division dependent decrease in telomere length ; however, oxidants are known to alter telomere structure. An appealing view of the role of oxidative stress in ageing is the trade-off principle which states that a phenotypic trait can be evolutionarily conserved because of its positive effects on development, growth or fertility, and despite its negative effect on somatic functions and ageing. It is likely that most cellular stresses which comprise adaptive and toxic functions follow such a rule[35].

Emerging pathological evidence indicates that major chronic aging-related diseases such as atherosclerosis, arthritis, dementia, osteoporosis, and cardiovascular diseases, are inflammation-related. A proposal for the molecular inflammation hypothesis of the aging views the redox derangement that occurs during aging as the major factor for increased risk for age-related inflammation. Accumulated data strongly indicate the activation of redox-sensitive transcription factors and dysregulated gene expression under the age-related oxidative stress seems to be the major culprits. Key players involved in the inflammatory process are the age-related upregulation of NF-kappaB, IL-1beta, IL-6, TNF-alpha, cyclooxygenase-2, adhesion molecules, and inducible NO synthase. Furthermore, data are presented on the molecular events involved in age-related NF-kappaB activation and phosphorylation by IkappaB kinase/NIK and MAPKs. Also, the involvement of another super family of transcription factors, PPARs (PPARalpha, gamma) as regulators of proinflammatory responses and NF-kappaB signaling pathway is involved on the physiological significance of a well-maintained balance between NF-kappaB and PPARs[36].

Some strategies aimed at reducing oxidative stress-related pathology have been performed in animals. However, only a few can be used and are efficient in humans, such as avoidance of unfavourable environmental conditions (radiation, dietary carcinogens, smoking...) and antioxidant dietary supplementation. Epidemiological data suggest that antioxidants may have a beneficial effect on many age-related diseases: atherosclerosis, cancer, some neurodegenerative and ocular diseases. However, the widespread use of supplements is hampered by several factors: the lack of prospective and controlled studies; insufficient knowledge on the pro-oxidant, oxidant and ant-oxidant properties of the various supplements; growing evidence that free radicals are not only by-products, but also play an important role in cell signal transduction, apoptosis and infection control. Although current data indicate that antioxidants cannot prolong maximal life span, the beneficial impact of antioxidants on various age-related degenerative diseases may forecast an improvement in life span and en-

hance quality of life. The current lack of sufficient data does not permit the systematic recommendation of anti-oxidants. Nevertheless, antioxidant-rich diets with fruit and vegetables should be recommended[37].

Even if the scientific discussion upon the OS hypothesis of ageing is still open, on the basis of the existing correlative information, it seems reasonable to state the involvement of OS as a causative factor in the ageing.

Eye Disorders

The eye is a unique organ because of its constant exposure to radiation, atmospheric oxygen, environmental chemicals and physical abrasion. Oxidative stress mechanisms in ocular tissues have been hypothesized to play a role in diseases such as glaucoma, cataract, uveitis, retrolental fibroplasias, age-related macular degeneration and various forms of retinopathy provides an opportunity for new approaches to their prevention and treatment. In the anterior uvea, both H_2O_2 and synthetic peroxides exert pharmacological/toxicological actions tissues of the anterior uvea especially on the sympathetic nerves and smooth muscles of the iris-ciliary bodies of several mammalian species. Effects produced by peroxides require the presence of trace amounts of extracellular calcium and the functional integrity of mitochondrial calcium stores. Arachidonic acid metabolites appear to be involved in both the excitatory action of peroxides on sympathetic neurotransmission and their inhibitory effect on contractility of the iris smooth muscle to muscarinic receptor activation. In addition to the peroxides, isoprostanes (free radical's products catalyzed peroxidation of arachidonic acid independent of the cyclo-oxygenase enzyme) can also alter sympathetic neurotransmission in anterior uveal tissues. In the retina, both H_2O_2 and synthetic peroxides produced an inhibitory action on potassium depolarization induced release of [3H] D-aspartate, *in vitro* and on the endogenous glutamate and glycine concentrations *in vivo*. Effects caused by peroxides in the retina are mediated, at least in part, by second messengers such as nitric oxide, prostaglandins and isoprostanes. The ability of H_2O_2 to alter the integrity of neurotransmitter pools from sympathetic nerves in the anterior uvea and glutaminergic nerves in the retina could underlie its role in the etiology of glaucoma[38].

Cataracts are the leading cause of blindness worldwide. Opacity of the lens is a direct result of oxidative stress. Cataracts occur primarily due to age, but also are common in diabetes where superoxide in the mitochondria is elevated as a result of hyperglycemia. The risk factors of cataract including diet (vitamins, fat and alcohol) as well as UV light and diabetes[39].

Age is by far the biggest risk factor for cataract, and it is sometimes assumed that cataract is simply an amplification of this aging process. This appears not to be the case, since the lens changes associated with aging and cataract are distinct. Oxidation is the hallmark of age-related nuclear (ARN) cataract. Loss of protein sulfhydryl groups, and the oxidation of methionine residues, are progressive and increase as the cataract worsens until > 90% of cysteine and half the methionine residues are oxidised in the most advanced form. By contrast, there may be no significant oxidation of proteins in the centre of the lens with advancing age, even past age 80. The key factor in preventing oxidation seems to be the concentration of nuclear GSH. Provided that nuclear GSH levels can be maintained above 2 mm, it appears that significant protein oxidation and post-translational modification by reactive small molecules, such as ascorbate or UV filter degradation products, is not observed. Adequate coupling of the metabolically-active cortex, the source of antioxidants such as GSH, to the quiescent nucleus, is crucial especially since it would appear that the cortex remains viable in old lenses, and even possibly in ARN cataract lenses. Therefore, it is important to understand the reason for the onset of the lens barrier. This barrier, which becomes apparent in middle age, acts to impede the flow of small molecules between the cortex and the nucleus. The barrier, rather than nuclear compaction (which is not observed in human lenses), may contribute to the lowered concentration of GSH in the lens nucleus after middle age. By extending the residence time within the lens centre, the barrier also facilitates the decomposition of intrinsically unstable metabolites and may exacerbate the formation of H_2O_2 in the nucleus. This hypothesis, which is based on the generation of reactive oxygen species and reactive molecules within the nucleus itself, shifts the focus away from theories for cataract that postulated a primary role for oxidants generated outside of the lens. Unfortunately, due to marked variability in the lenses of different species, there appears at present to be no ideal animal model system for studying human ARN cataract[40].

Varma et al, examined the feasibility of inhibiting cataract formation by treatment with

pyruvate, a metabolite known to effectively scavenge reactive species of oxygen and inhibit protein glycation, both known to be involved in the genesis of diabetic cataracts. In addition, pyruvate stimulates tissue metabolism, which is depressed with the onset of cataract formation. The objective of these experiments was to determine if this compound could be effective in offsetting the progress of cataract, specifically if administered after the diabetes-induced lens changes have begun, as opposed to the previous reports wherein it has been reported to delay cataract formation if administered prophylactically with the immediate onset of diabetes. Diabetes was induced by intraperitoneal administration of streptozotocin to mice. Lens transparency was assessed by slit lamp examination and its photography. ATP was determined enzymatically by reacting it with luciferin-luciferase mixture and measuring the fluorescence intensity. The findings described herein are in accordance with this possibility. The incidence of cataract in the group of diabetic animals, where treatment with pyruvate was initiated after the initial lens changes set in, was significantly lower at all times of observation in comparison to the untreated diabetic group. In addition, the severity of opacities in the pyruvate-treated group, when present, was much minor, the transparency of these cases being close to that in the control animals. The ophthalmic findings are supported biochemically by ATP levels, which were significantly higher in the pyruvate group in comparison to the untreated group. The present findings emphasize the clinical usefulness of initiating treatment with anti-oxidants and metabolic agonists even when the lens changes are detected at the time of the diabetes diagnosis. The latter usually comes much later than the onset of visual aberrations. Prophylaxis is not an absolute requirement[41].

The aetiology of AMD (Age-related Macular Degeneration) is multi-factorial with well established risk factors, i.e. age (> 60 years), genetic, hypertension, cigarette smoking and a low plasma amount of antioxidants. Female sex, exposure to sunlight, light-coloured iris, cardiocascular diseases, diet and alcoholism have also been associated with AMD. The macula is especially prone to direct light exposure, infact its photreceptor cells mediate light transduction into neuronal impulses. Moreover, their plasma membrane have the highest polyinsatured fatty acid amount of any body tissue. In additional, retinal oxygen turnover is very high and its cellular mitochondria are abundant. As a result, the lens and macula are strongly sensitive to OS, particularly without adequate eye protection. In addition, the eye requires an efficient internal antioxidant system.

Because AMD results partially from cumulative oxidative damages, antioxidants seem to exert a protective effect against this patology. Particularly, lutein and zeaxanthin, which are primarily obtained from dark green leafy vegetables, were specifically effective. There is no effective therapy for AMD. Therefore, the identification, in a given population, of lifestyle-related risk factors is the only strategy available to delay the disease progression.

Several pharmaceutical agents have been associated with rare but serious retinopathies, some resulting in blindness. Little is known of the mechanism(s) that produce these injuries. Mechanisms proposed thus far have not been embraced by the medical and scientific communities. However, preclinical and clinical data indicate that oxidative stress may contribute substantially to iatrogenic retinal disease. Retinal oxidative stress may be precipitated by the interaction of putative retinal toxins with the ocular redox system. The retina, replete with cytochromes P450 and myeloperoxidase, may serve to activate xenobiotics to oxidants, resulting in ocular injury. These activated agents may directly form retinal adducts or may diminish ocular reduced glutathione concentrations. Data are reviewed that suggest that indomethacin, tamoxifen, thioridazine, and chloroquine all produce retinopathies via a common mechanism-they produce ocular oxidative stress[42].

Retinopathy, a severely disabling complication of diabetes mellitus, is today the leading cause of acquired blindness among young adults in developed countries. Good glycaemic control can attenuate the development of diabetic retinopathy but such metabolic control is often difficult to achieve and maintain and additional therapies need to be identified by which retinopathy can be prevented or arrested. Hyperglycaemia plays a critical role in the development and progression of retinopathy, but the mechanism by which hyperglycaemia results in the development of retinopathy is not clear. Oxidative stress is increased in the retina in diabetes. The possible sources of increased oxidative stress might include increased generation of free radicals or impaired anti-oxidant defence system. Dietary supplementation with anti-oxidants in animal mod-

els of diabetic retinopathy inhibits retinal metabolic abnormalities and retinal histopathology, suggesting that oxidative stress is associated with the development of retinopathy. The mechanism by which anti-oxidants inhibit retinopathy in diabetes warrants further investigation, but animal studies show that increasing the diversity of anti-oxidants provides significantly more protection than using any single anti-oxidant. Thus, supplementation with anti-oxidants represents an achievable adjunct therapy to help preserve vision in diabetic patients[43].

Photoageing/Skin Pathologies

Superimposed on the physiological and innate process of ageing, photoageing (or extrinsic ageing) is related to enviromental. Exposure of skin to ultraviolet (UVA ed UVB) radiation can result in both acute (sunburn, erythema and melanogenesis) and long-term adverse effects such as inflammation, pigmentation, immuno-modulation, photo-ageing, carcinogenesis and ophthalmological diseases such as cataract and age-related macular degeneration.

Since the skin is always in contact with oxygen in the presence of surface lipid, it is one of the best target organs of environmental oxidative damage. Among the different insults induced by UV irradiation, free radicals and lipid peroxides are one of the most reliable candidates for explaining damages. Moreover direct absorption of UVB photons by DNA and subsequent structural changes, production of ROS following irradiation with UVA and UVB requires the absorption of photons by endogenous molecules called photosensitizers[44]. When a molecule adsorbs UV radiation, it become electronically excited and becomes a short-lived free radical. This mechanism is called photosensitization; several cellular components (e.g., porphyrins, flavins, quinones and others) and biologically active drugs (e.g., tetracyclines,thiazides) can act as photosensitizers within skin cells. Because most photosensitized reactions are oxygen dependent, UV irradiation absorption results in the production of ROS. Free radicals are also produced by neutrophils (white blood cells having immune functions) that are increased in photodamaged skin and contribute to the overall prooxidant state. Therefore, UV-induced production of ROS in the skin develops OS, when their formation exceeds the anti-oxidant defence capacity of the target cell. Fortunately, the skin possesses a wide range of interlinked anti-oxidant mechanisms including melanin and carotenoids, which act as a UV-absorbing optical filter as well as a free radical scavenger.

However, it is necessary prevent the side effects of UV light on skin. Effective and appropriate photoprotection seems to be physical protection by clothes and physicochemical protection by sunscreens. It is also reasonable to administer anti-oxidants for this purpose and recent advances in free radical research have opened the door to clinical application of therapeutic anti-oxidants, such as retinoic acid. It is known for its capacity to repair photoaged skin, infact it has been shown to infere with the UV/ROS initiated pathways.

Oral antioxidants that scavenge reactive oxidants and modulate the cellular redox status may be useful; systemic photoprotection overcomes some of the problems associated with the topical use of sunscreens. Preclinical studies amply illustrate the photoprotective properties of supplemented antioxidants, particularly RRR-alpha-tocopherol, L-ascorbate and beta-carotene. However, clinical evidence that these antioxidants prevent, retard or slow down solar skin damage is not yet convincing[45].

Cancer

Cancer is generally believed to result from one or more permanent genetic changes within a cell. In most tumors, carcinogenesis is a very complex multi-factorial process that can be widely grouped into five components: genetic, viral, chemical, physical and inflammatory. Even if some rare congenital conditions lead to cancer in early childhood, most of tumors arise in adulthood from a complex interaction between genetic and enviromental factors including lifestyle, nutrition, radiations, metals and so on. There is now to clarify evidence that free radicals are relevant factors in carcinogenesis. ROS increase in inflammation and in exposure to exogenous sources including pollutants, smoking, some drugs and radiation can induce cancer-causing mutations, oxidized lipids and proteins and modify signal transduction pathways that enhance cancer risk.

The development of carcinogenic cells from normal ones is a multiple steps process involving initiation, promotion and progression. Initiation is an irreversible, specific modification in the DNA of a target cell. Promotion involves the reversible stimulation of the expansion of the initiated cell or the reversible alteration of gene expression in

that cell or its progeny. ROS can act as both initiator and promoter of tumors by damaging critical cellular macromolecules and by acting as stimulator or inducer of cell-signaling molecules. Conversely, some antioxidants are anti-promoters and anti-carcinogens. The role of ROS in cancer is also supported by showing that dietary anti-oxidants as well as endogenous anti-oxidants act as cancer preventive agents. Human epidemiological studies provide that OS increase with clinical progression of some cancer and that a diet rich in anti-oxidant-containing foods reduces the risk of these cancer. New data, however, show that some dietary anti-oxidants may have potential as adjuvant in cancer therapy contributing to reduce painful side effects associated with treatment[46].

Chronic exposure of UV radiation to the skin is the leading cause of the most of cutaneous tumors. The capacity of UV radiation to induce immune toxicity has been also associated to its ability to induce skin cancer[47].

Current treatment of fibrosarcoma, an aggressive cancer of the connective tissue, is generally associated with poor prognosis. Matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), and constituents of the extracellular matrix (ECM), such as fibronectin, play a critical role in angiogenesis and underlie neoplastic invasion and metastasis. This and anticancer properties of lysine, proline, arginine, ascorbic acid, and green tea extract (NM) were analyzed to investigate the effect of these nutrients in vitro on human fibrosarcoma cells HT-1080 by measuring cell proliferation, modulation of MMP-2 and MMP-9, and invasive potential. *In vivo*, Roomi et al[48] studied the growth of human fibrosarcoma HT-1080 cells in athymic nude mice and the expression of MMPs and VEGF. Cell proliferation was evaluated by MTT assay, MMP expression by gelatinase zymography, and invasion through Matrigel and migration by scratch assay. Tumors were excised, weighed, and processed for histology in both the control and nutrient-supplemented groups. Results showed NM inhibited the growth and reduced the size of tumors in nude mice; decreased MMP-9 and VEGF secretion was found in the supplemented group tissues. NM inhibited invasion through Matrigel and migration with total inhibition at 1,000 microg/mL. These results offer promise in the therapeutic use of the nutrient mixture of lysine, proline, arginine, ascorbic acid, and green tea extract tested in the treatment of fibrosarcoma.

Mantovani et al[49] have tested the efficacy and safety of an integrated treatment based on a pharmaconutritional support, antioxidants and drugs, all given orally, in a population of advanced cancer patients with cancer-related anorexia/cachexia and oxidative stress. An open early-phase II study was designed according to the Simon two-stage design. The integrated treatment consisted of diet with high polyphenols content (400 mg), antioxidant treatment (300 mg/d alpha-lipoic acid + 2.7 g/d carbocysteine lysine salt + 400 mg/d vitamin E + 30,000 IU/d vitamin A + 500 mg/d vitamin C), and pharmaconutritional support enriched with 2 cans per day (n-3)-PUFA (eicosapentaenoic acid and docosahexaenoic acid), 500 mg/d medroxyprogesterone acetate, and 200 mg/d selective cyclooxygenase-2 inhibitor celecoxib. The treatment duration was 4 months. The following variables were evaluated: (a) clinical (Eastern Cooperative Oncology Group performance status); (b) nutritional [lean body mass (LBM), appetite, and resting energy expenditure]; (c) laboratory [proinflammatory cytokines and leptin, reactive oxygen species (ROS) and antioxidant enzymes]; (d) quality of life (European Organization for Research and Treatment of Cancer QLQ-C30, Euro QL-5D, and MFSI-SF). From July 2002 to January 2005, 44 patients were enrolled. Of these, 39 completed the treatment and were assessable. Body weight increased significantly from baseline as did LBM and appetite. There was an important decrease of proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha and a negative relationship worthy of note was only found between LBM and IL-6 changes. As for quality of life evaluation, there was a marked improvement in the European Organization for Research and Treatment of Cancer QLQ-C30, Euro QL-5D(VAS), and multidimensional fatigue symptom inventory-short form scores. At the end of the study, 22 of the 39 patients were "responders" or "high responders". The minimum required was 21; therefore, the treatment was effective and more importantly was shown to be safe. The efficacy and safety of the treatment have been shown by the study; therefore, a randomized phase III study is warranted.

An alternative approach for skin cancer prevention and treatment is the topical application of herbal antioxidants. Plant-derived antioxidants are often extracts and therefore contain a complex mixture of constituents, like flavonoids and polyphenols, which contribute to the overall ac-

tivity of the extract. In an Hinneburg's study[50] an extract from buckwheat herb was compared to rutin, which is the main constituent of the extract, regarding their antioxidant and radical scavenging activity. Additionally, the photoprotective properties of the extract were compared to those of a commercial UV absorber. The antioxidant activity was quantified regarding the reactivity versus the 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH). The photoprotective properties of the extract were examined by the inhibition of the photosensitized lipid peroxidation of linolic acid. In the DPPH assay, the extract had significantly better antioxidant activity than pure rutin. The extract prevented more effectively the UV-induced peroxidation of linolic acid than rutin itself or the commercial UV absorber. The use of the extract from buckwheat herb seems to be more beneficial than the use of pure rutin. This can be referred to the presence of minor phenolic compounds in the extract. The results indicate that it is advisable to use antioxidants rather than only UV absorber to obtain a maximum of photo protection.

Yuccaols (A, B, C) are phenolic constituents isolated from Yucca schidigera bark characterized by unusual spirostructures made up of a C15 unit and a stilbenic portion closely related to resveratrol. These novel compounds are of particular interest for their antioxidant and anti-inflammatory properties. However, their effects on cell proliferation, migration, and platelet-activating factor (PAF) biosynthesis remain unknown. PAF, a potent mediator of inflammation, is known to promote angiogenesis and in vitro migration of endothelial cells and Kaposi's sarcoma (KS) cells. The objective of a Balestrieri's[51] study was to determine the effect of Yuccaols and resveratrol on the vascular endothelial growth factor (VEGF)-induced proliferation, migration, and PAF biosynthesis in KS cells. The results indicated that Yuccaols (25 microM) were more effective than resveratrol (25 microM) in inhibiting the VEGF-induced KS cell proliferation. Western blot analysis revealed that Yuccaols reduced the VEGF-induced phosphorylation of p38 and p42/44, thus indicating a possible interference with the mechanism underlying the VEGF-stimulated cell proliferation. Furthermore, Yuccaols completely inhibited the VEGF-stimulated PAF biosynthesis catalyzed by the acetyl-CoA:lyso-PAF acetyltransferase and enhanced its degradation through the PAF-dependent CoA-independent transacetylase (250% of control). In addition, Yuccaol C abrogated the PAF-induced cell motility whereas Yuccaol A and Yuccaol B reduced the cell migration from 7.6 microm/h to 6.1 microm/h and 5.6 microm/h, respectively. These results indicate that the anti-inflammatory properties attributed to Yucca schidigera can be ascribed to both resveratrol and Yuccaols and provide the first evidences of the anti-tumor and anti-invasive properties of these novel phenolic compounds.

Because of its antioxidant properties, alpha-tocopherol (vitamin E) has been used in oxidative stress associated to diseases prevention. Navarra et al[52] explored whether alpha-tocopherol modulates some cell responses induced by angiogenic and proliferative stimuli. For this purpose, they evaluate the effect in human vein endothelial cells (HUVECs), of alpha-tocopherol treatment (5-40 μmol/L) for 72 h on the production of ROS, induction of matrix metalloproteinases (MMPs), expression of vascular endothelial-cadherin (VE-cadherin) and alpha(2)-integrin, cell migration, cell proliferation, and tube formation. alpha-Tocopherol significantly inhibits intracellular ROS production induced by TNF-alpha ($p < 0.01$) or PMA ($p < 0.001$). However, alpha-tocopherol does not interfere with mRNA expression of VE-cadherin, alpha^2-integrin, MMP-1, MMP-2, and MMP-9. Similarly, alpha-tocopherol does not modulate cell migration and capillary-like tube formation although at the concentration of 20 and 40 μmol/L it potentiated PMA-induced DNA synthesis ($p < 0.05$). These results suggest that although alpha-tocopherol supplementation reduces endothelial cell oxidative stress, it does not alter the cell response to angiogenic stimuli.

Cardiovascular Diseases

Common vascular risk factors, including hyperlipidemia (cholesterol, low-density lipoproteins, etc.), hypertension, cigarette smoking, diabetes, overweight, physical inactivity, age, male sex and familial predisposition, only partially explain the excess risk of developing cerebrovascular and Coronary heart Disease (CHD) and a lot of studies support today the role of OS in their pathogenesis.

Paradoxically, even if moderate excercise develops an acute oxidant stress, regular endurance exercise is associated with improved cardiovascular function and a reduction in traditional CHD risk factors. These findings are consistent with the theory that adaptations induced by acute exposures to exercise-induced oxidative stress lead

to long-term vascular protection. This occurs through activation of signalling pathways that lead to increased synthesis of intracellular anti-oxidants and anti-oxidants enzymes and decreased ROS production during exercise[53].

Since nitric oxide (NO) is a cell-signaling molecule that plays a relevant role in the regulation of vasomotor tone and blood flow, regarding vascular diseases NO is interestingly and a decrease in NO bioactivity has been related with impaired endothelial function. Endothelial cells produce NO in response to various stimuli, including shear stress, acetylcholine, bradykinin and circulating factors in plasma such as estrogen, insulin and lipids. NO, in turn, diffuses to neighbouring smooth muscle cells and platelets, where NO induces vasorelaxation, inhibits platelet activation and adhesiveness and modulate inflammatory responses. An increase in ROS results in a decrease in NO bioactivity by diverting NO away from anti-inflammatory reactions to those that promote injury[54].

Previous clinical studies with prostaglandin I^2 (PGI^2) analogue beraprost sodium suggested the potential effects on protection of cardiovascular events in patients with peripheral artery disease. Although the mechanism is not well known, experimental studies have shown protective effects of endothelial cells. Ohata et al[55] evaluated the effects of beraprost sodium on vascular endothelial function in the forearm of patients with coronary artery disease. Beraprost sodium (120 μg/day) was orally administered to 14 coronary artery disease patients for 4 weeks and then stopped for 4 weeks. Eleven control patients did not receive beraprost sodium treatment. Reactive hyperemia was induced in the forearm, endothelium-dependent vasodilatation was assessed by plethysmography, and urinary 8-iso-prostaglandin F(2alpha) (8-iso-PGF(2alpha)) was measured at baseline, 4 weeks and 8 weeks. Both groups had similar reactive hyperemic responses at baseline. In the control group, reactive hyperemic response and urinary 8-iso-PGF(2alpha) remained unchanged for 8 weeks. In the beraprost group, maximum forearm blood flow increased significantly (p = 0.01) after 4 weeks of treatment and returned to baseline at 8 weeks. Duration of hyperemia increased significantly (p = 0.003) after 4 weeks, and remained greater than baseline at 8 weeks (p = 0.02). Urinary 8-iso-PGF(2alpha) decreased significantly (p = 0.03) after 4 weeks, and tended to be lower at 8 weeks (p = 0.07). Changes in reactive hyperemia correlated weakly but significantly with changes in 8-iso-PGF(2alpha) (p < 0.001). Beraprost sodium decreased oxidative stress and improved forearm endothelium-dependent vasodilatation in coronary artery disease patients. The favorable effects on vascular endothelium could potentially lead to a decrease in vascular events.

Cocoa and chocolate have recently been found to be rich plant-derived sources of antioxidant flavonoids with beneficial cardiovascular properties. These favorable physiological effects include: antioxidant activity, vasodilation and blood pressure reduction, inhibition of platelet activity, and decreased inflammation. Increasing evidence from experimental and clinical studies using cocoa-derived products and chocolate suggest an important role for these high-flavanol-containing foods in heart and vascular protection[56].

Atherosclerosis

Atherosclerosis is a chronic pathology involving the deposition of plasma lipoproteins and the proliferation of cellular components in the artery wall that provide a barrier to arterial blood flow. A relevant evidence has been supported the theory that free-radical-mediated oxidative processes and specific products arising therefore play a key role in atherogenesis[57]. At the base of this hypothesis are low-density lipoproteins (LDL), which as part of the normal circulation, occasionally leave the anti-oxidant-replete plasma, entering the subendothelial space of arteries: here LDL lipids are oxidized. The oxidized form of LDL (oxLDL) is able of beginning mechanisms leading to the formation of atherosclerotic lesions: is taken up by macrophages and induces the release of factors that recruit other cells and stimulate smooth muscle cell proliferation. oxLDL may also up-regulate expression of cellular adhesion molecules that facilitate leukocyte binding. High levels of oxLDL can also down-regulate the expression of endothelial nitric oxide synthase (eNOS), enzyme synthetizing most of vascular NO.

Oxidative modification of LDL caused by ROS triggers to initiate endothelial inflammation which is the initial lesion of atherogenesis leading to atherosclerosis and vascular thrombosis. Moreover, oxidative mechanisms are proposed to play a role in lesions maturation and degeneration , causing heart attack and stroke. The oxidation theory is supported by the presence of oxLDL within atherosclerotic lesions and the correlation between the sensitivity of LDL to oxidation and risk of CVD.

Moreover, LDL oxidation can be inhibited by nutritional anti-oxidants. Several epidemiological evidences and interventional studies correlate higher level of anti-oxidant-rich food uptake with lower incidence of CHD (Coronary Heart Disease)[58].

LDL is protected from oxidation by antioxidants, as well as by a second line of defense-paraoxonase 1 (PON1), which is a high-density lipoprotein-associated esterase that can hydrolyze and reduce lipid peroxides in lipoproteins and in arterial cells. Cellular paraoxonases (PON2 and PON3) may also play a relevant protective role against oxidative stress at the cellular level. Many epidemiological studies have indicated a protective role for a diet rich in fruits and vegetables against the development and progression of cardiovascular disease. Basic research provides plausible mechanisms by which dietary antioxidants might reduce the development of atherosclerosis. These mechanisms include inhibition of LDL oxidation, inhibition of cellular lipid peroxidation and consequently attenuation of cell-mediated oxidation of LDL. An additional possible mechanism is preservation/increment of paraoxonases activity by dietary antioxidants (vitamin E, carotenoids and polyphenolic flavonoids)[59].

Evidence is now emerging that some dietary "antioxidants" influence signaling pathways and the expression of genes relevant in atherosclerosis by mechanisms other than antioxidative ones. By concrete examples Brigelius-Flohe et al[60] show that (1) vitamin E has gene regulatory functions which might be more important than acting as an antioxidant in vivo, (2) selenium itself is not an antioxidant at all, and even not in general when incorporated into glutathione peroxidases, and (3) a moderate oxidative stress is beneficial rather than detrimental since it can induce defense mechanisms counteracting xenobiotic and oxidative stress.

Consumption of soy protein is associated with a lower risk of cardiovascular disease in man, and reduced atherosclerosis in a variety of experimental animals. Although a portion of the cardiovascular protective effects appears to be due to reductions in plasma lipoprotein concentration, in most people the magnitude of this effect is relatively small. In many, but not all studies using animal models, the reduction in atherosclerosis is in part independent of changes in plasma lipids and lipoproteins. This implies that there may be a direct effect on the arterial wall of one or more of the components in soyprotein that reduces susceptibility to atherosclerosis. The most actively studied components of soy protein that may be responsible for these anti-atherogenic effects are the isoflavones and various protein fractions. Extraction of isoflavones and other alcohol-soluble components from soy protein lowers, but does not eliminate its ability to reduce atherosclerosis. Surprisingly, in most studies, adding back the isoflavone-rich alcohol extract to the previously extracted soy protein, or to another protein, does not restore its lipoprotein lowering or anti-atherogenic properties. This implies that alcohol extraction either destroys an active component of soy, alters the structural integrity of the soy proteins, or disassociates a required isoflavone-soy protein complex. The sites of action on the arterial wall, and the mechanisms by which various soy components act to reduce atherosclerosis are just now being studied. The recent demonstration that expression of estrogen receptor alpha is required for atheroprotection by soy protein provides important new mechanistic insight. Other properties of soy, including antioxidant, anti-inflammatory and potentially antithrombogenic properties need to be explored more mechanistically before the full potential of dietary soy protein for the protection from cardiovascular disease will be known[61].

Leptin, a 167-amino acid peptide hormone produced by white adipose tissue, is primarily involved in the regulation of food intake and energy expenditure. Leptin receptors are expressed in many tissues including the cardiovascular system. Recent studies suggest that hyperleptinemia may play a relevant role in obesity-associated cardiovascular diseases including atherosclerosis. Leptin exerts many potentially atherogenic effects such as induction of endothelial dysfunction, stimulation of inflammatory reaction, oxidative stress, decrease in paraoxonase activity, platelet aggregation, migration, hypertrophy and proliferation of vascular smooth muscle cells. Leptin-deficient and leptin receptor-deficient mice are protected from arterial thrombosis and neointimal hyperplasia in response to arterial wall injury. Several clinical studies have demonstrated that high leptin level predicts acute cardiovascular events, restenosis after coronary angioplasty, and cerebral stroke independently of traditional risk factors. In addition, plasma leptin correlates with markers of subclinical atherosclerosis such as carotid artery intima-media thickness and coronary artery calcifications. Inhibition

of leptin signaling may be a promising strategy to slow the progression of atherosclerosis in hyperleptinemic obese subjects[62].

Treatment of hypertension (HT) can reduce the risk for cardiovascular diseases. Tomato extract contains carotenoids such as lycopene, beta carotene, and vitamin E, which are known as effective antioxidants, to inactivate free radicals and to slow the progression of atherosclerosis. Engelhard et al[63] wanted to evaluate the effect of tomato extract on systolic and diastolic blood pressure in grade-1 HT, on serum lipoproteins, plasma homocysteine, and oxidative stress markers. Their study is a single-blind, placebo-controlled trial. Thirty-one subject with grade-1 HT, without concomitant diseases, who required no anti-hypertensive or lipid-lowering drug therapy, who were recruited from primary care clinics, completed the trial. Subjects entered a 4-week placebo period, then an 8-week treatment period with tomato extract, 250 mg Lyc-O-Mato, and a 4-week control period with placebo. Systolic blood pressure decreased from 144 (SE ± 1.1) to 134 mm Hg (SE ± 2, $p < .001$), and diastolic blood pressure decreased from 87.4 (SE ± 1.2) to 83.4 mm Hg (SE ± 1.2, $p < .05$). No changes in blood pressure were demonstrated during placebo periods. Thiobarbituric acid-reactive substances (TBARS), a lipid peroxidation products marker, decreased from 4.58 (SE ± 0.27) to 3.81 nmol/mg (SE ± 0.32, $p < .05$). No significant changes were found in lipid parameters. A short-term treatment with antioxidant-rich tomato extract can reduce blood pressure in patients with grade-1 HT, naive to drug therapy.

Hatzigeorgiou et al[64] studied 865 consecutive patients, 39-45 years of age, without known coronary artery disease and presenting for a periodic physical examination. Antioxidant intake was assessed with the Block Dietary Questionnaire, and coronary atherosclerosis was identified by measuring coronary artery calcification using electron beam computed tomography. The mean age was 42 (± 2), 83% were male, and the prevalence of coronary artery calcification was 20%. Vitamin supplements were used by 56% of the participants, and the mean (± SD) daily intake (dietary plus supplemental) of vitamins A, C, and E were 1683 mg (± 1245), 371 mg (± 375), and 97 mg (± 165), respectively. There was no significant correlation between coronary artery calcification score and individual vitamin or total antioxidant vitamin intake, even after adjusting for traditional cardiac risk factors. The highest quartile of vitamin E was positively associated with calcification (odds ratio = 1.77; 95% confidence interval, 1.02-3.06). Antioxidant vitamin intake is not significantly related to coronary artery calcification, implying that there is no effect on the development of early coronary atherosclerosis. High doses of vitamin E may confer an increased risk of calcified atherosclerosis.

Stroke/Ischemia-Reperfusion (I-R)

Stroke is the main cause of disability and mortality in Western countries. Particularly the condition of ischemia and reperfusion occurring after stroke has been shown to be associated with free radical-mediated reactions probably leading to cell death[65]. Even if ischemic and haemorrhagic stroke have different risk factors and pathophysiological mechanisms, there is evidence of an increased production of free radicals and other reactive species in both conditions, leading to oxidative stress. Ischemic stroke is the result of the interruption or severe reduction of blood flow into brain arteries followed by physiological and metabolic changes that occur few seconds after the interruption of blood flow. When anoxia is followed subsequently by reperfusion, tissue can be saved but reperfusion might probably have harmful consequences: on reoxygeneration, OS is rapidly developed and numerous non-enzymatic oxidation reactions occurs both in the cytoplasm and/or in cellular organelles. Independently of the processes responsible for ischemic stroke, ischemia actives a cascade of events that can increase free radical generation through several different pathways, including inflammatory cells, xanthine oxidase, ciclooxygenase and mitochondria. Moreover the large increases in glutamate and aspartate that follow ischemia may cause free radical production by excitotoxic mecahnisms. During ischemia and reperfusion the modified function of the mitochondrial electron transport chain is a likely source of OS. Upon reperfusion, the accumulation of bloodborne inflammatory cells, such as neutrophils and monocytes/macrophages might further activate oxidative stress. Consequently, a great amount of O_2-derived free radicals appears during the first minute of reperfusion and peaks some 4 to 7 minutes after the begin of reperfusion. In ischemic tisues, a lot of researches have established a direct role for ROS in oxidative damage to lipids, proteins and nucleic acids.

To assess the effect of vitamin C-in conjunction to aspirin-in ischemic stroke-related lipid

peroxidation, Polidori et al[66] measured plasma levels of ascorbate, of 8,12-isoprostanes F2alpha-VI (8,12-iPF2alpha-VI) and activities and levels of a broad spectrum of antioxidant enzymes and micronutrients in stroke patients randomized to receive, from stroke onset and up to three months, either vitamin C (200 mg/day) plus aspirin (300 mg/day) or only aspirin (300 mg/day). By the end of the first week, patients treated with vitamin C plus aspirin had higher vitamin C levels ($p = 0.02$) and lower 8,12-iPF2alpha-VI levels ($p = 0.01$) than patients treated with aspirin alone. The significance was maintained for the increase of vitamin C after three months of therapy ($p < 0.01$). The clinical functional outcome for both groups of patients similarly ameliorated after three months of treatment. These autors conclude that vitamin C, at the dose of 200 mg/day and in conjunction with aspirin, significantly decreases ischemic stroke-related lipid peroxidation in humans. Further studies are warranted to clarify whether the use of vitamin C may add clinical long-term beneficial effects in patients with stroke.

Bailey et al[67] have developed a clinical study to evaluate the effects of ascorbate prophylaxis on ROS exchange kinetics in 22 patients scheduled for elective abdominal aortic aneurysm (AAA) or infra-inguinal bypass (IIB) repair. Complex surgery involving AAA and IIB repair requires obbligatory I-R which has been associated with an increase in the circulating concentration of ROS[68] and, thus, I-R injury is considered the most important cause of the systemic inflammatory response syndrome that can, at last, lead to a multiple organ dysfunction[69].

Clinical studies have therefore focused on some antioxidant strategies to scavenge ROS with the only aim of improving functional recovery following surgical revascularization[70]. Ascorbic acid seems to be of crucial importance since its depletion during surgical I-R has been demonstrated in peripheral blood[71-73] and local samples taken from the coronary sinus[74], evidence for increased ROS production.

The benefits of vitamin C prophylaxis remain equivocal with human studies demonstrating an improvement[75], no change[76] or increase in ROS and subsequent deterioration in vascular function[77-79].

Measurement of peripheral arterial and local coronary sinus bloods during cardiopulmonary bypass (CPB) surgery indicated a net transcardiac release of TBARS and depletion of peripheral ascorbate pool as evidenced by a decrease in the EPR signal intensity of the dymethyl sulfoxide-supplemented A^- during reperfusion. Prevoius CPB studies have also demonstrated increased ascorbate consumption as early as the ischemic phase of surgery and independent of perioperative changes in blood volume and differences in the partial pressure of oxygen (PO_2). These well-controlled studies identified that oxidative stress is not simply exclusive to reperfusion with important redox-reactive events initiated during the ischemic phase of surgery. They also confirm the critical importance of ascorbate as a major plasma antioxidant during human surgical I-R.

In Bailey' s study, patients were assigned double-blind to receive intravenous sodium ascorbate (2 g vitamin C, n = 10) or placebo (0.9% saline, n = 12) administered 2 h prior to surgery. Blood samples were obtained from the arterial and venous circulation proximal to the respective sites of surgical repair (local) and from an antecubital vein (peripheral) during cross-clamping (ischemia) and within 60 s of clamp release (reperfusion). Ascorbate supplementation increased the venoarterial concentration difference (v-adiff) of lipid hydroperoxides (LH), interleukin (IL)-6 and vascular endothelial growth factor (VEGF) protein during ischemia. This increased the peripheral concentration of LH, total creatine phosphokinase (CPK), and VEGF protein during reperfusion ($p < 0.05$ vs placebo). Electron paramagnetic resonance (EPR) spectroscopy confirmed that free iron was available for oxidative catalysis in the local ischemic venous blood of supplemented patients. An increased concentration of the ascorbate radical (A.-) and alpha-phenyl-tert-butylnitrone (PBN) adducts assigned as lipid-derived alkoxyl (LO.) and alkyl (LC.) species were also detected in the peripheral blood of supplemented patients during reperfusion ($p < 0.05$ vs ischemia). In conclusion, these findings suggest that ascorbate prophylaxis may have promoted iron-induced oxidative lipid damage via a Fenton-type reaction initiated during the ischemic phase of surgery. The subsequent release of LH into the systemic circulation may have catalyzed formation of second-generation radicals implicated in the regulation of vascular permeability and angiogenesis.

In women, cardiovascular disease is highly prevalent and is costly in terms of mortality and morbidity. Women should routinely consume a diet known to prevent coronary heart disease,

stroke, and other cardiovascular risk factors. Essential aspects of good nutrition for women include diets rich in fiber, whole grains, fresh fruits, vegetables, fish, nuts, antioxidants, minerals, vegetable protein, marine and plant omega-3 fatty acids, and vitamins of the B group. Some foods known to provide cardiac protection in women, such as potatoes and citrus fruit juices, may not be cardioprotective in men. Thus, it is important to continue research efforts in women to determine the best diet for cardiovascular health[80].

Based on the above cited rationale, clinical trials evaluating the efficacy of anti-oxidant treatment or of antioxidant-rich nutritional intervention in stroke are in progress. And these should always include the measurement of biomarkers of oxidative stress to define their capacity for predicting long-term clinical outcome and therapeutic response.

Obesity

Although obesity is related to traditional CVD risk factors, relatively little is known regarding the relationship between obesity and OS. Associations between obesity and markers of oxidative stress and the sensitivity of lipid to oxidative modification have been observed in humans[81,82]. The processes that underlie observed associations between obesity and oxidative stress are unclear, even if several theroy have been proposed. For example, it has been suggested that oxidative stress in obesity may result, partly, from the accumulation of intracellular triglycerides[83]. Specifically, intracellular triglycerides are supposed to elevate superoxide radical generation within the electron transport chain by inhibiting the mitochondrial adenosine nucleotide transporte. The inhibition of this transporter leads to a diminuish in intra-mitochondrial adenosine diphosphate (ADP) that, in turn, reduces the protons's flux trough the adenosine triphosphate-synthase reaction (i.e., the adenosine triphosphate-synthase reaction requires ADP as substrate). As a result, electrons build up within the electron transport chain, which can then reduce O_2 to form O_2-. Another hypothetical source of increased oxidative stress may be the presence of excessive adiposity *per se* because adipocytes and preadipocytes have been identified as sources of inflammatory citokines[84]. Citokines are potent stimuli fro the generation of ROS/RNS by macrophages and monocytes. Particularly, cytokines up-regulate the activity of oxidant generating enzymes, including NAD(P)H-oxidase, inducible NOS and myeloperoxidase.

If the storage of intracellular triglycerides or fat tissue promotes oxidative stress, then reduction of total body fat through diet and/or exercise may be an effective means of reducing systemic inflammation and OS. Consistent with this hypothesis, reductions in plasma markers of oxidative stress and in ROS production by isolated leukocytes have been observed after 4 weeks of energy restriction and weight loss[85]. However, the evidence is far from conclusive and the independent contributions of energy restriction and fat loss *per se* remain unclear.

Since chronic hyperglycemia is more prevalent in obese individuals, oxidative stress is also believed to play a relevant role in the development of obesity-related disorders including diabetes and hypertension, even if the cause and effect relationship are not always clear.

Crujeiras et al[86] want to estimate the ability of two hypocaloric diets with different fruit contents to improve antioxidant biomarkers related to lipid peroxidation in obese women. Fifteen obese women (age 32 ± 6 y, body mass index 34.9 ± 2.9 kg/m^2) were assigned to two different dietary treatments for 8 wk. The subjects received a hypocaloric diet (600 kcal/d restriction from the measured individual energy expenditure) containing 5% (n = 8) or 15% (n = 7) energy supplied by fructose from fruits. Anthropometric measurements, blood lipid profile, plasma oxidative markers, total antioxidant capacity, and malondialdehyde (MDA) were evaluated before and after the nutritional intervention in addition to some relations among them. No differences in weight loss were observed between diets (5% energy from fructose in the low fruit diet –6.9 ± 2% versus 15% energy from fructose in the high fruit diet –6.6 ± 2%; $p = 0.781$). Low-density lipoprotein cholesterol levels significantly decreased ($p = 0.048$) in obese women who followed the high fruit diet, which was accompanied by a statistical ($p = 0.046$) diet-related decrease (–30%) in the ratio of MDA to antioxidant capacity. There was a positive association between MDA diet-related change and low-density lipoprotein cholesterol ($r = 0.665$, $p = 0.003$), with antioxidant capacity directly proportional to the fiber plus fructose content associated with fruit consumption ($r = 0.697$, $p = 0.025$). A fruit-enriched hypocaloric diet appears to be more effective against oxidative stress. Consumption of antioxidant substances contained in fruit could be a useful strategy in the

design of hypocaloric diets that, with the weight reduction, could increase the improvement of cardiovascular risk factors related to obesity.

The effects of tea on obesity and diabetes have received increasing attention. Tea catechins, particularly (-)-epigallocatechin gallate (EGCG), appear to have antiobesity and antidiabetic effects. While few epidemiological and clinical studies show the health benefits of EGCG on obesity and diabetes, the mechanisms of its actions are emerging based on the various laboratory data. These processes may be related to various pathways, such as through the modulations of energy balance, endocrine systems, food intake, lipid and carbohydrate metabolism, the redox status, and activities of different types of cells (i.e., fat, liver, muscle, and beta-pancreatic cells)[87].

Diabetes

It has been suggested that in diabetes the production of oxidative stress may be mostly due to hyperglycaemia[88,89]. The generation of free radicals correlated to chronic hyperglycaemia may result from non-enzymatic glycation[90] and glucose auto-oxidation[91].

It has been suggested that insulin, at least hyperinsulinaemia, may directly induce intracellular generation of free radicals[92].

Ceriello et al[93] wanted to evaluate the effects of an acute increase in glycaemia on plasma antioxidant defences.

Some studies have reported that the use of anti-oxidants can neutralize some effects acutely induced by hyperglicaemia, such as vasoconstriction[94,95], activation of coagulation[96] and the increase in ICAM-1 (intercellular adhesion molecule 1) plasma level[97].

In this study, during the oral glucose tolerance test (OGTT), plasma concentration of protein-bound sulphydryl (SH) groups, vitamin C, vitamin E and uric acid significantly decreased in normal as well as non-insulin-dependent diabetes mellitus (NIDDM) subjects. The oxidative destruction of essential sulphydryl groups of proteins in the presence of oxidative stress has been riported[98]. Sulphydryl groups are the most relevant source of anti-oxidants in plasma and are preferentially utilized when exposing to free radicals[99]. Ascorbic acid is one of the most relevant antioxidant defences in human plasma[100]. Virtually every water-soluble free radical can be scavenged by ascorbate and its capacity to regenerate the lipid-soluble antioxidant vitamin E is an additive evidence of its important antioxidant role in blood. Uric acid has preventive antioxidant activity and stabilize vitamin C in human plasma. All the water-soluble antioxidants seem to regenerate or spare the major lipid-soluble antioxidant, vitamin E. In Ceriello' experience, the first line of defence against oxidative damage seems to be provided by the plasma sulphydryl groups and ascorbic acid, uric acid and α-tocopherol being spared during the initial phases of the reaction and used thereafter. A prevoius study of a negative correlation between urate and post-prandial glucose[101] supports these findings.

Total plasma radical-trapping activity, which evaluates plasma antioxidant capacity due to known and unknown antioxidants present in the plasma as well as their mutual co-operation, was also significantly reduced. This finding supports the hypothesis that hyperglycaemia may, even acutely, induce an oxidative stress.

Even lipid alterations[102] have been hypothesized as contributory factors to oxidative stress production in diabetes.

The life of type 2 diabetic patients is marked by very frequent rapid rise, especially in the postprandial phase, in their blood glucose, insulin and lipid (principally triglyceride) levels. If blood glucose and/or insulin and/or lipid levels are relevant determinants of OS in diabetes, then their postprandial increases should play a major role in the generation of OS. To verify this theory, the same Authors[103], in another study, administered a standardized meal to 10 type 2 diabetic patients and 10 healthy matched normal subjects to estimate its effects on plasma OS production. In diabetic patients, at baseline and after the meal, plasma malondialdehyde (MDA), vitamin C, protein SH groups, uric acid, vitamin E, and total plasma radical-trapping parameter (TRAP), which evaluates plasma antioxidant capacity due to known and unknown antioxidants present in the plasma as well as their mutual cooperation, were measured. After the meal, plasma MDA and vitamin C increased, while protein SH groups, uric acid, vitamin E, and total plasma radical-trapping parameter decreased more significantly in the diabetic subjects than in control subjects. Thus, it seems that meals in diabetic patients induce an OS that leads on one side to increased generation of lipid peroxides and on the other to consumption of antioxidant capacity.

In this study, only one antioxidant behaved differently, i.e., ascorbic acid. This standardized meal contained 100 mg of ascorbate, while other antioxidants were not so abundant. This may ex-

plain the post-prandial increase in vitamin C. The TRAP decrease might have been greater in absence of ascorbic acid supplementation. However, available data suggest that ascorbate contributes only ~5% to the total antioxidant power of plasma. On the other hand, another explanation could be that ascorbic acid circulates only in aqueous compartments, while the target of OS measured in this study is essentially related to lipid compartments.

In this study, there was no difference in the after-meal insulin variation between normal and diabetic subjects (the latter, being type 2, were insulin-resistance), so that insulin does not appear to play a causative role in the postprandial OS production.

Lipids, particularly triglycerides, may also contribute to meal-associated OS in diabetes. This theory is supported by the different after-meal profile of tryglycerides in diabetic and normal subjects and is convincingly confirmed by the amount of MDA produced. However, it should be kept in mind that tryglyceride generation itself is closely dependent on plasma glucose level[104].

This finding shows that in the absorptive phase, free radicals are produced in diabetic patients.

Moreover, the possibility that an acute rise of glycemia is accompained by an increase of free radical generation is a well-known condition[105]).

Because OS seems to be involved in the pathogenesis of diabetic complications, evidence that is generated during the postprandial phase may contribute to the debate on the managment of diabetes by underscoring the importance of controlling postprandial glycemic spikes, additionally to mean blood glucose concentrations as described by glicated hemoglobin.

When endothelial cells are exposed to high glucose, RNS and ROS production occurs[106]. In endothelial cells, intracellular hyperglycemia induces overproduction of the radical superoxide (O_2-) at the mitochondrial level. Overproduction of superoxide is thought to be the first and key process in the action of all other pathways involved in the pathogenesis of diabetic complications. It has been shown also a simultaneous increase in the production of nitric oxide (NO) (107), that is a particularly harmful because NO and O_2- react to produce peroxynitrite ($ONOO^-$), a potent oxidant with a long time emivita. The peroxynitrite action is cytotoxic because it inhibits mitochondrial electron transport, oxidizes sulphydryl groups in protein, initiates lipid peroxidation without requirement of transition metals, and nitrates aminoacids such as tyrosine, which in turn affect many signal transduction pathways. Chronic hyperglycemia promotes non-enzymatic glycation of proteins and glycated proteins can increase oxidant generation by activating phagocytes or by directly releasing O_2- and hydrogen peroxide. Additionally, advanced glycation endproducts stimulate oxidant production through specific interactions with receptors present on vascular cells.

Type 2 diabetes (T2DM) is an increasing disease resulting from the interaction between genetic predisposition and lifestyle. In genetically predisposed subjects, the combination of excess caloric intake and reduced physical activity induces a state of insulin resistance. When beta cells are no longer able to compensate for insulin resistance by adequately increasing insulin production, impaired glucose tolerance appears (IGT), characterized by excessive postprandial hyperglycemia[108]. This condition is characterized by an excessive blood glucose level in the postprandial phase with fasting glucose being in the normal range. Impaired glucose tolerance may evolve into overt diabetes, characterized by high glicemia in any condition whether fasting or postprandial. These 3 conditions, ie, insulin resistance, impaired glucose tolerance, and overt diabetes, are associated with an increased risk of cardiovascular disease[109,110]. Because all these conditions are also accompanied by the presence of an oxidative stress[111-113], OS may be the pathogenic mechanism linking insulin resistance with dysfunction of both β-cells and endothelium, eventually leading to overt diabetes and cardiovascular disease.

The most important tissues involved in the pathogenesis of insulin resistance are muscle and adipose tissue. When caloric intake exceeds the energy expenditure, the substrate-induced increase in Krebs cycle activity produces an excess of mitochondrial NADH (mNADH) and ROS[114]. Prevention of ROS formation occurred by preventing the build-up of mNADH by inhibiting insulin-stimulated nutrient uptake and preventing the entrance of energic substrates (pyruvic acid, fatty acids) into the mitochondria. An influx of substrates into the citric acid cycle produces mitochondrial acetyl-CoA and NADH. The production of excessive NADH may be avoided in some ways, one of which is the inhibition of FFA (free fatty acids) oxidation[115]. An increase in intracel-

lular FFA, in turn, leads to reduced GLUT4 translocation to the plasma membrane, resulting in resistance to insulin-stimulated glucose intake in muscle and adipose tissue[116-118]. In this setting, insulin resistance may be considered a compensatory mechanism that protects the cells against further insulin-stimulated glucose and fatty acid uptake and therefore oxidative damage.

Many studies support this theories: *in vitro* and in animal models, antioxidants have been shown to improve insulin sensibility[119]. Some clinical trials have demonstrated that treatment with vitamin E, C or glutathione improves insulin sensibility in insulin-resistance. The finding that insulin resistance is associated in humans with reduced intracellular antioxidant defense also support this theories[120].

Available evidence leads to the theories that OS can be considered the clue to the association of overnutrition with the development of diabetes. It may also link the progressive β-cell failure to an increased cardiovascular risk, a relevant association in the clinical setting. This hypothesis, moreover, may also contribute to explaining why treating cardiovascular risk with drugs, such as calcium channel blockers (CCBs), ACE inhibitors, AT-1 receptor antagonists, and statins, all compounds showing intracellular preventive antioxidant activity, results in the onset of new cases of diabetes possibly being reduced[121]. If OS is the pathogenic mechanism leading from insulin resistance to overt diabetes, a drug capacity to prevent or reverse OS can account for its clinical usefulness[122-126]. Furthermore, the beneficial effect of controlling postprandial hyperglycemia on both the development of diabetes[127] and the prevention of cardiovascular disease[128] also support this theories, because it has been shown that in the postprandial state there is an OS production, which is closely dependent on the level of glycaemia reached[129].

However, even convincing evidence is now available supporting the hypothesis that OS may play a relevant role in the development of both diabetes and CVD clinical trials with antioxidants, particularly with vitamin E, have failed to demonstrate any beneficial effect[130]. It has been suggested that antioxidant therapy with vitamin E or other antioxidants is limited to scavenging already formed oxidants and may, therefore, be considered a more "symptomatic" rather than a causal treatment for OS[131].

Boshtam et al[132] have developed a triple-blind, placebo-controlled clinical trial to determine the effect of the vitamin E on fasting blood sugar (FBS), serum insulin, and glycated hemoglobin (GHb) in type 11 diabetic patients (NIDDM). A total of 100 patients, with no complications, aged 20-60 years old were chosen from those consulting the Isfahan Social Security Service Diabetes Clinic and divided randomly into two treated and placebo groups, and matched for age, sex, level of education, and occupation. The treated and placebo groups were given vitamin E tablets (200 IU/day) and placebo respectively. Serum vitamin E, total cholesterol (TC), triglycerides (TG), FBS, insulin, and GHb were measured at the beginning and at the end of the study (a period of 27 weeks); FBS, GHb and insulin levels were also determined several times during the period. Blood lipids and FBS were measured using the ELAN 2000 autoanalyzer at the Isfahan Cardiovascular Research Center, while for measuring insulin the enzyme-linked immunosorbent assay (ELISA) method was used; GHb was determined calorimetrically (thiobarbituric acid), and for vitamin E measurements the Hansen and Warwick method was used, by which the vitamin E was determined fluorometrically. The findings of this study show no effect of vitamin E supplementation in the patients: GHb did not change appreciably, FBS was reduced nonsignificantly (–4.3% in the treated group vs. –14.0% in the placebo group, $p < 0.05$). In the case of insulin, no increase was seen; instead, a decrease was observed (slightly more than 17% in the two groups, $p = 0.15$). No changes were observed in the levels of blood lipids. It was concluded that a daily vitamin E supplement of 200 IU for a period of 27 weeks does not affect insulin, GHb, or FBS in type II diabetic patients. However, since this antioxidant vitamin is beneficial in other ways in these patients, it would seem justified to recommend its use. Certainly, more extensive research is necessary to draw definite conclusions.

Neurodegenerative Disorders

OS has been implicated as a common pathogenetic mechanism in various neurodegenerative diseases. Central nervous system (CNS) is particularly exposed to free radical injury, given its high metal content, which can catalyze the formation of ROS and the relatively low content of anti-oxidant defences. Indeed, a lot of studies show markers of oxidative damage. Lipid peroxidation, protein oxidation, DNA oxidation and glycoxidation markers in brain areas affected by neurodegenerative diseases. OS damage is also

intimately correlated to glutamate neurotoxicity, known as "excitotoxicity". An excessive concentration of extracellular glutamate over-activates glutamate receptors, leading to very high intracellular calcium levels and a cascade of events resulting in neural cell death[133].

Alzheimer Disease (AD)

AD represents the most prominent cause of dementia in the elderly and is clinically characterised by memory disfunction, loss of lexical access, spatial and temporal disorientation and impairment of judgement. Histopathologically, AD is charcterised by synaptic and nerve cell loss, extracellular deposition of β-amyloid protein forming senile plaques and intracellular precipitation of tau protein. The exact biochemical mechanism of the pathogenesis of AD is still unknown, but much attention is given to the role of the massive loss of the neurotransmitter acetylcholine (necessary for cognition and memory) and to the possible involvement of OS in its development[134].

The complex nature and genesis of oxidative damage in AD can be partly due to mitochondrial and redox-active metal anomalies. AD is essentially an acceleration of the ageing mechanism in affected brain regions that become progressively more damaged by free radicals. In the initial stage of pathology development, β-amyloid deposition and protein tau precipitation may function as compensatory responses and downstream adaptations to ensure that neuronal cells don't succumb to oxidative injuries. However, during the progession of the disease, the anti-oxidant activity of both β-amyloid and tau evolves into prooxidant activity representing a typical gain-of-function transformation, which can result from an increase in reactive species and a decrease in clearance mechanisms.

Agents that show promise in helping prevent AD include: (1) aged garlic extract, (2) curcumin, (3) melatonin, (4) resveratrol, (5) Ginkgo biloba extract, (6) green tea, (7) vitamin C, and (8) vitamin E. While the clinical value of antioxidants for the prevention of AD is often ambiguous, some can be recommended based upon: (1) epidemiological evidence, (2) known benefits for prevention of other dieases, and (3) benign nature of the substance. Long-term, prospective studies are recommended[135].

Five vegetables traditionally consumed among South-Asian migrants in Bradford (Yorkshire, UK) were tested for their free radical scavenging activity (FRSA) in the DPPH (1,1-diphenyl-2-picrylhydrazil radical) screening assay (using extracts prepared both by cold maceration and also by boiling the plant in the solvent under reflux) and for their in vitro non-enzymatic inhibition of bovine brain lipid peroxidation. In both antioxidant assays a strong activity was shown by extracts derived from okra (Abelmoschus esculentus, Malvaceae) fruits and charungli (Caralluma edulis, Asclepiadaceae) aerial parts. Extracts from bitter melon (Momordica charantia, Cucurbitaceae) and angular loofah (Luffa acutangula) showed a significant difference in the FRSA between the extract obtained by using cold maceration and that prepared by boiling the plant in the solvent under reflux, suggesting the chemical composition of the plant changed during the heating process, leading to an increase in the amount of antioxidant components. These findings confirm the great interest of the nutraceutical sciences in extracts of Caralluma edulis, whose phytochemistry and phytopharmacology should be investigated further in order to detect possible phytotherapeutic uses in the prevention of ageing related diseases and Alzheimer disease[136].

Several epidemiological studies indicate that moderate consumption of wine is associated with a lower incidence of Alzheimer's disease. Wine is enriched in antioxidant compounds with potential neuroprotective activities. However, the exact molecular mechanisms involved in the beneficial effects of wine intake on the neurodegenerative process in Alzheimer's disease brain remain to be clearly defined. Marmbaud et al[137] showed that resveratrol (trans-3,4',5-trihydroxystilbene), a naturally occurring polyphenol mainly found in grapes and red wine, markedly lowers the levels of secreted and intracellular amyloid-beta (Aβ) peptides produced from different cell lines. Resveratrol does not inhibit Aβ production, because it has no effect on the Aβ – producing enzymes beta – and gamma-secretases, but promotes instead intracellular degradation of Aβ via a mechanism that involves the proteasome. Indeed, the resveratrol-induced decrease of Aβ could be prevented by several selective proteasome inhibitors and by siRNA-directed silencing of the proteasome subunit beta[5]. These findings demonstrate a proteasome-dependent anti-amyloidogenic activity of resveratrol and suggest that this natural compound has a therapeutic potential in Alzheimer's disease.

The anti-oxidating vitamins C and E could therefore have a beneficial effect and reduce the damage caused by beta-amyloid. Several observational studies in mostly healthy, elderly individuals have indicated that vitamin C and E, mainly from food as well as the combination of high doses of the same vitamins, may have beneficial effect on the development of Alzheimer dementia. One clinical controlled trial in patients with manifest Alzheimer dementia, in which vitamin E 2000 mg/day was given as the only vitamin, has to a certain extent confirmed these results. A causal relationship between intake of the vitamins and Alzheimer dementia has not been clarified. The correct dosages are not known, but a diet rich in these vitamin could probably reduce the risk of dementia. With a high intake of vitamin E, the addition of vitamin C is necessary[138].

Parkinson Disease (PD)

PD is clinically characterised by bradykinesia, postural inability, gait difficulty and tremor. It's the result of neurodegeneration occurring in specific brain areas (*substantia nigra* and *striatum*) and of dopamine depletion. The processes of cell death in PD have not yet been fully elucidated, but increased OS, abnormal mitochondrial function and excitotoxicity are perhaps among the most relevant initiators or mediators of neuronal damage.

The evidence of an involvement of free radicals in PD comes from the observation that oxidation of dopamine yields potentially toxic compounds, called semiquinones, and the accelerated metabolism of dopamine may induce an excessive formation of ROS. Infact, PD was found to be associated with increased oxidative damage to DNA[139] and also lipid peroxidation resulted increased[140]. Other signs are elevated iron brain levels and reduced levels of ferritin[141].

Twenty-six metals and the oxidative status in 71 patients affected by Parkinson's disease and 44 healthy individuals were compared in order to identify potential biomarkers of the disease. In the patients, the following significant imbalances were found ($p <$ or $= 0.05$): (1) in serum, an increment of Ca, Mg, Ni, Si and V, and a decrement of Cd, Co, Fe, Li, Sn, Zn and Zr; (2) in blood, raised levels of Co, Li, Ni and Si and decreased of Al, Be, Ca, Cd, Fe, Mg, Mo, Sn, Zn and Zr; (3) increased formation of oxidant species and lowered anti-oxidant capacity ($p <$ or $= 0.001$ for both). Barium, Bi, Cr, Cu, Hg, Mn, Pb, Sb, Sr, Tl and W did not change with the disease. The best discriminating variables between patients and controls were Cd, Co, Fe, Ni and Si in serum (91.2% of cases correctly classified), and Al, Cd, Co, Fe, Mo and Si in blood (98.2% of cases properly classified)[142].

Mitochondrial dysfunction has been well established to occur in PD and appears to play a role in the pathogenesis of the disorder. A key component of the mitochondrial electron transport chain (ETC) is coenzyme Q(10), which not only serves as the electron acceptor for complexes I and II of the ETC but is also an antioxidant. In addition to being crucial to the bioenergetics of the cell, mitochondria play a central role in apoptotic cell death through a number of mechanisms, and coenzyme Q(10) can affect certain of these processes. Levels of coenzyme Q(10) have been reported to be decreased in blood and platelet mitochondria from PD patients. A number of preclinical studies in vitro and in vivo models of PD have demonstrated that coenzyme Q(10) can protect the nigrostriatal dopaminergic system. A phase II trial of coenzyme Q(10) in patients with early, untreated PD demonstrated a positive trend for coenzyme Q(10) to slow progressive disability that occurs in PD[143].

Amyotrophic Lateral Sclerosis (ALS)

ALS o Lou Gehrig's disease is a progressive, fatal neurodegenerative disease chracterized by gradual degeneration of motor meurons in the cortex, brainstem and spinal cord. Motor neurons are responsible for supplying electrical stimulation to the muscles necessary for the movement of body parts. The cause of sporadic ALS is unknown; however, in about 10% of all ALS cases, the disease is inherited and familiar (FALS). About 20% of FALS cases are associated with mutations and lowered activity of CuZnSOD. CuZnSOD catalyzed the formation of hydrogen peroxide through the dismutation of superoxide radical anions playing a relevant role in regulating oxidative damage to cells. There is evidences of oxidative damage to both DNA and protein as well as lipids.

Free radical accumulation and oxidative stress have been proposed as contributing to the progression of amyotrophic lateral sclerosis (or motor neuron disease). A range of antioxidant medications are available and have been studied. From the literature, in the individual studies no significant effect was observed of vitamin E 500 mg twice daily; acetylcysteine 50 mg/kg daily subcutaneous infusion; or a combination of L-

methionine 2 g, vitamin E 400 International Units, and selenium 3 × 10-5 g three times daily (Alsemet). There is insufficient evidence of efficacy of individual antioxidants, or antioxidants in general, in the treatment of people with amyotrophic lateral sclerosis: only one study reported a mild positive effect. Generally the studies were poorly designed and underpowered, with low numbers of participants and of short duration. Further well-designed trials of medications such as vitamin C and E are unlikely to be performed. If future trials of antioxidant medications are performed, careful attention should be given to sample size, outcome measures and duration of the trial. The high tolerance, safety, relatively low cost of vitamins C and E and other considerations related to the lack of other effective treatments for amyotrophic lateral sclerosis, explain the continuing use of these vitamins by physicians and patients. While there is no substantial clinical trial evidence to support their clinical use, there is no clear contraindication[144].

References

1) Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Method Enzymol 1990; 186: 1-85.
2) Saran M, Michel C, Bors W. Reactions of NO with O2-. implications for the action of endothelium-derived relaxing factor. Free Radic Res Commun 1989; 83: 1705-1715.
3) Stubbe J. Ribonucleotide reductase: amazing and confusing. J Biol Chem 1990; 265: 5329-5332.
4) Reichard P, Ehrenberg A. Ribonucleotide reductase: a radical enzyme. Science 1983; 221: 514-519.
5) Babior BM. Oxygen-dependent microbial killing by phagocytes. N Engl J Med 1978; 298: 659-668.
6) Slater TF. Necrogenic action of carbon tetrachloride in the rat: a speculative mechanism based on activation. Nature 1966; 209: 36-40.
7) Cheeseman KH, Albano E, Tomasi A, Slater TF. Biochemical studies on the metabolic activation of haloalkanes. Environ Health Perspect 1985; 64: 85-101.
8) Ross D. Glutathione, free radicals and chemoterapeutic agents. Mechanisms of free radical-induced toxicity and glutathione-dependent protection. Pharmacol Ther 1988; 37: 231-243.
9) Ross D, Moldeus PR. Antioxidant defense systems and oxidative stress. In. Vigo-Pelfrey C, ed. Membrane Lipid Oxidation, Vol. II Boca Raton: CRC, 1991: pp 151-170.
10) Cheeseman KH. Lipid peroxidation in biological systems. In: Halliwell B, Aruoma OI, ed. DNA and Free Radicals. London: Ellis Horwood, 1993.
11) Porter NA. Autoxidation of polyunsaturated fatty acids: initiation, propagation and product distribution (basic chemistry). In: Vigo-Pelfrey C, ed. Membrane Lipid Oxidation. Boca Raton: CRC, 1990: pp 33-62.
12) Esterbauer H, Zollner H, Schaur RJ. Aldehydes formed by lipid peroxidation: mechanisms of formation, occurence and determination. In: Vigo-Pelfrey C, ed. Membrane Lipid Oxidation. Boca Raton: CRC, 1990: pp 239-83.
13) Esterbauer H, Zollner H, Schaur RJ. Hydroxyalkenals: cytoyoxic products of lipid peroxidation. ISI Atlas Sci 1988; 1: 311-317.
14) Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radic Biol Med 1991; 11: 81-128.
15) Poli G, Albano E, Dianzani MU. The role of lipid peroxidation in liver damage. Chem Phys Lipids 1987; 45: 117-142.
16) Lang J, Esterbauer H. Oxidised LDL and atherosclerosis. In: Vigo-Pelfrey C ed. Membrane Lipid Oxidation. Boca Raton: CRC, 1991: pp 265-82.
17) Marx G, Chevion M. Site-specific modification of albumin by free radicals. Reaction with copper (II) and ascorbate. Biochem J 1986; 236: 397-400.
18) Stadtman ER, Oliver CN. Metal-catalyzed oxidation of proteins. Physiological consequences. J Biol Chem 1991; 266: 2005-2008.
19) Simpson JA, Narita S, Gieseg S, Gebicki S, Gebicki JM, Dean RT. Long-lived reactive species on free-radical-damaged proteins. Biochem J 1992; 282: 621-624.
20) Stadtman ER. Metal-ion catalyzed oxidation of proteins: biochemical mechanism and physiological consequences. Free Radic Biol Med 1990; 9: 315-325.
21) Fraga CG, Shigenaga MK, Park JW, Degan P, Ames BN. Oxidative damage to DNA during aging:8-hydroxy-2'-guanosine in rat organ DNA and urine. Proc Natl Acad Sci USA 1990; 87: 4533-4537.
22) Kasai H, Nishimura S. Formation of 8-hydroxyguanosine in DNA by oxygen radicals and its biological significance. In: Sies H ed. Oxidative Stress: Oxidants and Antioxidants,. London: Academic Press 1991; pp 99-116.
23) Cotgreave I, Moldeus P, Orrenius S. Host biochemical defense mechanims against prooxidants. Annu Rev Pharmacol Toxicol 1988; 28: 189-212.
24) Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 1984; 219: 1-14.
25) Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidants and human disease:where are we now? J Lab Clin Med 1992; 19: 598-620.

26) WENDEL A. Glutathione peroxidase. In: Jakoby WB, Bend JR, Caldwell J ed. Enzymatic Basis of Detoxication. New York: Academic Press, 1980; 119: 598-620.

27) BURTON GW, CHEESEMAN KH, DOBA T, INGOLD KU, SLATER TF. Vitamin E as an antioxidant in vitro and in vivo. In: Biology of Vitamin E. Ciba Foundation Symposium 101. London: Pitman Press 1983: pp 4-18.

28) BURTON GW, JOYCE A, INGOLD KU. Is vitamin E the only lipid-soluble chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch Biochem Biophys 1983; 221: 281-290.

29) BURTON GW, INGOLD KU. Vitamin E: application of the principles of physical organic chemistry to the exploration of its structure and function. Accounts Chem Res 1986; 19: 194-201.

30) STOCKER R, BOWRY VW, FREI B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does a-tocopherol. Proc Natl Acad Sci USA 1991; 88: 1646-1650.

31) STOCKER R, FREI B. Endogenous antioxidant defences in human blood plasma. In: Sies H ed. Oxidative Stress: Oxidants and Antioxidants. London: Academic Press, 1991: pp 213-242.

32) PACKER JE, SLATER TF, WILSON RL. Direct observation of a free radical interaction between vitamin E and vitamin C. Nature 1979; 278: 737-738.

33) HALLIWELL B. How to characterise a biological antioxidant. Free Radic Res Commun 1990; 9: 1-32.

34) PARTHASARATHY S, YOUNG SG, WITZUM JL, PITTMAN RC, STEINBERG D. Probucol inhibits oxidative modification of low density lipoprotein. J Clin Invest 1986; 77: 641-644.

35) BAROUKI R. Ageing free radicals and cellular stress. Med Sci (Paris) 2006; 22: 266-272.

36) CHUNG HY, SUNG B, JUNG KJ, ZOU Y, YU BP. The molecular inflammatory process in aging. Antioxid Redox Signal 2006; 8: 572-581.

37) BONNEFOY M, DRAI J, KOSTKA T. Antioxidants to slow aging, facts and perspectives. Presse Med 2002; 31: 1174-1184.

38) OHIA SE, OPERE CA, LEDAY AM. Pharmacological consequences of oxidative stress in ocular tissues. Mutat Res 2005; 579: 22-36.

39) VINSON JA. Oxidative stress in cataracts. Pathophysiology 2006; 13: 151-162.

40) TRUSCOTT RJ. Age-related nuclear cataract-oxidation is the key. Exp Eye Res 2005; 80: 709-725.

41) VARMA SD, HEGDE KR, KOVTUN S. Attenuation and delay of diabetic catarcts by antioxidants: effectiveness of pyruvate afetr onset of cataract. Ophthalmologica 2005; 219: 309-315.

42) TOLER SM. Oxidative stress plays an inportant role in the pathogenesis of drug-induced retinopathy. Exp Biol Med 2004; 229: 607-615.

43) KOWLURU RA, KENNEDY A. Therapeutic potential of anti-oxidants and diabetic retinopathy. Expert Opin Investig Drugs 2001; 10: 1665-1676.

44) RAVANAT JL, DOUKI T, CADET J. Direct and indirect effects of UV radiation on DNA and its components. J Photochem Photobiol B 2001; 63: 88-102.

45) FUCHS J. Potentials and limitations of the natural antioxidants RRR-alpha-tocopherol, L-ascorbic acid and beta-carotene in cutaneous photoprotection. Free Radic Biol Med 1998; 25: 848-873.

46) MANTOVANI G, MADEDDU C, MACCIO A, GRAMIGNANO G, LUSSO MR, MASSA E, ASTARA G, SERPE R. Cancer-related anorexia/cachexia sindrome and oxidative stress: an innovative approach beyond current treatment. Cancer Epidemiol Biomarkers Prev 2004; 13: 1651-1659.

47) KELFKENS G, DE GRUIJL FR, VAN DER LEUN JC. Ozone depletion and increase in annual carcinogenic ultraviolet dose. Photochem Photobiol 1990; 52: 819-823.

48) ROOMI MW, IVANOV V, KALINOVSKY T, NIEDZWIECKI A, RATH M. In vivo and in vitro antitumor effect of ascorbic acid, lysone, proline, arginine, and green tea extract on human fibrosarcoma cells HT-1080. Med Oncol 2006; 23: 105-111.

49) MANTOVANI G, MACCIO A, MADEDDU C, GRAMIGNANO G, LUSSO MR, SERPE R, MASSA E, ASTARA G, DEIANA L. A phase II study with antioxidants, both in the diet and supplemented, pharmaconutritional support, progestagen, and anti-cyclooxygenase-2 showing efficacy and safety in patients with cancer-related anorexia/cachexia and oxidative stress. Cancer Epidemiol Biomarkers Prev 2006; 15: 1030-1034.

50) HINNEBURG I, KEMPE S, RUTTINGER HH, NEUBERT RH. Antioxidant and photoprotective properties of an extract from buckwheat herb (fagopyrum esculentum MOENCH). Pharmazie 2006; 61: 237-240.

51) BALESTRIERI C, FELICE F, PIACENTE S, PIZZA C, MONTORO P, OLESZEK W, VISCIANO V, BALESTRIERI ML. Relative effects of phenolic constituents from Yucca schidigera Roezl. Bark on Kaposi's sarcoma cell proliferation, migration, and PAF synthesis. Biochem Pharmacol 2006; 71: 1479-1487.

52) NAVARRA T, DEL TURCO S, PAPA A, BATTAGLIA D, LAZZERINI G, BASTA G. Lack of effect of alpha-tocopherol on in vitro angiogenesis. Microvasc Res 2006; 72: 12-19.

53) YAO EH, YU Y, FUKUDA N. Oxidative stress on progenitor and stem cells in cardiovascular diseases. Curr Pharm Biotechnol 2006; 7: 101-108.

54) SPEIKER LE, LUSCHER TF. Protection of endothelial function. Handb Exp Pharmacol 2005: 619-644.

55) OHATA S, ISHIBASHI Y, SHIMADA T, TAKAHASHI N, SUGAMORI T, SAKANE T, HIRANO Y, OYAKE N, MURAKAMI Y, HIGAMI T. Effects of oral beraprost sodium, a prostaglandin I2 analogue, on endothelium dependent vasodilatation in the forearm of patients with coronary artery disease. Clin Exp Pharmacol Physiol 2006; 33: 381-387.

56) ENGLER MB, ENGLER MM. The emerging role of flavonoid-rich cocoa and chocolate in cardiovascular health and disease. Nutr Rev 2006; 64: 109-118.

57) STEINBERG D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997; 272: 20963-20966.

58) TRIBBLE DL. AHA Science Advisory Antioxidant consumption and risk of coronary heart disease: emphasison vitamin C, vitamin E, and beta-carotene: a statement for healthcare professionals from the American heart association. Circulation 1999; 99: 591-595.

59) AVIRAM M, KAPLAN M, ROSENBLAT M, FUHRMAN B. Dietary antioxidants and paraoxonases against LDL oxidation and atherosclerosis development. Handb Exp Pharmacol 2005; 170: 263-300.

60) BRIGELIUS-FLOHE R, KLUTH D, BANNING A. Is there a future for antioxidants in atherogenesis? Mol Nutr Food Res 2005; 49: 1083-1089.

61) CLAIR RS, ANTHONY M. Soy, isoflavones and atherosclerosis. Handb Exp Pharmacol 2005; 170: 301-323.

62) BELTOWSKI J. Leptin and atherosclerosis. Atherosclerosis 2006; 189: 47-60.

63) ENGELHARD YN, GAZER B, PARAN E. Natural antioxidants from tomato extract reduce blood pressure in patients with grade-1 hypertension: a double blind, placebo-controlled pilot trial. Am Heart J 2006; 151: 100.

64) HATZIGEORGOIU C, TAYLOR AJ, FEUERSTEIN IM, BAUTISTA L, O'MALLEY PG. Antioxidant vitamin intake and subclinical coronary atherosclerosis. Prev Cardiol 2006; 9: 75-81.

65) ALEXANDROVA M, BOCHEV P, MARKOVA V, BECHEV B, POPOVA M, DANOVSKA M, SIMEONOVA V. Dynamics of free radical processes in acute ischemic stroke: influence on neurological status and outcome. J Clin Neurosci 2004; 11: 501-506.

66) POLIDORI MC, PRATICO D, INGEGNI T, MARIANI E, SPAZZAFUMO L, DEL SINDACO P, CECCHETTI R, YAO Y, RICCI S, CHERUBINI A, STAHL W, SIES H, SENIN U, MECOCCI P, AVASAS STUDY GROUP. Effects of vitamin C and aspirin in ischemic stroke-related lipid peroxidation: results of the AVASAS (Aspirin versus ascorbic acid plus aspirin in stroke) Study. Biofactors 2005; 24: 265-274.

67) BAILEY DM, RAMAN S, MCENENY J, YOUNG IS, PARHAM HL, HULLIN DA, DAVIES B, MCKEEMAN G, MCCORD JM, LEWIS MH. Vitamin C prophylaxis promotes oxidative lipid damage during surgical ischemia-reperfusion. Free Radic Biol Med 2006; 40: 591-600.

68) MCCORD JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985; 312: 159-163.

69) EDREES WK, LAU LL, YOUNG IS, SMYE MG, GARDINER KR, LEE B, HANNON RJ, SOONG CV. The effect of lower limb ischaemia-reperfusion on intestinal permeability and the systemic inflammatory response. Eur J Vasc Endovasc Surg 2003; 25: 330-335.

70) NAGEL E, MEYER ZU, VILSENDORF M, BARTELS M, PICHLMAYR R. Antioxidative vitamins in prevention of ischemia/reperfusion injury. Int J Vitam Nutr Res 1997; 67: 298-306.

71) LASSNIGG A, PUNZ A, BARKER R, KEZNICKL P, MANHART N, ROTH E, HIESMAYR M. Influence of intravenous vitamin E supplementation in cardiac surgery on oxidative stress: a double-blinded, randomized, controlled study. Br J Anaesth 2003; 90: 148-154.

72) CHRISTEN S, FINCKH B, LYKKESFELDT J, GESSLER P, FRESE-SCHAPER M, NIELSEN P, SCHMID ER, SCHMITT B. Oxidative stress precedes peak systemic inflammatory response in pediatric patients undergoing cardiopulmonary by pass operation. Free Radic Biol Med 2005; 38: 1323-1332.

73) PIETRI S, SEGUIN JR, D'ARBIGNY P, CULCASI M. Ascorbyl free radical: a non invasive marker of oxidative stress in human open-heart surgery. Free Radic Biol Med 1994; 16: 523-528.

74) PIETRI S, SEGUIN JR, D'ARBIGNY P, DRIEU K, CULCASI M. Ginko biloba extract (EGB 761) pretreatment limits free radical oxidative stress in patients undergoing coronary bypass surgery. Cardiovasc Drugs Ther 1997; 11: 121-131.

75) WIJNEN MH, VADER HL, VAN DEN WALL BAKE AW, ROUMEN RM. Can renal dysfunction after infra-renal aortic aneurysm repair be modified by multi-antioxidant supplementation? J Cardiovasc Surg 2002; 43: 483-488.

76) WATTERS JM, VALLERAND A, KIRKPATRICK SM, ABBOTT HE, NORRIS S, WELLS G, BARBER GG. Limited effects of micronutrient supplementation on strength and physical function after abdominal aortic aneurysmectomy. Clin Nutr 2002; 21: 321-327.

77) CHILDS A, JACOBS C, KAMINSKI T, HALLIWELL B, LEEUWENBURGH C. Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. Free Radic Biol Med 2001; 31: 745-753.

78) GALLEY H, DAVIES M, WEBSTER N. Ascorbyl radical formation in patients with sepsis: effect of ascorbate loading. Free Radic Biol Med 1996; 20: 139-143.

79) REHMAN A, COLLIS C, YANG M, KELLY M, DIPLOCK AT, HALLIWELL B, RICE-EVANS C. The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. Biochem Biophys Res Commun 1998; 246: 293-298.

80) ALBERT NM. We are what we eat: women and diet for cardiovascular health. J Cardiovascular Nurs 2005; 20: 451-460.

81) VAN GAAL LF, VERTOMMEN J, DE LEEUW IH. The in vitro oxidizability of lipoprotein particles in obese and non-obese subjects. Atherosclerosis 1998; 137: 39-44.

82) VINCENT HK, POWER SK, STEWART DJ, SHANELY RA, DEMIREL H, NAITO H. Obesity is associated with increased myocardical oxidative stress. Int J Obes Relat Metab Disord 1999; 23: 67-74.

83) BAKKER SJ, IJZERMAN RG, TEERLINK T, WESTERHOFF HV, GANS RO, HEINE RJ. Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and beta-cell failure? Atherosclerosis 2000; 148: 17-21.

84) COPPACK SW. Pro-inflammatory citokines and adipose tissue. Proc Nutr Soc 2001; 60: 349-356.

85) DANDONA P, MOHANTY P, GHANIM H, ALJADA A, BROWNE R, HAMOUDA W, PRABHALA A, AFZAL A, GARG R. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. J Clin Endocrinol Metab 2001; 86: 355-362.

86) CRUJEIRAS AB, PARRA MD, RODRIGUEZ MC, MARTINEZ DE MORENTIN BE, MARTINEZ JA. A role for fruit content in energy-restricted diets in improving antioxidant status in obese women during weight loss. Nutrition 2006; 22: 593-599.

87) KAO YH, CHANG HH, LEE MJ, CHEN CL. Tea, obesity, and diabetes. Mol Nutr Food Res 2006; 50: 188-210.

88) BAYNES JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40: 405-412.

89) CERIELLO A, GIUGLIANO D. Oxidative stress and diabetic complications. In: Alberti KGMM, Zimmet P, De Fronzo RA, eds. International Textbook of Diabetes Mellitus, 2nd edn. Chichester: John Wiley & Sons; 1997: pp 1453-1461.

90) CERIELLO A, QUATRARO A, GIUGLIANO D. New insight on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications. Diabetic Med 1992; 9: 297-99.

91) WOLFF SP, DEAN RT. Glucose auto-oxidation and protein modification. The potential role of "autoxidative glycosylation" in diabetes. Biochem J 1987; 245: 243-250.

92) CERIELLO A, PIRISI M. Is oxidative stress the missing link between insulin resistance and atherosclerosis?. Diabetologia 1995; 38: 1484-1485.

93) CERIELLO A, BORTOLOTTI N, CRESCENTINI A, MOTZ E, LIZZIO S, RUSSO A, EZSOL Z, TONUTTI L, TABOGA C. Antioxidant defences are reduced during the oral glucose tolerance test in normal an non-insulin-dependent diabetic subjects. Eur J Clin Invest 1998; 28: 329-233.

94) MARFELLA R, VERRAZZO G, ACAMPORA R, LA MARCA C, GIUNTA R, LUCARELLI C, PAOLISSO G, CERIELLO A, GIUGLIANO D. Glutathione reverses systemic hemodynamic changes by acute hyperglycaemia in healthy subjects. Am J Physiol 1995; 268: 1167-1173.

95) CERIELLO A, MOTZ E, CAVARAPE A, LIZZIO S, RUSSO A, QUATRARO A, GIUGLIANO D. Hyperglycaemia counterbalances the anti-hypertensive effect of glutathione in diabetic patients. Evidence linking hypertension and glycemia through the oxidative stress in diabetes mellitus. J Diab Compl 1997; 11: 250-255.

96) CERIELLO A, GIACOMELLO R, STEL G, MOTZ E, TABOGA C, TONUTTI L, PIRISI M, FALLETI E, BARTOLI E. Hyperglycemia-induced thrombin formation in diabetes. The possible role of oxidative stress. Diabetes 1995; 44: 924-928.

97) CERIELLO A, FALLETI E, BORTOLOTTI N, MOTZ E, CAVARAPE E, RUSSO A, GONANO F, BARTOLI E. Increased circulating ICAM-1 levels in Type-2 diabetic patients: the possibile role of the oxidative stress and metabolic control. Diabetologia 1995; 388: 154.

98) SORIANI M, PIETRAFORTE D, MINETTI M. Antioxidant potential of anaerobic human plasma: role of serum albumin and thiols as scavengers of carbon radicals. Arch Biochem Biophys 1994; 312: 180-188.

99) FREI B, STOCKER R, AMES BN. Antioxidant defences and lipid peroxidation in human blood plasma. Proc Natl Acad Sci USA 1988; 85: 9748-9752.

100) HALLIWELL B, GUTTERIDGE JMC. The antioxidants of human extracellular fluids. Arch Biochem Biophys 1990; 280: 1-8.

101) ISHIHARA M, SHINODA T, AIZAWA T, SHIROTA T, NAGASAWA Y, YAMADA T. Hypouricaemia in NIDDM patients. Diabetes Care 1988; 11: 796-797.

102) OBERLEY LW. Free radicals and diabetes. Free Rad Biol Med 1988; 5: 113-124.

103) CERIELLO A, BORTOLOTTI N, MOTZ E, PIERI C, MARRA M, TONUTTI L, LIZZIO S, FELETTO F, CATONE B, TABOGA C. Meal-generated oxidative stress in type 2 diabetic patients. Diabetes Care 1998; 21: 1529-1533.

104) WOLFE RR, SHAW JH, DURKOT MJ. Effects of sepsis on VLDL kinetics: responses in basal state and during glucose infusion. Am J Physiol 1985; 248: 732-740.

105) CERIELLO A. Acute hyperglycaemia and oxidative stress generation. Diabet Med 1997; 14: 45-49.

106) SORIANO FG, VIRAG L, SZABO C. Diabetic endothelial dysfunction role of reactive oxygen and nitrogen species production and poly(ADP-ribose) polymerase activation. J Mol Med 2001; 79: 437-448.

107) BECKMAN JS, KOPPENOL WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996; 271: 1424-1437.

108) KAHAN SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia 2003; 46: 3-19.

109) LAKKA HM, LAAKSONEN DE, LAKKA TA, NISKANEN LK, KUMPUSALO E, TUOMILEHTO J, SALONEN JT. The meta-

bolic syndrome and total and cardiovascular disease in middle-aged men. JAMA 2002; 288: 2709-2716.

110) Balkau B, Bertrais S, Ducimetiere P, Eschwege E. Is there a glycaemic threshold for mortality risk? Diabetes Care 1999; 22: 696-699.

111) Heillbronn LK, Ravussin E. Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr 2003; 78: 361-369.

112) Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and b-cell dysfunction? Diabetes 2003; 52: 1-8.

113) Griendling KK, Fitzgerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation 2003; 108: 1912-1916.

114) Maddux BA, See W, Lawrence JC Jr, Goldfine AL, Goldfine ID, Evans JL. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of a-lipoic acid. Diabetes 2001; 50: 404-410.

115) Williamson JR, Cooper RH. Regulation of the citric acid cycle in mammalian systems. FEBS Lett 1980; 117: 73-85.

116) Tretter L, Adam-Vizi V. Inhibition of Krebs cycle enzymes by hydrogen peroxide: a key role of alpha-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. J Neurosci 2000; 20: 8972-8979.

117) Rudich A, Tirosh A, Potashnik R, Hemi R, Kanety H, Bashan N. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. Diabetes 1998; 47: 1562-1569.

118) Talior I, Yarkoni M, Bashan N, Eldar-Fielman H. Increased glucose uptake promotes oxidative stress and PKC delta activation in adipocytes of obese, insulin-resistance mice. Am J Physiol 2003; 285: 295-302.

119) Paolisso G, Giugliano D. Oxidative stress and insulin action. Is there a relationship? Diabetologia 1996; 39: 357-363.

120) Bruce CR, Carrey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defence mechanism. Diabetes 2003; 52: 2338-2345.

121) Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004; 24: 816-823.

122) Mason RP, Marche P, Hintze TH. Novel vascular biology of third-generation L-type calcium channel antagonists: ancillary actions of amlodipine. Arterioscler Thromb Vasc Biol 2003; 23: 2155-2163.

123) Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Arterioscler Thromb Vasc Biol 2001; 21: 1712-1719.

124) Munzel T, Keaney JF Jr. Are ACE inhibitors a "magic bullet" against oxidative stress? Circulation 2001; 104: 1571-1579.

125) Ceriello A, Motz E. Angiotensin-receptor blockers, type 2 diabetes, and renoprotection. N Engl J Med 2002; 346: 705-707.

126) Da Ros R, Assaloni R, Ceriello A. The preventive anti-oxidant action of thiazolidines: a new therapeutic prospect in diabetes and insulin resistance. Diabet Med 2004; 21: 1249-1252.

127) Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M; STOP-NIDDM Trial Research Group. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomized trial. Lancet 2002; 359: 2072-2077.

128) Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M; STOP-NIDDM Trial Research Group. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA 2003; 290: 486-494.

129) Ceriello A, Quagliaro L, Catone B, Pascon R, Piazzola M, Bais B, Marra G, Tonutti L, Taboga C, Motz E. Role of hyperglycaemia in nitrotyrosine postprandial generation. Diabetes Care 2002; 25: 1439-1443.

130) Marchioli R, Schweiger C, Levantesi G, Gavazzi L, Valgussa F. Anti-oxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. Lipids 2001; 36: 53-63.

131) Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev 2001; 53: 135-159.

132) Boshtam M, Rafiei M, Golshadi ID, Ani M, Shirani Z, Rostamshirazi M. Long term effects of oral vitamin E supplement in type II diabetic patients. Int J Vitam Nutr Res 2005; 75: 341-346.

133) Rascol O, Payoux P, Ory F, Ferreira JJ, Brefel-Courbon C, Montastruc JL. Limitations of current Parkinson's disease therapy. Ann Neurol 2003; 53: S3-15.

134) Zhu X, Smith MA, Perry G, Aliev G. Mitochondrial failures in Alzheimer's disease. Am J Alzheimer Dis Other Demen 2004; 19: 345-352.

135) Frank B, Gupta S. A review of antioxidants and Alzheimer's disease. Ann Clin Psychiatry 2005; 17: 269-286.

136) Ansari NM, Houlihan L, Hussain B, Pieroni A. Antioxidant activity of five vegetables traditionally consumed by South-Asian migrants in Bradford, Yorkshire, UK. Phytother Res 2005; 19: 907-911.

137) MARAMBAUD P, ZHAO H, DAVIES P. Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. J Biol Chem 2005; 280: 37377-37382.

138) LANDMARK K. Could intake of vitamins C and E inhibit development of Alzheimer dementia? Tidsskr Nor Laegeforen 2006; 126: 159-161.

139) BEAL MF. Aging, energy, and oxidative stress in neurodegenerative diseases. Ann Neurol 1995; 38: 357-366.

140) YORITAKA A, HATTORI N, UCHIDA K, TANAKA M, STADTMAN ER, MIZUNO Y. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc Natl Acad Sci USA 1996; 93: 2696-2701.

141) DEXTER DT, CARAYON A, JAVOY-AGID F, AGID Y, WELLS FR, DANIEL SE, LEES AJ, JENNER P, MARSDEN CD. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 1991; 114: 1953-1975.

142) FORTE G, ALIMONTI A, PINO A, STANZIONE P, BRESCIANINI S, BRUSA L, SANCESARIO G, VIOLANTE N, BOCCA B. Metals and oxidative stress in patients with Parkinson's disease. Ann Ist Super Sanità 2005; 41: 189-195.

143) SHULTS CW. Therapeutic role of coenzyme Q^{10} in Parkinson's disease. Pharmacol Ther 2005; 107: 120-130.

144) ORRELL RW, LANE RJ, ROSS M. Antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev 2005: CD002829.

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