

# ENZOGENOL

## Three-Part Scientific Review

### Managing Oxidative Stress in Disease – *professionally*

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# ENZO:PROFESSIONAL –

## Managing Oxidative Stress in Disease – *professionally*

### Part I: Oxidative Stress in Inflammation

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**Oxidative stress is involved in diverse pathologies, including the spectrums of inflammatory, cardiovascular, and neurodegenerative diseases. Excellent reviews are available on these topics that greatly expand the short summary given below.**

#### **Oxidative Stress in Inflammation**

Inflammation itself produces oxidative stress since sites of inflammation are sites of increased free radical production. The free radical production by activated monocytes, neutrophils, eosinophils and macrophages is part of the defence mechanism to destroy invasive pathogens and remove damaged tissue.

The types of radicals produced are the reactive oxygen and nitrogen species (ROS and RNS), also called oxygen free radicals or nitrogen free radicals. Superoxide anion ( $O_2^{\cdot-}$ ) is produced by NADPH oxidase in activated neutrophils to kill pathogens. It exerts its toxicity by conversion to other more toxic ROS.  $O_2^{\cdot-}$  is converted to hydrogen peroxide ( $H_2O_2$ ) by action of superoxide dismutase, and further to the hydroxyl radical ( $OH^{\cdot}$ ) by reaction with transition metal ions such as  $Fe^{2+}$ . The  $OH^{\cdot}$  radical is extremely reactive and is one of the strongest oxidizing agents. The enzyme myeloperoxidase (MPO) provides another bacterial killing mechanism in neutrophils by catalyzing the oxidation of chloride ions by  $H_2O_2$ ; this reaction results in the formation of hypochlorous acid (HOCl), a powerful bactericidal agent. The ROS are highly toxic to the target pathogens but also for producing and neighbouring cells. Therefore neutrophils have to contain large reserves of endogenous antioxidants such as glutathione and ascorbate. Their ability to maintain these antioxidants in the reduced state during phagocytosis may prevent death from oxidative suicide. RNS are nitric oxide ( $NO^{\cdot}$ ) and peroxynitrite ( $ONOO^{\cdot-}$ ).  $NO^{\cdot}$  is a very important signalling molecule with critical functions for many body systems including the cardiovascular and nervous system and is produced throughout the body by nitric oxide synthases in many cell types. In inflammation,  $NO^{\cdot}$  is produced by the inducible nitric oxide synthase (iNOS). When induced by signals like bacterial membrane components or inflammatory cytokines iNOS produces large amounts of  $NO^{\cdot}$ .  $NO^{\cdot}$  may react with  $O_2^{\cdot-}$  to form highly toxic  $ONOO^{\cdot-}$  which may contribute to the microbicidal activity of neutrophils. The main role of neutrophil-derived  $NO^{\cdot}$

may be to facilitate the migration of neutrophils from blood vessels to surrounding tissue by causing vasodilatation. Due to its important signalling functions and the ability to act as an antioxidant in itself,  $NO^{\cdot}$  is in most situation regarded as a “good radical”, but its overproduction in conjunction with large amounts of ROS may turn this friendly molecule into a toxin. The balance between  $NO^{\cdot}$  and  $O_2^{\cdot-}$  as well as their overall levels are the important determinants of oxidative stress levels, and the ROS, or oxygen free radicals, are considered to be the harmful, unwanted species in this balanced interplay.

In the acute inflammatory response the production of this entire battery of free radicals is an important mechanism that is necessary to fight of invading pathogens. However, the problems begin when the inflammatory process persists and becomes chronic, because the inherently high level of oxidative stress turns against the healthy body tissues. In diseases, such as the different forms of **arthritis** or in **autoimmune disorders** like **lupus** or **multiple sclerosis**, where inflammation manifests chronically, free radicals are permanently produced and have the capacity to cause tissue destruction. It is in particular in these types of diseases that managing oxidative stress levels should be considered as part of the general therapy.

Evidence for the role of oxidative stress in disorders of **chronic inflammation** comes from many studies on the consequences of the elevated oxidant levels. Biomarkers of cellular damage indicative of oxidative stress, including lipid peroxides, free F<sub>2</sub>-isoprostane, carbonylated proteins, oxidised DNA bases, thioredoxin levels and advance glycation end products were found to be elevated in the plasma or the effected tissues of patients with diverse inflammatory diseases. These diseases include **rheumatoid and osteoarthritis**<sup>8,12,13,14</sup>, **juvenile chronic arthritis**<sup>10</sup>, **asthma**<sup>6,7</sup>, **severe chronic kidney disease**<sup>4</sup>, **amyloidosis**<sup>5,11</sup>, **inflammatory bowel disease**<sup>9</sup>, and others.

In addition to what is known as inflammatory disorders, inflammation is increasingly being recognised as a fundamental part of many different disease aetiologies as highlighted by a recent TIME magazine cover story entitled “*The secret killer - The surprising link between inflammation and heart attacks, cancer, Alzheimer’s and other diseases*” (Feb 24, 2004). Increasing evidence

suggests that inflammation in the brain is closely associated with the pathogenesis of several **neurodegenerative diseases** including **Parkinson's** and **Alzheimer's** disease, **multiple sclerosis**, and **amyotrophic lateral sclerosis**. The hallmark of brain inflammation is the activation of glial cells that then produce a variety of proinflammatory and neurotoxic factors which include oxygen free radicals<sup>1</sup>. Inflammatory processes involving macrophage invasion with the propagation of the inflammatory milieu including the generation of oxygen free radicals in the arterial wall are recognised as major contributors to the development of **atherosclerosis** and **cardiovascular disease**. Inflammation within the atherosclerotic plaques seems to contribute the propensity of the plaques to rupture and cause a heart attack<sup>2,3</sup>.

### **Other therapeutic targets in inflammation**

A range of enzymatic reactions that mediate the inflammatory response without directly generating free radicals and oxidative stress are also very important therapeutic targets in inflammation. Several kinases, including protein kinase C and different MAP kinases play important roles in the signal transduction events that take place during the inflammatory response resulting in the production of proinflammatory cytokines and the activation of other enzymes. Phospholipase A2, an enzyme involved in many cell activation processes, is an important intra- and extracellular mediator of inflammation catalysing the release of arachidonic acid. This is subsequently metabolised via the cyclooxygenase and lipoxygenase pathways to prostaglandins and leukotrienes. These molecules are intimately involved in inflammation, asthma, and allergy, as well as in multiple other physiologic and pathologic processes.

**In summary, oxidative stress is an integral part of the inflammatory process. However, when inflammation persists and becomes chronic, as is the case in many diseases, the proper management of the elevated oxidative stress levels should be a part of the therapy. In addition to oxidative stress many other therapeutic targets need consideration when treating chronic inflammation.**

### **Why to use flavonoids in the therapy of inflammatory disorders?**

Flavonoids are uniquely suited as a support or single therapy in the treatment of inflammatory disorders due to their combination of antioxidant and anti-inflammatory properties. The antioxidant and anti-inflammatory properties of flavonoids have been well established. A comprehensive review by Middleton *et al.* has discussed the effects of flavonoids on mammalian cells and their implications for inflammation in great detail<sup>15</sup>, and the knowledge on anti-inflammatory effects of flavonoids has been further broadened by many recent studies.

The reasons to use flavonoid/proanthocyanidin based antioxidants in the management of inflammatory disorders include:

1. The radical scavenging capacity of flavonoids, and particularly proanthocyanidins is several times higher than that of the vitamin antioxidants for a wide range of radicals<sup>16</sup>. Flavonoids are chemically a diverse range of compounds containing different ring structures with different side chains and polymerisation states. This diversity greatly contributes to their radical scavenging ability and other effects on enzyme systems the body<sup>17</sup>. The potency as antioxidants and the chemical diversity of multi-compound flavonoid extracts, like ENZO Professional, strongly recommend flavonoids in therapeutic efforts to lower oxidative stress levels.
2. Flavonoids have been shown to inhibit the production of ROS, the activity of the ROS generating enzymes MPO and NADPH oxidase, and several signal transducing enzymes involved in the cell activation in neutrophils<sup>15</sup>.
3. Flavonoids have been shown to reduce biomarkers of oxidative cell damage in cell culture systems, animal models and human studies<sup>19, 20, 21, 22, 23</sup>.
4. Flavonoids inhibit lipid peroxidation this can be by direct radical scavenging or by chelation properties of transition metals<sup>18</sup>.
5. Flavonoids have direct anti-inflammatory properties that have been documented in many studies. The anti-inflammatory properties of flavonoids include cyclooxygenase (COX2) inhibition, lowering prostaglandin E2 production, inhibition of several signal transducing molecules and enzymes, such as inhibition of NFκB activation, inhibition of nitric-oxide synthase (iNOS) induction and subsequent NO<sup>•</sup> production, and inhibition of proinflammatory cytokines, such as interleukin-1β and interleukin 2<sup>15, 24, 25, 26, 27, 28</sup>.
6. In addition to anti-inflammatory properties flavonoids may also have beneficial effects in arthritic diseases by positively influencing the balance between synthesis and degradation of extra-cellular cartilage constituents. Evidence for this action comes from inhibition of proteoglycan and type II collagen breakdown and/or stimulation of synthesis, and inhibition of aggrecan degrading activity of the ADAMTS group of enzymes by some flavonoids.<sup>27, 29, 30</sup>

**In conclusion, flavonoids are very promising agents for the management of oxidative stress. In addition, flavonoids possess multiple anti-inflammatory activities making them an excellent choice as a support agent in the treatment of inflammatory disorders.**

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# ENZO:PROFESSIONAL –

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### Part II: Oxidative Stress in Cardiovascular Disease

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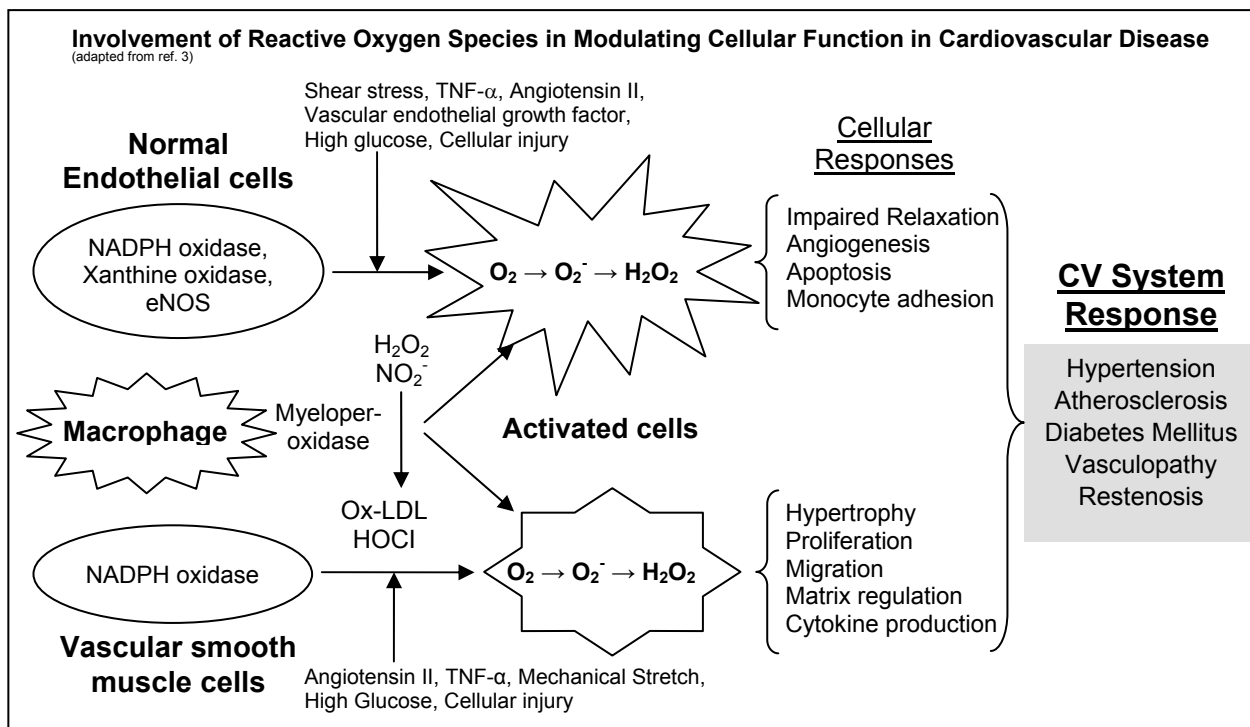
#### **Oxidative Stress in Cardiovascular Disease<sup>1,2,3</sup>**

Reactive oxygen and nitrogen species (ROS, RNS) play a crucial role in the normal vascular physiology, but also in the development and progression of vascular pathologies. The main ROS that are produced in the vessel wall are the superoxide anion ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $OH^{\cdot}$ ); the RNS are nitric oxide ( $NO^{\cdot}$ ) and peroxynitrite ( $ONOO^{\cdot-}$ ). Each of these molecules derives from specific enzymatic or chemical reactions.  $NO^{\cdot}$  is produced by endothelial cells through endothelial nitric oxide synthase (eNOS).  $NO^{\cdot}$  is the most important signalling molecule responsible for endothelium-dependent vascular relaxation. In addition, it also mediates inhibition of platelet activation, adhesion, and aggregation, prevention of vascular smooth muscle proliferation, and adhesion of leukocytes to the endothelium.  $O_2^{\cdot-}$  and  $H_2O_2$  are produced by virtually all types of vascular cells in the mitochondria by enzymes like cytochrome P450 and the membrane-associated NADPH oxidases, and play a critical role in vascular homeostasis and function, mediating growth, apoptosis, and survival of endothelial and vascular smooth muscle cells (VSMC). Normal endothelial function is characterized by a dynamic balance between  $NO^{\cdot}$  and the ROS, particularly  $O_2^{\cdot-}$ .

Oxidative stress in the vasculature occurs when the finely tuned interplay of these oxidants as intra- and inter-cellular signalling molecules is disturbed, and oxidants, particularly the ROS, are overproduced. In general, this can occur when endogenous antioxidant defence mechanisms, such as superoxide dismutase, glutathione peroxidase, chain terminators (vitamins A and C), hemoglobin, and catalase, are unable to balance oxidant stress. At the cellular level, ROS-mediated injury occurs through oxidation of macromolecules, including peroxidation of lipids, and oxidative modification of proteins and nucleic acid. The downstream vascular consequences of these oxidative events include vasoconstriction, inflammation, vascular remodeling, and thrombosis. Initial vascular injury elicits a number of

maladaptive phenomena that compromise the normal anti-atherogenic mechanisms of a properly functioning blood vessel. Yet, in response to oxidant stress and accompanying alterations in the local redox state, the normal profibrinolytic vascular environment is lost, exposing the vascular lumen to the threat of thrombotic occlusion. Impaired modulation of cellular growth results in abnormal proliferation and remodelling of the vascular wall. Oxidant stimulation of inflammatory adaptations produce increased monocyte adhesiveness and permeability to plasma lipoproteins. The vasorelaxant effects of endogenously produced  $NO^{\cdot}$  are overwhelmed by a preponderance of vasoconstricting substances: the resulting impairment in dynamic, endothelium-dependent vasodilation is the physiologic hallmark of endothelial dysfunction.

Several biochemical consequences of unbalanced ROS production in the vessel wall contribute to the initiation and progression of pathology. Of utmost importance is the fact that oxidant stress, particularly from  $O_2^{\cdot-}$ , reduces  $NO^{\cdot}$  bioavailability. eNOS can become “uncoupled” leading to generation of  $O_2^{\cdot-}$  and  $H_2O_2$  instead of  $NO^{\cdot}$ . Angiotensin II is known to stimulate  $O_2^{\cdot-}$  overproduction by VSMC through enhanced NADPH oxidase activity. Consequently, the highly reactive molecule  $ONOO^{\cdot-}$  forms from the reaction of  $O_2^{\cdot-}$  and  $NO^{\cdot}$ , and acts highly pro-atherogenic through lipid peroxidation and protein nitration that are some of the earliest atherogenic events. Pathophysiological expression of inducible iNOS in both macrophages and VSMC leads to overproduction of  $NO^{\cdot}$ , elevates cytokine levels and results in localized inflammation. Macrophages are likely also the major vascular source of  $O_2^{\cdot-}$  in disease states. Extracellular release of ROS by macrophages can activate matrix metalloproteinases that degrade the collagen-based extra cellular matrix, contributing to weakening of the fibrous cap and plaque rupture.  $O_2^{\cdot-}$  and  $H_2O_2$  or their products modulate the activity of signalling pathways that, in turn, control gene expression. ROS regulate several general classes of genes, including adhesion molecules and chemotactic factors, antioxidant enzymes, and vasoactive substances. Upregulation of adhesion and chemotactic molecules by oxidant-sensitive mechanisms is of particular relevance to vascular pathology since these molecules promote adhesion and migration of monocytes into the vessel wall.



Strong evidence for a fundamental role of ROS in CVD comes from numerous animal studies. Animal models of **atherosclerosis** have documented that all the constituents of the plaque produce and use ROS. Lesion formation is associated with the accumulation of lipid peroxidation products, induction of inflammatory genes, inactivation of  $\text{NO}^*$  resulting in endothelial dysfunction, activation of matrix metalloproteinases, and increased smooth muscle cell growth. Abundant evidence supports a role for  $\text{O}_2^-$  in various animal models of **hypertension**. Vascular  $\text{O}_2^-$  is increased in several hypertension models, and appears to be functionally important because administration of antioxidants partially normalizes the blood pressure of the animals. There is also evidence that oxidative stress is increased in the vascular dysfunction that accompanies **diabetes mellitus**. In animal models of diabetes, antioxidant defence capacity is diminished in certain tissues. Increased vascular  $\text{O}_2^-$  production, decreased tissue glutathione, impaired endothelial-dependent relaxation, and increased NADPH oxidase activity leading to uncoupling of eNOS have all been demonstrated which clearly indicates an association between ROS and diabetes. On the basis of animal studies, a role for ROS in the intimal hyperplasia that results in **restenosis** after arterial balloon angioplasty has been proposed, and a variety of antioxidants reduced neointimal proliferation and promoted vessel remodelling.

In human studies, contemporary biomarkers of increased ROS generation, such as levels of isoprostanes (iPs) and lipid peroxides, oxidised LDL and their antibodies, protein oxidation and DNA adduct levels, suggest that elevated oxidant stress is a characteristic of the spectrum of cardiovascular diseases. Patients with

**hypercholesterolemia** have evidence of increased ROS generation. Urinary iP excretion is increased, as are circulating antibodies to oxidised LDL, and levels of iPs in circulating LDL. Many of the risk factors and some of the consequences of **atherosclerosis** have been associated with evidence of oxidant stress in vivo. For example, elevated levels of iP, oxidised proteins and 8-oxodeoxyguanosine levels in DNA were all found in smokers. Impaired endothelial cell function is one ROS-related symptom in atherosclerosis patients. Urinary iP are increased in patients with **hyperhomocysteinemia**, **diabetes mellitus**, **alcoholism**, **lupus** anticoagulant, and **renovascular hypertension**. Elevated levels of certain iPs have been reported in pericardial fluid and the urine of patients with **heart failure**. Urinary iPs confirm, in patients, the oxidative burst that accompanies reperfusion after a period of ischemia. Thus, urinary iPs increase coincident with reperfusion in patients subject to coronary angioplasty or therapeutic thrombolysis. The **ischemic episodes** in patients with unstable angina also are characterized by increased lipid peroxidation.

Despite the fact that proof of principle of the efficacy of antioxidants has been obtained in animal models of **atherogenesis**, **atherosclerosis regression**, and **reperfusion injury**, most, but not all, clinical trials of antioxidants have reached disappointing conclusions. How can this be? Several possibilities exist relating to the endpoint chosen, the selection of patients and the efficacy and dosage of the antioxidants in those studies. It is possible that ROS generation is more relevant to initiation of the process, rather than its culmination. Most trials are initiated when atherosclerosis is established and chose endpoints such as myocardial infarction, persistent angina,

stroke, and vascular death. It is also possible that the patients included in these trials were inappropriate for testing the hypothesis. Thus, no clinical trials have been performed in which patient inclusion was based on biochemical evidence of elevated ROS generation. Most trials have been conducted with vitamins E and C. However, both are incompletely effective at suppressing elevated levels of biomarkers in humans, and much higher doses of these vitamins have been used in model systems than are administered in clinical trials.

**In summary, the evidence for the involvement of oxidative stress in a whole range of CVDs is compelling and calls for the recognition of the potential benefit to the patient that may be achieved by managing levels of oxidative stress professionally through the controlled application of suitable potent antioxidants.**

### **Why to use flavonoids or, in particular, proanthocyanidins in the therapy of CVD?**

An excellent introductory review by A.M. Fine on the benefits of proanthocyanidins can be found in *Alternative Medicine Review*<sup>4</sup>. There are several good reasons to use flavonoid/proanthocyanidin based antioxidants as a support therapy to prevent and treat cardiovascular disease conditions.

7. The radical scavenging capacity of flavonoids, and particularly proanthocyanidins is several times higher than that of the vitamin antioxidants for a wide range of radicals<sup>5</sup>.
8. Numerous studies in cell culture systems, animal models and humans have documented diverse therapeutic effects of proanthocyanidins that can be beneficial in CVD patients. These effects include vasodilatory, anti-hypertensive, and anti-inflammatory activities; inhibition of lipid and LDL oxidation; inhibition of platelet aggregation, and capillary permeability and fragility; effects on enzyme systems including phospholipase A<sub>2</sub>, cyclooxygenase, and lipoxygenase; protection of Vitamin C and E from oxidation; and cardio protective effects after ischemic reperfusion injury<sup>6-11</sup>.
9. The well documented cardio-protective effects of red wine, epidemiologically known as the “French Paradox”, attribute the beneficial effects to the flavonoids in the red wine which are mainly proanthocyanidins<sup>10</sup>. Several other epidemiological studies also provide evidence that flavonoid intake is inversely correlated with, and may reduce the risk of, several factors of CVDs like mortality from coronary heart disease<sup>12</sup>, incidence of myocardial infarction<sup>12-14</sup>, incidence of stroke<sup>15</sup>, CVD risk based on a range of conditions<sup>13</sup>, ischemic heart disease mortality<sup>16,17</sup>, incidence of cerebrovascular disease<sup>17</sup>, and overall CVD-related mortality<sup>18</sup>.

10. Our own research in a small human clinical study found positive effects on risk factors of CVD. In this study dietary supplementation with Enzogenol<sup>®</sup> was shown to improve endothelial function and reduce systolic blood pressure and plasma viscosity<sup>19</sup>.

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# ENZO:PROFESSIONAL – Managing Oxidative Stress in Disease – *professionally* Part III: Oxidative Stress in Neurodegenerative Diseases

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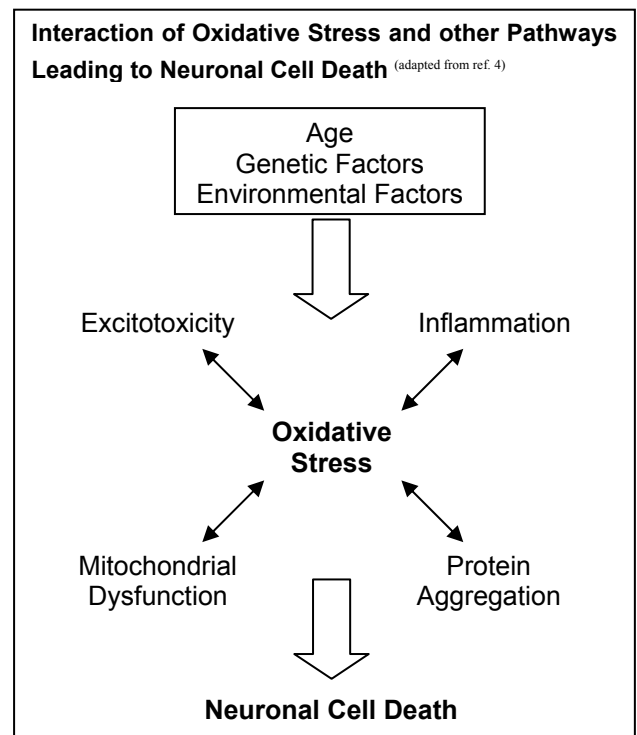
## Oxidative Stress in Neurodegeneration

The formation of free radicals and thereby the occurrence of oxidative stress is a common component of many, if not all, neurodegenerative disorders. Irrespective of the primary cause of the individual neurodegenerative disorder, the onset of oxidative stress is a common mechanism by which neuronal death occurs and which contributes to disease progression.

Both the reactive oxygen species (ROS) superoxide ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $OH^{\cdot}$ ), and the reactive nitrogen species (RNS) nitric oxide ( $NO^{\cdot}$ ) and peroxynitrite ( $ONOO^{\cdot-}$ ) are generated in the brain and are involved in neuronal cell death. These reactive species can damage and cause functional alterations in lipids, proteins and DNA. This, in turn, can cause functional alterations in cells ultimately leading to apoptotic and necrotic cell death. Specific intracellular pathways that mediate oxidative stress in neurodegenerative diseases include mitochondrial electron transport,  $NO^{\cdot}$ -associated pathways, arachidonic acid (AA) metabolism, and protease activation. As part of the degenerative process functional alterations in these pathways occur that are intimately linked to increased levels of oxidative stress. Mitochondrial dysfunction leads to electron leakage from the electron transport chain and results in increased ROS production. Excitotoxicity occurs through excessive activation of glutamate receptors which leads to neuronal damage via mechanisms that involve the disruption of calcium homeostasis. This, in turn, influences mitochondria and can lead to increased ROS production and further promote neuronal cell death. Nitric oxide toxicity can occur under pathologic conditions when  $NO^{\cdot}$  may react with  $O_2^{\cdot-}$  to form the highly reactive  $ONOO^{\cdot-}$ . Inflammation initiated by activation of microglial cells, which are the resident macrophages in the brain, is generally considered a consequence of neuronal cell death and results in the generation of ROS and RNS by the activated microglial cells. Although it is difficult to determine whether oxidative stress is the cause or the

consequence of these dysfunctions, it certainly contributes to the final outcome which is neuronal cell death<sup>1,2,3,4</sup>.

An overwhelming sum of evidence for the involvement of oxidative stress in neurological pathologies has been obtained from **Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, diabetic neuropathy, mild cognitive impairment** and other neurological disorders. In many of these diseases free radical generation, hence oxidative stress, seems to become a self accelerating system. Initiated by biochemical and cellular changes that include oxidative stress in the central nervous system, disease progression is accompanied by further changes in cellular metabolism that cause more free radical production and thereby fuel the neurodegenerative process.



**Alzheimer's disease** is the most common neurodegenerative disorder, and the role of oxidative stress in Alzheimer's pathology has been well established. Factors that contribute to increased radical formation in Alzheimer's are the alteration of mitochondrial function, leading to electron leakage in the respiratory chain and the consequent formation of  $O_2^{\cdot-}$ ; the unbalanced high activity

of superoxide dismutase and monoamine oxidase B which causes the production of more H<sub>2</sub>O<sub>2</sub>; the alteration of iron homeostasis with increased iron levels in the brain causing increased OH<sup>•</sup> generation; the increased lipid peroxidation and membrane alterations; and the pro-aggregating effect of ROS on β/A4 protein and the C-terminal fragment of amyloid precursor<sup>5</sup>. Evidence for high levels of oxidative stress has been documented in Alzheimer's patients in countless publications. The biomarkers of oxidative stress that are found to be elevated in these patients include increased lipid oxidation in the frontal cortex, oxidative damage of glutamine oxidase, increased concentration of aluminium, elevated lipid peroxides in multiple brain regions and ventricular fluid, increased concentration of 3-nitrotyrosine, 8-hydroxydeoxyguanosine and isoprostanes in cerebrospinal fluid, increased DNA-damage in lymphocytes<sup>6,7,8,9,10,11,12</sup>.

**Parkinson's disease** is the second most common neurodegenerative disorder. Equally strong evidence has implicated oxidative stress in the pathogenesis of Parkinson's. In the substantia nigra in Parkinson's disease key alterations occur, in iron handling, mitochondrial function and in the antioxidant protective systems, particularly superoxide dismutase (SOD) and reduced glutathione. These indices of oxidative stress are accompanied by evidence of free radical mediated damage in the form of increased lipid peroxidation, oxidative protein modification and oxidation of DNA bases<sup>2,6,12,13,14</sup>.

**Huntington's disease (HD)**, a hereditary type of brain atrophy, is caused by mutation of the huntingtin gene. The mutant huntingtin protein that normally resides in the cytoplasm migrates into the nucleus where it forms protein aggregates. Studies of the involvement of oxidative stress in HD have found elevated levels of oxidative damage products such as malondialdehyde, 8-hydroxydeoxyguanosine, 3-nitrotyrosine and heme-oxygenase in areas of degeneration in HD brain; and increased free radical production in animal models. This indicates the involvement of oxidative stress either as a causative event, or as a secondary constituent of the cell death cascade in the disease<sup>15</sup>.

**Amyotrophic lateral sclerosis (ALS)**, also known as **Lou Gehrig's disease** and **motor neuron disease**, is inherited in 10% of cases, with a genetic defect in the SOD1 gene accounting for 2.5% of these, and sporadic in 90% of cases. It is characterised by adult onset progressive degeneration of motor neurons in the spinal cord and motor cortex. Multiple mechanisms contribute to pathogenesis in ALS, and recent advances suggest that oxidative stress may play a significant role in the amplification, and possibly the initiation, of disease. The evidence includes biomarkers of oxidative damage, i.e. lipid peroxidation and DNA damage in the cortex, spinal

cord and plasma of patients with ALS. Further evidence comes from a SOD1-mutant mouse model, where protein and lipid oxidation are elevated at both presymptomatic and symptomatic ages, and antioxidant supplementation improves survival. Excitotoxicity and free radical production are interlinked in a complex network of signalling interaction between the motor neurons, microglia and astrocytes that may lead to accelerated ROS production and promote neuronal cell death in ALS. Immune activation also plays an important role in ALS with activated microglia generating large numbers of toxic free radicals that contribute to the killing motor neurons. High levels of oxidative stress, however, are not only the consequence of inflammation but also contribute to its initiation. Oxidative stress in ALS also plays a role in pathways related to apoptosis, mitochondrial damage, and protein aggregation – intracellular events that are capable of generating oxidative stress *de novo*<sup>4</sup>.

### **Age as a risk factor in neurodegenerative diseases**

An established risk factor for many neurodegenerative diseases is age. This is in accordance with the fact that these diseases only become symptomatic when considerable neuronal damage has accumulated. Hence, the older someone the more likely they will show signs of neurodegeneration. Oxidative stress plays a major role in the slow accumulation of neuronal loss with age, and naturally decreasing antioxidant defences in the CNS with age potentially exacerbate or accelerate this progression. The fact that neurological symptoms only appear relatively late poses great difficulties for therapeutic intervention, and therefore, preventative measures in form of antioxidant supplementation should be considered early.

**In summary, the widespread distribution of oxidative stress in patients with neurodegenerative disorders, and its intimate interaction with the many mechanisms implicated in the pathogenesis of these diseases suggest that oxidative stress is likely a dominant process in the initiation of events leading to neurodegeneration, and is certainly a major factor in its progression. For this reason it is a rational choice to use therapeutic interventions with antioxidants to manage oxidative stress levels in neurodegenerative pathologies.**

### **Why to use flavonoids in the therapy of neurodegenerative diseases?**

Therapeutic intervention with antioxidants in neurodegenerative diseases has been suggested by many researchers and physicians in the field. Some clinical trials using Vitamin E, Vitamin C, deprenyl, and selegiline have been carried out in Alzheimer's and Parkinson's disease patients with mixed results<sup>12</sup>. So far, flavonoid preparations have not been trialled. Yet, there are several good reasons to use flavonoids in an adjunct therapy to

lower oxidative stress levels in patients with neurodegenerative disease<sup>16</sup>.

11. The radical scavenging capacity of flavonoids, and particularly proanthocyanidins is several times higher than that of the vitamin antioxidants for a wide range of radicals<sup>17</sup>. Flavonoids are chemically a diverse range of compounds containing different ring structures with different side chains and polymerisation states. This diversity greatly contributes to their radical scavenging ability and other effects on enzyme systems the body<sup>18,19</sup>. The potency as antioxidants and the chemical diversity of multi-compound flavonoid extracts, like ENZO Professional, strongly recommend flavonoids in therapeutic efforts to lower oxidative stress levels.
12. The anti-inflammatory properties of flavonoids that arise through influencing cellular signal transduction and enzyme systems may be even more important than the beneficial effect of radical scavenging by actually reducing free radical production<sup>20</sup>.
13. The metal chelating properties of flavonoids may be of particular benefit when considering the alterations in iron homeostasis that are common in Alzheimer's and Parkinson's disease leading to increased iron levels and contributing to increased levels of toxic hydroxyl radicals and peroxynitrite.
14. Many studies of flavonoids carried out in cell culture and animal models have documented neuroprotective effects and support the possibility of therapeutic benefit in the treatment of neurodegenerative disease. Ishige and colleagues have found in a cell culture system that flavonoids protect from oxidative stress by three distinct mechanisms: directly affecting glutathione metabolism, acting as antioxidants, and maintaining low calcium levels despite high levels of ROS<sup>16,21,22,23,24,25</sup>.
15. Studies following the effects of dietary consumption of flavonoids have shown improvements in motor and cognitive function which may be attributed to inhibition of oxidative and inflammatory processes in the brain, or reflect a more direct enhancement of brain function<sup>19,26</sup>.

**In conclusion, flavonoids are promising agents and should be considered for an adjunct therapy to combat oxidative stress and brain inflammatory processes in neurodegenerative disorders. High dose flavonoid supplementation may also have great benefits in the prevention of neurodegenerative diseases and should therefore be considered early, even before the onset of symptoms.**

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