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Protection Against Oxidative Stress and “IGF-I Deficiency Conditions”

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1. Introduction

Oxidative stress is thought to contribute to the development of a wide range of diseases including neurodegenerative (Alzheimer, Parkinson, Amyotrophic Lateral Sclerosis...), diabetes, cancer, rheumatoid arthritis, cardiovascular and liver diseases several of them are related with low levels of IGFs such as degenerative and aging disorders [1-8].

Oxidative stress represents an imbalance between the production of ROS/RNS and a biological system's ability to detoxify the reactive intermediates or to repair the resulting damage. In normal conditions ROS are reduced into water. For these reason cells are protected against oxidative stress by an interacting network of antioxidant enzymes. This detoxification pathway is the result of several enzymes, with Superoxide Dismutase (SOD, EC 1.15.1.1) catalyzing the first step O_2 into H_2O_2 and Catalase (CAT; EC. 1.11.1.6) and Glutathione Peroxidase (GSH-Px; EC 1.11.1.9) removing H_2O_2 into H_2O by two different pathways. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals exert deleterious effects on cell through direct attack on DNA, proteins or membrane lipids (including mitochondrial lipids). These oxidative damages lead to the cellular death. The mechanisms of oxidative cellular damage are summarized in Fig. 1.

The main sources of ROS/RNS are: exogenous (γ irradiation, UV irradiation, drugs, xenobiotics, and toxin metabolism) and endogenous (metabolic pathways, mitochondrial respiration, oxidative burst, fagocytosis, enzyme activities, aging and diseases).

Although the exposure of the organism to ROS/RNS is extremely high from exogenous sources, the exposure to endogenous sources is much more important and extensive, because it is a continuous process during the life span of every cell in the organism.

Mechanisms of oxidative cellular damage

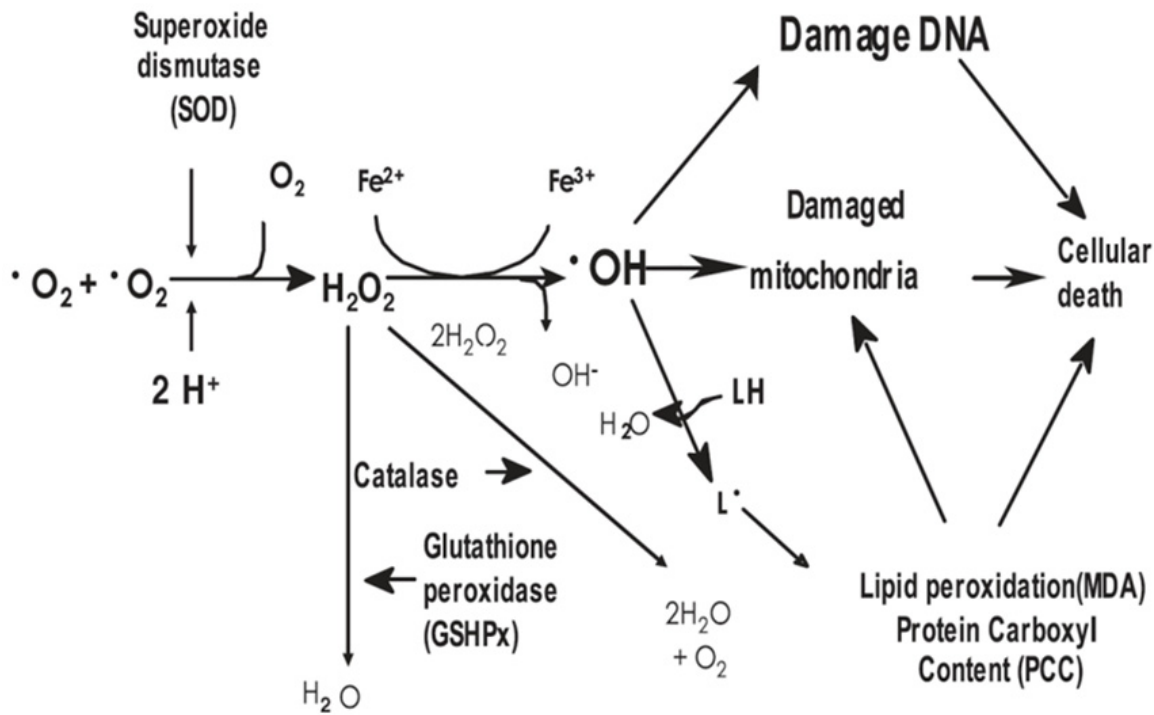


Figure 1. Mechanisms of oxidative cellular damage. Free radicals are reduced into water with the cooperation of the three main antioxidant enzymes: SOD, Catalase, and GSHPx. The generation of hydroxyl radicals from hydroperoxide produces the development of oxidative cell injury: DNA damage; carboxylation of proteins; and lipid peroxidation, including lipids of mitochondrial membranes. By these pathways, oxidative damage leads to cellular death. [5]

Mitochondria are the major endogenous source of ROS underphysiological conditions, because 2% to 3% of the O_2 consumed is converted to $\text{O}_2\cdot^-$. Intramitochondrial ROS production increases after peroxidation of intramitochondrial membrane lipids. Furthermore mitochondria are particularly sensitive to ROS/RNS-induced injury in the pathogenesis of disease. Oxidative stress exerts deleterious effects on mitochondria function by directly impairing oxidative phosphorylation through direct attack of proteins or membrane lipids. ROS/RNS can also induce mitochondrial DNA deletions and mitochondrial membrane permeability transition (MMPT). MMP pore opening activates caspases, which is an endpoint to initiate cell death. Recently, a large number of studies have associated mitochondrial dysfunction caused by ROS/RNS to both accidental cell death (necrosis) and programmed cell death (apoptosis).

Continuous exposure to various types of oxidative stress from numerous sources has led the cell and the entire organism to develop defense mechanisms for protection against reactive metabolites. Mitochondrial like a source of ROS are summarized in Fig. 2.

Damaged mitochondria like an intracellular source of ROS

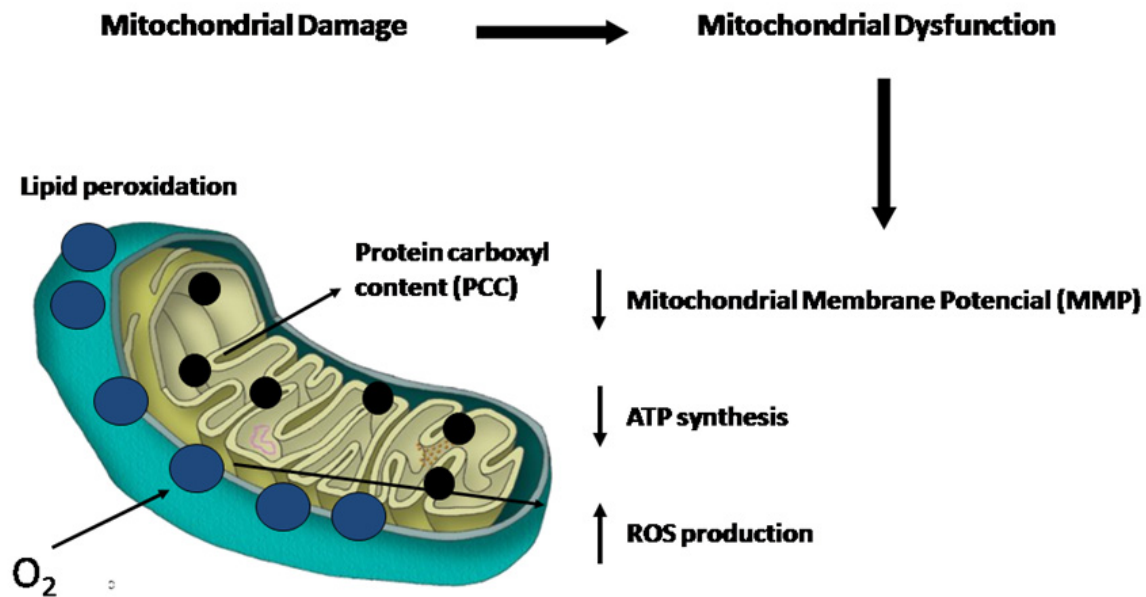


Figure 2. Mitochondrial damage is an intracellular source of ROS. Oxidative stress exerts deleterious effects on mitochondria function by directly impairing oxidative phosphorylation through direct attack of proteins or membrane lipids. Mitochondrial damage produces mitochondrial dysfunction decreasing MMP and ATP synthesis and increasing ROS production.

Understanding that mitochondria are the most important cellular targets of IGF-I [5, 6, 7] and it is the main intracellular source of ROS our results show a novel mechanism for oxidative stress regulation through mitochondrial protection and normalization of antioxidants enzymes activities by IGFs signaling pathways.

2. Oxidative Stress: Mitochondrial damage and antioxidant defences

2.1. Mitochondrial damage

Mitochondria play a central role in many cellular functions including energy production, respiration, heme synthesis, and lipids synthesis, metabolism of amino acids, nucleotides, and iron, and maintenance of intracellular homeostasis of inorganic ions, cell motility, cell proliferation and apoptosis [8, 9]. Mitochondria contain their own DNA (mtDNA). The mtDNA occurs in small clusters called nucleoids or chondriolites. The number of mtDNA molecules in nucleoids varies in size and numbers in response to physiological conditions. While nuclear DNA encodes the majority of the mitochondrial proteins only a few of these proteins are encoded by mitochondrial DNA. A recent mitochondrial proteomic study in *S. cerevisiae* identified at least 750 mitochondrial proteins that perform mitochondrial function

[10]. The last decade has witnessed an increased interest in mitochondria, not only because mitochondria were recognized to play a central role in apoptosis but also since mitochondrial genetic defects were found to be involved in the pathogenesis of a number of human diseases [9,11].

A variety of cellular systems, including NADPH oxidase, xanthine oxidase, uncoupled eNOS (endothelial NO synthase) and cytochrome P450 enzymes, can generate ROS, but, in most mammalian cells, mitochondria are the principal organelles for ROS production. The production of mitochondrial ROS is a consequence of oxidative phosphorylation at the respiratory chain complexes I and III where electrons derived from NADH and FADH can directly react with oxygen or other electron acceptors and generate free radicals [12-14]. Indeed, the increase of the redox potential at complex I and complex III induces ROS generation [15, 16]. Mitochondria are also a major site for the accumulation of low molecular weight Fe^{2+} complexes, which promote the oxidative damage of membrane lipids [17-19].

Mitochondria do not only represent the major source of ROS production but they are also the major targets for their damaging effects. Mitochondrial DNA (mtDNA) seems to be highly vulnerable to oxidative challenges compared to nuclear DNA for three main reasons: 1) its close proximity to the electron transport chain (ETC), 2) it is continuously exposed to ROS generated during oxidative phosphorylation (it is estimated that up to 4% of the oxygen consumed by cells is converted to ROS under physiological conditions) [20] and 3) its limited capacity of DNA repair strategies and the lack of protection by histones [21]. ROS also produce more than 20 types of mutagenic base modifications in DNA [22]. These DNA lesions cause mutations in mtDNA that can lead to impairment of mitochondrial function [23]. Taken together, this makes clear that mtDNA is extremely susceptible to mutation by ROS-induced damage. Given that mitochondria are the major producer of ATP, it is also likely that mitochondrial dysfunction leads to the reduction in ATP level that may affect ATP-dependent pathways involved in transcription, DNA replication, DNA repair, and DNA recombination. Mitochondria are intimately involved in deoxyribose nucleoside triphosphate (dNTP) biosynthesis [24]. It is conceivable that mitochondrial damage contributes to mutagenesis of the nuclear genome in part due to impaired nucleotide biosynthesis. In fact, it is well established that an imbalance in the dNTP pool is mutagenic to cells [25]. Studies demonstrate that a dNTP pool imbalance can induce nucleotide insertion, frame-shift mutation [25] sister chromatid exchange, recombination and double-strand break [26].

Mitochondria also play a key role in regulation of apoptosis under a variety of pathological conditions, including ischemia, hypoxia, and myocardial infarction [27-30]. The electrochemical potential across mitochondrial membrane, MMP, is known to be highly sensitive to apoptotic stimulation. As an index of mitochondrial function in living cells, MMP can be measured with an indicator dye, e.g., rhodamine 123 (Rh123), which fluoresces in direct proportion to MMP [31]. Decreased MMP occurs in cells undergoing apoptosis induced by oxidative agents, such as H_2O_2 in primary neuronal cultures [32]. Injured mitochondria can release cytochrome-c into the cytoplasm when cells are treated with

proapoptotic stimuli [33]. Cytochrome-c activates the apoptosome containing the caspase-activating protein Apaf-1 and subsequently the caspase cascade that induce apoptosis [34].

Because macromolecules in mitochondria (including mtDNA) are particularly susceptible to oxidative damage of mitochondrial turnover is critical for the maintenance of a healthy mitochondrial phenotype, normal energy production, and the promotion of healthy aging [35]. Mitochondria are highly dynamic organelles, and deregulation of mitochondrial turnover is likely one of the intrinsic causes of mitochondrial dysfunction, which contributes to deregulation of cell metabolism, oxidative stress, and altered signal transduction during the aging process.

Autophagy is a catabolic process that contributes to the maintenance of cellular homeostasis through the degradation of damaged mitochondria in lysosomes. The available evidence suggests that there is an age-dependent decline in autophagic function, which likely contributes to the accumulation of damaged non-functional mitochondria. In addition, dysfunction of the proteasomes [36] may be also contributed to the accumulation of damaged mitochondrial proteins in the age diseases.

Mitochondria are important cellular targets of IGF-I [5, 6], different groups have described that IGF-I decreases mitochondrial superoxide production [37] and low levels of IGF-I have been linked to increase in oxidative stress damage [3, 5, 40-42]. IGF-I also acts as an anti-apoptosis factor of multiple cell types, and its anti-apoptotic effects occur through engagement with IGF-I receptor (IGF-IR) and thought to activate an intracellular signal transduction pathway that may modulate the mitochondria, cytochrome c and caspase pathway [38, 39]. Recent studies show that treatment of aged rodents with IGF-I confer mitochondrial protection, including an attenuation of mitochondrial ROS generation in the liver [40-42]. The available data suggest that treatments that increase circulating IGF-I levels exert citoprotective effects in aging and degenerative diseases [1-3, 5-7, 42]. Thus, further studies are necessary to determine the role of mitochondrial mechanisms in beneficial effects of IGF-I treatment, including the effects of IGF-I on autophagy of dysfunctional mitochondria and apoptosis.

2.2. Antioxidant defenses

ROS and RNS consist of radicals and other reactive oxygen/nitrogen factors that can react with other substrates. Examples of ROS and RNS are superoxide, nitric oxide, peroxynitrite and hydrogen peroxide. Under physiological conditions, these are counterbalanced by an array of defense pathways, and it needs to be emphasized that ROS and RNS have many physiological roles that include signaling. In excess, or in situations where defenses are compromised, ROS and RNS may react with fatty acids, proteins and DNA, thereby causing damage to these substrates. Under normal conditions, antioxidant defenses include the enzymatic and non-enzymatic defense systems regulate the ROS and RNS produced. Antioxidants regulate oxidative and nitrosative reactions in the body and may remove ROS and RNS through scavenging radicals, decreasing the production of ROS and RNS, thus preventing the damage caused by ROS and RNS.

a. Enzymatic defense systems.

Enzymatic defense systems such as Superoxide Dismutases (SOD), Catalases (CAT), Glutathione Peroxidases (GPx), Glutathione Reductases (GSR or GR; EC 1.8.1.7) and Glutathione Transferases (GST; EC 2.5.1.18) protect mitochondria and DNA from oxidative stress. It has been proposed that polymorphisms in these enzymes are associated with DNA damage and subsequently the individual's risk of wide range of diseases susceptibility [42, 43].

Superoxide Dismutases catalyze the dismutation of O_2^- to H_2O_2 and O_2 , rendering the potentially harmful superoxide anion less hazardous. SODs require a metal cofactor for function and can be grouped by the bound metal ion [44-46]. Iron containing SODs have been found in prokaryotes and some plants. Manganese SODs (MnSOD) are found in both prokaryotes and eukaryotes and are localized primarily to the mitochondria. MnSOD is encoded by the *SOD2* gene mapping to chromosome 6 in human. The third class represents SODs, which require both copper and zinc as cofactors (Cu-Zn SODs). Cu-Zn SODs are homodimers in eukaryotes and are located predominantly in the cytoplasm. In most prokaryotes, Cu-Zn SODs exist as homodimers found in the periplasm. Human Cu-Zn SOD, encoded by the *SOD1* gene mapping to chromosome 21, has been implicated as the source of some cases of familial Amyotrophic Lateral Sclerosis (ALS) [47, 48]. The eukaryotic extracellular SOD is a subset of the Cu-Zn variety but functions as a tetramer rather than the usual dimer found in eukaryotes. Human extracellular Cu-Zn SOD, encoded by the *SOD3* gene mapping to chromosome 4 [49], *SOD3* protein can be measured in plasma, lymph, cerebrospinal, and synovial fluids [50, 51]. Almost all human tissues contain measurable levels of *SOD3* and at least eight different tissues; including heart, lungs, and placenta, synthesize *SOD3* mRNA. *SOD3* is the primary enzymatic antioxidant defense of the vascular wall. The physiopathological role of *SOD3* has been examined in vascular-related diseases, atherosclerosis, hypertension, diabetes, ischemia-reperfusion injury, lung disease, various inflammatory conditions, and neurological diseases.

Catalase catalyzes the reduction of H_2O_2 to water using either an iron or manganese cofactor [52, 53]. CAT is a common antioxidant enzymes found in nearly all-living organisms that are exposed to oxygen and they can also remove organic H_2O_2 to oxidize toxins including phenols, formic acid, and hydroperoxides. CAT is present only or primarily in the peroxisome fraction and is absent in mitochondria of mammalian cells, except rat heart mitochondria [54]. Therefore, the only enzymatic defense system against hydrogen peroxide in mitochondria is the glutathione redox cycle system.

CAT is a homotetramer encoded by *ctt1* gene mapping in chromosome 11 [55].

Genetic polymorphisms in catalase and its altered expression and activity are associated with oxidative DNA damage and subsequently the individual's risk of cancer susceptibility [56]. A few polymorphisms have been described for the catalase-encoding gene; these are normally related with the development of mental disorders [57]. Humans with low catalase levels (acatalasemia) have an increased risk for diabetes mellitus; while the clinical features of acatalasemia are oral gangrene, altered lipid, carbohydrate, homocysteine metabolism

and the increased risk of diabetes mellitus [58] and lower levels of catalase activity in other tissues, seem to be asymptomatic.

Glutathione Peroxidase (GPx) are critical intracellular enzymes involved in the reduction of hydrogen peroxide H_2O_2 to water and lipid peroxides to their corresponding alcohols using selenium cofactor in the majority of the cases. GPx are present both in the cytosol and in mitochondria [59]. There are at least eight GPx enzymes: GPx1–GPx8 [60, 61]. The *GPx 1-8* genes mapping to chromosomes 3, 14, 5, 19, 6, 6, 1 and 5 respectively. Whereas GPx1 is the most abundant selenoperoxidase and is ubiquitously expressed in almost all tissues [60, 61], GPx2 expression is most prominent in the gastrointestinal tract [62]. Expression of GPx3 is greatest in the kidney, although this enzyme is expressed in various tissues and is secreted into extracellular fluids as a glycoprotein [63, 64]. Different from other glutathione peroxidases, GPx4, or phospholipid hydroperoxide GPx, is not a tetramer, but rather a monomer, and is the only GPx enzyme that reduces phospholipid hydroperoxides [65]. In addition, GPx4 contains a mitochondrial isoform that mediates the apoptotic response to oxidative stress [66, 67] and has a peroxidase independent structural role after sperm maturation [68]. GPx6 was identified as a selenoprotein in the human genome by homology search [69]. However, GPx6 from rodents and GPx5 from both humans and rodents do not contain Sec or Se [69]. Recently, GPx7 and GPx8 were also identified as selenium-independent GPx can act as true H_2O_2 scavengers, as expected of the selenium-dependent members [70-73]. Lower GPx activity predispose towards an impaired antioxidant protection and consequently stress oxidative damage to membrane fatty acids and functional proteins and, by inference, to neurotoxic damage, and hence the process of neuroprogression that accompanies severe or persistent illness [74].

Glutathione Reductase, also known as *GSR* or *GR*, is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant [75, 76]. GSR is a homodimer found are present both in the cytosol and in mitochondria.

For every mole of oxidized glutathione (GSSG), one mole of NADPH is required to reduce GSSG to GSH. The enzyme forms a FAD-bound homodimer. Human *GSR* gene mapping to chromosome 8. The *GR* is conserved between all kingdoms. In bacteria, yeasts, and animals, one *gr* gene is found; however, in plant genomes, two *gr* genes are encoded. *Drosophila* and *Trypanosomes* do not have any *GR* at all [76]. In these organisms, glutathione reduction is performed by either the thioredoxin or the trypanothione system, respectively [77, 78]. In cells exposed to high levels of oxidative stress, like red blood cells, up to 10% of the glucose consumption may be directed to the pentose phosphate pathway (PPP) for production of the NADPH needed for this reaction. In the case of erythrocytes, if the PPP is non-functional, then the oxidative stress in the cell will lead to cell lysis and anemia [79].

Glutathione Transferases have also been called glutathione *S*-transferases, *GST*. These enzymes catalyze nucleophilic attack by reduced glutathione (GSH) on nonpolar compounds that contain an electrophilic carbon, nitrogen, or sulphur atom. Their substrates include halogenonitrobenzenes, arene oxides, quinones, and α,β -unsaturated carbonyls [80-84]. Three major families of proteins that are widely distributed in nature exhibit glutathione

transferase activity. Two of these, the cytosolic and mitochondrial GST, comprise soluble enzymes that are only distantly related [85, 86]. The third family comprises microsomal GST and is now referred to as membrane-associated proteins in eicosanoid and glutathione (MAPEG) metabolism [87].

Cytosolic GSTs of mammals have been particularly well characterized, and were originally classified into Alpha, Mu, Pi, Theta, Zeta and Omega classes on the basis of a substrate/inhibitor specificity, primary and tertiary structure similarities and immunological identity [88]. The alpha class genes (*GSTA1-5*), located in a cluster mapped to chromosome 6, play an important role in cellular protection against oxidative stress and they are the most abundantly expressed glutathione S-transferases in liver. Decreased in alpha class GSTs has been observed in stomach and liver tumors. A significant decrease of glutathione transferase activity was described in amygdala, hippocampus and inferior parietal lobule in patients with AD [89]. The Mu class of GSTs has five genes (*GSTM1-5*) [90] that are found in a gene cluster on chromosome 1 [91]. The *GSTM1* gene contains four alleles that have been associated with a decreased risk of bladder cancer [92]. Neurodegenerative diseases such as PD and schizophrenia are characterized by the degeneration of dopaminergic neurons. *GSTM2-2* has been shown to catalyze the conjugation of GSH to aminochrome, a ROS generated in the redox cycling of orthoquinones within dopaminergic neurons [93]. Hence, *GSTM2-2* has been proposed to play a protective role against neurodegenerative diseases. The Theta class of GST has a null phenotype whereby individuals do not express catalytically active protein. The lack of enzyme activity, and therefore, an inability to detoxify carcinogens is associated with an increased risk toward a variety of cancers. The null phenotype is associated with an increased risk of tumors of the head and neck, oral cavity, pharynx, and larynx [94, 95]. The chromosomal locations of the genes that encoded for the different types of GST and the related diseases are summarized in table 1.

b. Non-enzymatic defense systems.

Examples of non-enzymatic defense systems are scavenger antioxidants (coenzyme Q10, vitamin C and E, and glutathione) and some proteins which act as antioxidants by binding ROS and RNS, e.g. thioredoxin (Trx), SS-peptides and acute phase proteins such as albumin, transferrin, haptoglobin and ceruloplasmin. These antioxidant systems thus protect the tissues against ROS and RNS.

Coenzyme Q10 (CoQ10) is an endogenous compound found in the inner mitochondrial membrane, is essential to electron transport and ATP production via the respiratory chain. CoQ10 is a strong anti-oxidant that confers resistance to mitochondrial damage by decreasing ROS/RNS production and that may suppress the production of proinflammatory substances, like nuclear factor κ B (NF κ B) gene expression and the production of pro-inflammatory cytokines [95-100]. CoQ10 and *vitamin E* are lipophilic antioxidants that are target to mitochondrial matrix by Triphenylphosphonium ion (TPP⁺) (mitoQ and mitovitamin E). These mitochondrial-targeted drugs can achieved concentrations in the mitochondrial matrix 100- to 1000-fold higher than those in the cytosol because of their strong positive charge, as mitochondria have a highly negative

Antioxidant Enzymes	E.C.	Subtypes	Subcellular Location	Chromosomal Location	Related Diseases
Superoxide Dismutases	SOD, ss EC 1.15.1.1	SOD1 (Cu-Zn SODs)	Cytosol	Chromosome 21	Familial Amyotrophic Lateral Sclerosis (ALS)
		SOD2 (MnSOD)	Mitochondrial	Chromosome 6	Cancer, asthma and degenerative disease
		SOD3 (Cu-Zn SODs)	Extracellular	Chromosome 4	Vascular-related diseases, atherosclerosis, hypertension, diabetes, ischemia-reperfusion injury, lung disease, various inflammatory conditions, and neurological diseases
Catalase	CAT, EC.1.11.1.6		Peroxisome fraction	Chromosome 11	Mental disorders, Diabetes mellitus.
Glutathione Peroxidases	GSH-Px, EC 1.11.1.9	GPx1 GPx2 GPx3 GPx4 GPx5 GPx6 GPx7 GPx8	Cytosol/Mitochondrial	Chromosome 3 Chromosome 14 Chromosome 5 Chromosome 19 Chromosome 6 Chromosome 6 Chromosome 1 Chromosome 5	Mental disorders
Glutathione Reductase	GSR or GR, EC 1.8.1.7		Cytosol/Mitochondrial	Chromosome 8	Red blood diseases
		Alpha, A1-1	Cytosol	Chromosome 6	
		Alpha, A2-2		Chromosome 6	
		Alpha, A3-3		Chromosome 6	
		Alpha, A4-4		Chromosome 6	
		Alpha, A5-5		Chromosome 6	
		Mu, M1-1		Chromosome 1	
		Mu, M2-2		Chromosome 1	
		Mu, M3-3		Chromosome 1	

Antioxidant Enzymes	E.C.	Subtypes	Subcellular Location	Chromosomal Location	Related Diseases
Glutathione Transferases	GST; EC 2.5.1.18	Mu, M4-4		Chromosome 1	Neurodegenerative, liver diseases and cancer
		Mu, M5-5		Chromosome 1	
		Pi, P1-1		Chromosome 11	
		Theta, T1-1		Chromosome 22	
		Theta, T2-2		Chromosome 22	
		Zeta, Z1-1		Chromosome 14	
		Omega, O1-1		Chromosome 10	
		Omega, O2-2		Chromosome 10	
		Kappa, K1-1	Mitochondrial/peroxisome	Chromosome 7	
		gp I, MGST2	Microsomal	Chromosome 4	
		gp I, FLAP		Chromosome 4	
		gp I, LTC4S		Chromosome 1	
		gp II, MGST3		Chromosome 1	
		gp IV, MGST1		Chromosome 12	
		gp IV, PGES1		Chromosome 9	

Table 1. Enzymatic defense systems

membrane potential between -150 mV and -180 mV. Both clinical and rodent studies have reported moderately beneficial actions of CoQ10 in reducing blood pressure, decreasing blood glucose, forestalling myocardial damage secondary to chemotherapeutic administration, limiting tumor growth, enhancing endothelial function and improving cognitive function in both Alzheimer’s and Parkinson’s disease patients [101]. However, one of the major limiting factors in the use of CoQ10 as a supplement is its bioavailability and delivery to the source of ROS generation.

Several other studies have used MitoQ10 in a variety of animal models of disease [102] and the results indicate that MitoQ10 protects against liver damage in an animal model of sepsis [103], contributes to the aetiology of the metabolic syndrome and atherosclerosis in a mouse model [104] protects pancreatic β -cells against oxidative stress and improves insulin secretion in glucotoxicity and glucolipotoxicity [105] and even protects against oxidative stress and cell death in the brain of rats exposed to the insecticide dichlorvos [106]. Importantly, the first clinical evidence of a potential benefit of MitoQ10 in humans comes from a study that MitoQ10 reduces liver damage induced by hepatitis virus infection [107].

Glutathione is formed in the liver from three amino acids, namely glycine, glutamine and cysteine. Cysteine is the rate-limiting step in the synthesis of reduced glutathione (GSH), the active form of glutathione. Glutathione has three major functions: 1) it is a strong antioxidant that protects cells against damage caused by free radicals and it recycles *vitamin C and E*, so that they again become active as antioxidants after been used in antioxidant processes. b) Glutathione is employed by the white blood cells as a source of energy used for lymphoproliferation. Therefore, glutathione may help increase the resistance to bacterial and viral infections. c) Glutathione is a natural purifier and therefore high concentrations are found in the liver.

SS-Peptides the Szeto-Schiller (SS) compounds are tetrapeptides with an alternating aromatic-cationic amino acids motif, and demonstrated in the inner mitochondrial membrane more than 1000-fold in comparison with the cytosolic concentration [108-110]. Although the positive charge might explain the mitochondrial-targing effect, the mitochondrial uptake of these SS peptides appears to be on mitochondrial potencial, as they are concentrated even in depolarized mitochondria [91, 92]. SS peptides are capable of scavenging H₂O₂ and ONOO⁻ and inhibiting lipid peroxidation. By reducing mitochondrial ROS production, these molecules inhibit MMPT and cytochrome *c* release and so prevent oxidant-induced cell death [111]. Using these peptides in an animal model of ischaemia/reperfusion injury improves cardiac function [112]. Whereas treatment with SS peptides attenuated mitochondrial H₂O₂ release induced by a high-fat diet and preserved insulin sensitivity in skeletal muscle [113]. Importantly, pre-clinical studies support the use of these peptides during ischaemia/reperfusion injury and neurodegenerative disorders [114].

Thioredoxin (Trx) is a multifunctional low-molecular weight protein containing an active thiol/disulphide site and possessing oxido reductase activity. Originally discovered in *E. coli*, Trx was later found in many prokaryotic and eukaryotic cells. The major Trx isoforms are cytosolic Trx1 and mitochondrial Trx2. Thioredoxins evolved similarly to chaperone-like proteins, whose function is maintenance of the dithiol/disulphide structure of proteins. A highly conservative amino acid sequence of the active centre (Trp-Cys-Gly-Pro-Cys- Lys) contains two active Cys residues (Cys32 and Cys35 in human Trx1 and Cys90 and Cys93 in human Trx2) that are oxidized into corresponding disulphides due to the transfer of two reducing equivalents from Trx to a disulphide-containing substrate. The disulphides formed in the active centres of Trx1 and Trx2 are reduced by thioredoxin reductase (TrxR). TrxR is an NADPH-dependent homodimer of oxidoreductase which reduces the active centre of disulphide in oxidized Trx and has two major isoforms, cytosolic (TrxR1) and mitochondrial (TrxR2). The Trx system plays a key role in cell function by limiting oxidative stress directly via antioxidant effects and indirectly by protein – protein interactions [115]. Cellular redox regulation of many processes is provided by the cooperation between the Trx and glutathione systems [115, 116]. Trx and GSH systems are involved in a variety of redox-dependent pathways such as supplying reducing equivalents for ribonucleotide reductase and peptide methionine sulfoxide reductase, the latter being involved in antioxidant defense and regulation of the cellular redox state [117]. The promoter of the Trx gene contains a series of stress responsive elements, various transcription factor binding sites, such as SP1, AP-1, NFkB and antioxidants response elements (ARE) [118].

c. Antioxidant defense systems against mitochondrial ROS formation.

The mitochondrial respiratory chain, located in the inner mitochondrial membrane (IM), is composed of four multimeric integral membrane proteins complexes (complexes I-IV), coenzymeQ (CoQ), and cytochrome c (cyt c). Complex I accepts electrons from NADH and complex II accepts electrons from succinate. Electrons then move down an electrochemical gradient through CoQ to complex III, from complex III to cyt c, and from cyt c to complex IV, which uses four electrons to reduce molecular oxygen to water (Fig. 3). The production of mitochondrial ROS is a consequence of oxidative phosphorylation at the respiratory chain complexes I and III where electrons derived from NADH can directly react with oxygen or other electron acceptors and generate free radicals [12-14]. Indeed, the increase of the redox potential at complex I and complex III induces ROS generation [15, 16]. Mitochondrial and cell cytosolic antioxidant systems can neutralize excess mitochondrial ROS under most conditions. With the exception of generation at complex III, ROS production in mitochondria is exclusively directed towards the matrix where MnSOD catalyses dismutation to H_2O_2 [119] which is then reduced to H_2O by GSH and Trx systems (Trx2) [120]. As the regeneration of GSH and reduced Trx2 depends on the NADPH/NADP⁺ redox state, an efficient mitochondrial bioenergetic function is required to maintain antioxidant activity. Matrix ROS can also pass through the MMTP, formed by voltage-dependent anion channel (VDAC), cyclophilin D (cyp D) and the adenine nucleotide translocator (ANT), directly to the cytosol where Cu-Zn SOD catalyses dismutation to H_2O_2 [119] which is then reduced to H_2O by catalase. Complex III generates ROS on both sides of the mitochondrial inner membrane and in the intermembrane space, where Cu-Zn SOD converts O_2^- into H_2O_2 , which diffuses in the cytosol where catalase reduces it into H_2O . Thus the efflux of H_2O_2 from the mitochondria is relatively modest, but may be modulated by either mitochondrial ROS themselves or changes in antioxidant defenses.

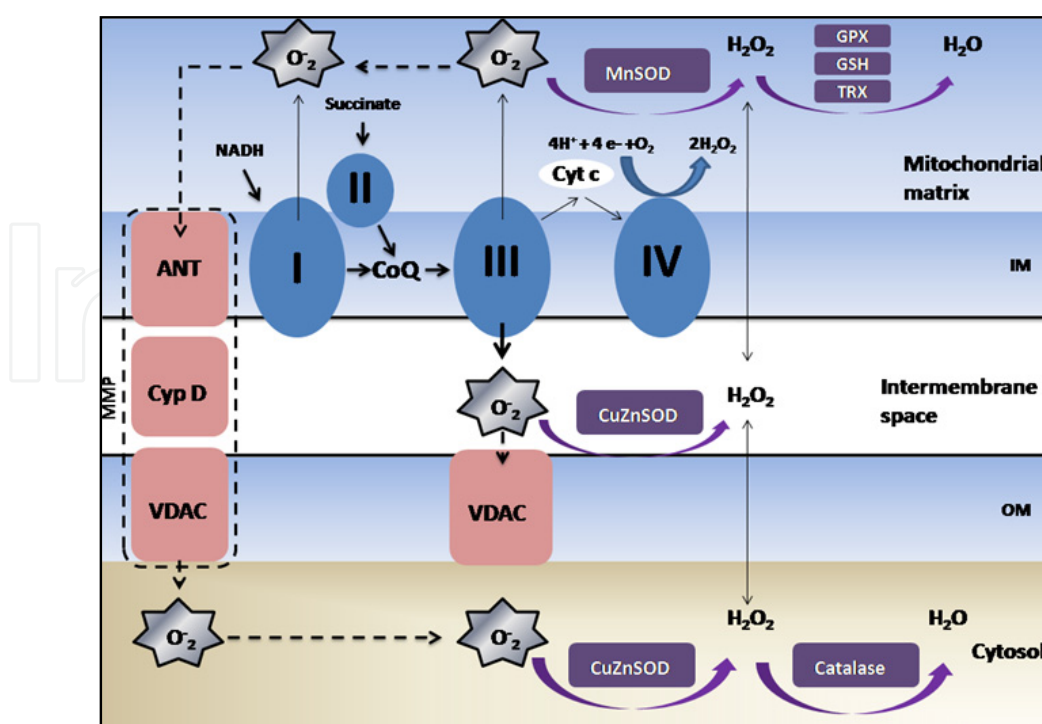


Figure 3. Antioxidant defense systems against mitochondrial ROS formation

d. Regulation of enzymatic antioxidant defense systems.

In order to prevent oxidative stress, the cell must respond to ROS by mounting an antioxidant defense system. Antioxidant enzymes play a major role in reducing ROS levels; therefore, redox regulation of transcription factors is significant in determining gene expression profile and cellular response to oxidative stress. There are different transcription factors involved in regulation of antioxidant enzymes and they can be regulated through IGFs signaling and other pathways related with receptors tyrosine kinases (RTKs). The most studied transcription factors are:

Nrf2 transcription factor: Phosphorylated Nrf2 translocates to the nucleus and binds the ARE. ARE driven expression of detoxifying and antioxidant enzymes and the cystine/glutamate transporter involved in GSH biosynthesis [121,122].

The PI3K/Akt/PP2A/GSK3 β and PKC/GSK3 play a role in regulation of Nrf2 and antioxidant gene regulation. PI3K pathway, consisting of p110 catalytic subunit and p85 regulatory subunit, is tightly coupled with RTKs activated by various growth factors such as IGFs, Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Nerve Growth Factor (NGF), and Vascular Endothelial Growth Factor (VEGF). PI3K is recruited to activate RTK dimers through a SH2 domain in the PI3K p85 regulatory subunit. PI3K catalyzes the synthesis of the second messenger phosphatidylinositol 3,4,5 triphosphate (PIP3) from phosphatidylinositol 4,5 bisphosphate (PIP2), wherein the membrane bound PIP3 serves as a signaling molecule to recruit proteins containing the pleckstrin homology (PH) domain. These PH domain proteins, such as the phosphoinositide-dependent protein kinase (PDK) and protein kinase B (AKT) serine/threonine kinases are thus activated and mediate further downstream signaling events [123]. The synthesis of PIP3 is negatively regulated primarily by the phosphatase and tensin homology (PTEN) phosphatase, which dephosphorylates PIP3 back to PIP2 [124]. Through PTEN, the PI3K pathway is subject to reversible redox regulation by ROS generated by growth factor stimulation. H₂O₂ was shown to oxidize and inactivate human PTEN through disulfide bond formation between the catalytic domain Cys-124 and Cys-71 residues [125, 126]. It was also demonstrated that endogenously generated ROS following treatment with peptide growth factors such as IGFs, EGF, or PDGF causes oxidation of PTEN leading to the activation of the PI3K pathway [127]. PTEN oxidation is reversed by peroxiredoxin II, a cytoplasmic peroxiredoxin isoform that eliminates H₂O₂ generated in response to growth factors [125]. It is noteworthy that various oxidants and ROS-producing chemicals activate transcription of a battery of antioxidant genes through a PI3K-NFE2-like 2 (Nrf2)-antioxidant response element (ARE) mechanism, where PTEN knockdown enhances transcription of ARE regulated antioxidant genes [128]. However, it is not known whether these oxidants induce PTEN oxidation and inhibition of phosphatase activity leading to gene activation. This leads to antioxidant gene expression that protects the cell. A role for NRF2 in drug resistance is suggested based on its property to induce detoxifying, drug transport, and antioxidant enzymes.

FOXO transcription factors: FOXO-mediated upregulation of MnSOD expression results in considerable lowering of cellular ROS [129]. Increase in ROS enhances FOXO transcriptional activity, and thus functions as a feedback mechanism. An increase in ROS levels induces activation of the small GTPase Ral, which will in turn lead to the phosphorylation and activation of the stress kinase JNK. Active JNK induces the phosphorylation of on FOXO. Phosphorylation of these residues is essential for FOXO transcriptional activity as shown by mutational analysis. Consistent with this, H₂O₂ treatment increases FOXO transcriptional activity and translocation of FOXO from the cytoplasm to the nucleus and activation of the transcription factor. Activation of FOXO through can now induce transcription of MnSOD and CAT, leading to a decrease in ROS levels. Thus, activation of FOXO by oxidative stress is part of a negative feedback loop to reduce the levels of oxidative stress in a cell, preventing damage to DNA, lipids and proteins.

Activation of PI3K/PKB signaling decreases FOXO activity and thus the levels of FOXO target genes like MnSOD and catalase [130]. Their regulation via PI-3K/PKB/FOXO signaling therefore implies that insulin, through this signaling cascade, may modulate the cellular ROS level.

Based on oxidative stress is an imbalance between the production of ROS and antioxidative defenses systems, IGF-I decreases mitochondrial ROS production and IGF-I/Akt pathway is involved in Nrf2 and FOXO activity, both transcription factors involved in the antioxidative enzymes regulation. We propose that IGF-I can exert direct effects on cells and can alter in opposite ways the expression of antioxidant enzymes depending on the ROS levels. This regulation may contribute to the citoprotective effects of treatment with low doses of IGF-I in experimental “IGF-I deficiency” conditions [2, 5, 6, 40-42]. This model is summarized in Fig. 4.

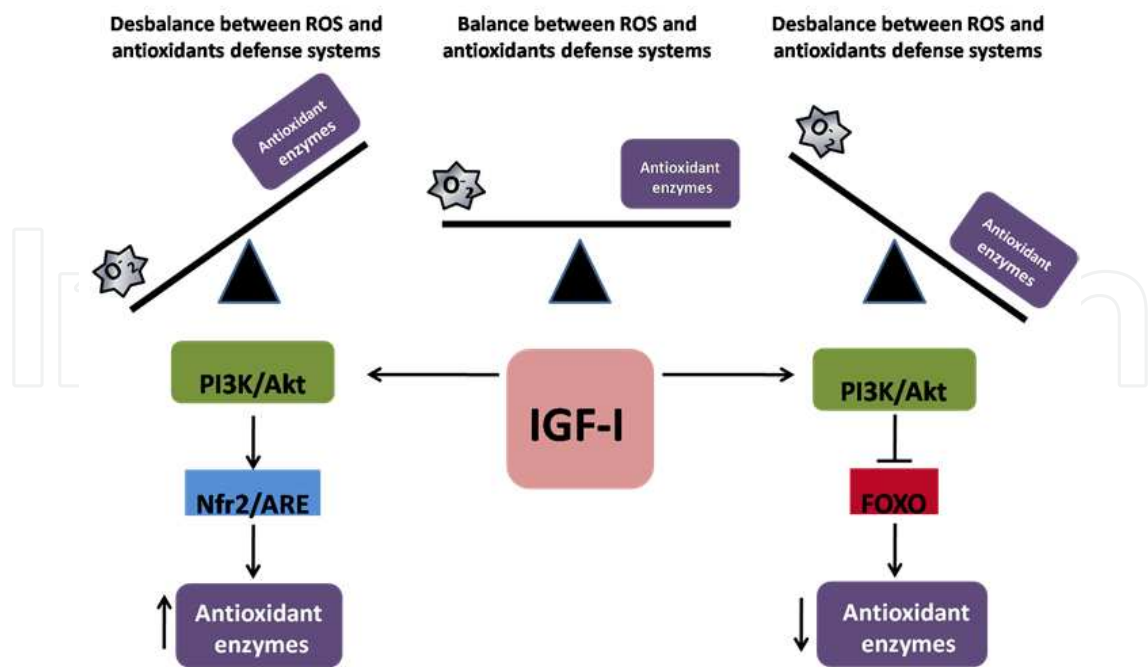


Figure 4. Regulation model of antioxidant enzymes mediated by IGF-I. IGF-I exerts a dual role depending on ROS concentration. High ROS levels increase antioxidant enzymes expression via Nrf2/ARE and low ROS levels decrease antioxidant enzymes expression via FOXO.

3. Aging and others conditions of "IGF-I deficiency" and oxidative stress

Mechanisms that cellular protection against oxidative injury are not well understood. It is known, however, that factors that promote the generation of ROS and/or impair antioxidative processes contribute to oxidative damage. Oxidative damage accumulates with aging and is likely responsible for the progressive decline in physiological systems. The identification of physiological regulators of antioxidative processes is critical to the understanding of degenerative diseases and aging processes. GH, IGF-I, IGF-II concentrations decline with age. The IGF-I is an anabolic hormone produced mainly in the liver in response to GH stimulation [131]. Circulating IGF-I serum levels decline by more than 50% in healthy older adults [132, 133]. Our team results show that exogenous administration of low doses of IGF-I restores IGF-I circulating levels and some age-related changes, improving glucose and lipid metabolisms, increasing testosterone levels and serum total antioxidant capability, and reducing oxidative damage in the brain and liver associated with a normalization of antioxidant enzyme activities and mitochondrial protection [5]. From these results we suggested that aging seems to be an unrecognized condition of "IGF-I deficiency." The best-known condition of "IGF-I deficiency" is Laron's dwarfism [134], characterized by an absence of GH receptors in the liver. Another condition of IGF-I deficiency is liver cirrhosis. In cirrhosis the reduction of receptors for GH in hepatocytes and the diminished ability of the hepatic parenchyma to synthesize cause a progressive decrease in serum IGF-I levels [135]. We have also shown previously that short courses of treatment with low doses of IGF-I in rats with carbon tetrachloride-induced cirrhosis had many systemic beneficial effects, and showed hepatoprotective and antioxidant properties, including mitochondrial protection [2, 3, 6, 41].

Oxidative Stress is one of the most important mechanisms of the cellular damage in aging [42,131] and in others "IGF-I deficiency" conditions such as liver cirrhosis where there are a diminution in IGF-I levels but not in GH levels followed of a decrease in liver biosynthetic capacity. In order to reproduce of "IGF-I deficiency" condition and the possible benefices of treatment with low doses of IGF-I, we used two different experimental models:

- a. Experimental model of cirrhosis: Male Wistar rats in which liver cirrhosis was induced using CCl₄. IGF-I therapy or saline was administrated the last 4 weeks [2, 6, 41, 137-139].
- b. Experimental model of aging: Healthy male Wistar rats were divided into two groups according to age: young control of 17 weeks, and aging control rats of 103 weeks. Old animals were randomly assigned to receive either saline or human IGF-I [5, 32,139,140].

In these experimental groups we measured the *oxidative stress* by determination of: Total serum antioxidant status [40], liver contents of pro-oxidative metals [138] (iron and copper), parameters of oxidative damage such as lipid peroxidation (MDA), protein carboxyl content (PCC) and activities of antioxidant enzymes in homogenates of brain and liver.

Understanding that mitochondria are one of the most important cellular targets of IGF-I [5, 6, 7] and they are the main intracellular ROS sources we studied the *mitochondrial function* by MMP, ATP synthesis, activities capase-3 processing and apoptosis in liver homogenates and isolated liver mitochondria [5, 41, 42] and intramitochondrial antioxidant capability of isolated liver mitochondria.

In these conditions of “IGF-I deficiency” low doses of IGF-I induced: a increase in the total serum antioxidant capacity closely related with serum IGF-I levels [40], a correlation between the SODs levels and MDA as shown in Fig. 5, a decrease of oxidative cell damage reducing MDA and PCC and improving antioxidant enzyme activities and a mitochondrial protection improving MMP, proton leak and reducing intramitochondrial ROS production and increasing ATP synthesis (Fig. 6), leading to reduced apoptosis [5, 41, 42].

All these data together provide evidence of the beneficial effect of IGF-I replacement therapy inducing anabolic [139] and antioxidant [5, 40, 41,139] actions in both experimental “IGF-I deficiency” conditions: cirrhosis and aging and experimental basis for further studies at exploring the potential IGF-I like a bioprotector due to its antioxidant, hepatoprotective and neuroprotective effects. Recently, we have also shown that IGF-II exerts similar effects [139,140]. The IGF-II is a peptide hormone that belongs to the family of IGFs. It plays an important role in the embryology development but the physiological function of IGF-II in the adult life are not fully understood [139,140]. IGF-II concentration decline with the age. Recently, we have also shown that low doses of IGF-II in aging rats exerts similar hepatoprotector and neuroprotector effects than IGF-I low doses therapy.

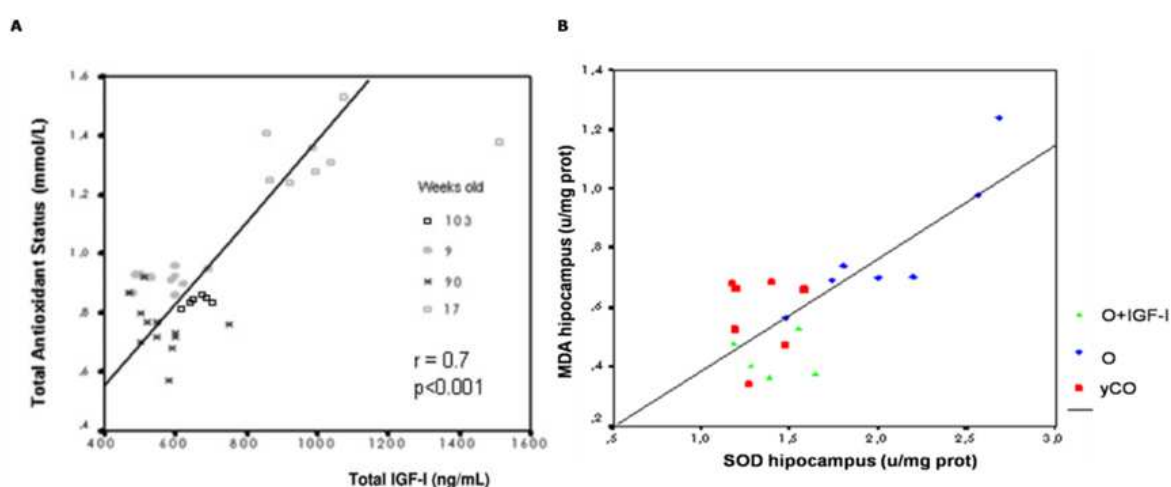


Figure 5. A) Correlation between TAS and IGF-I levels B) Correlation between SOD activity and MDA concentration

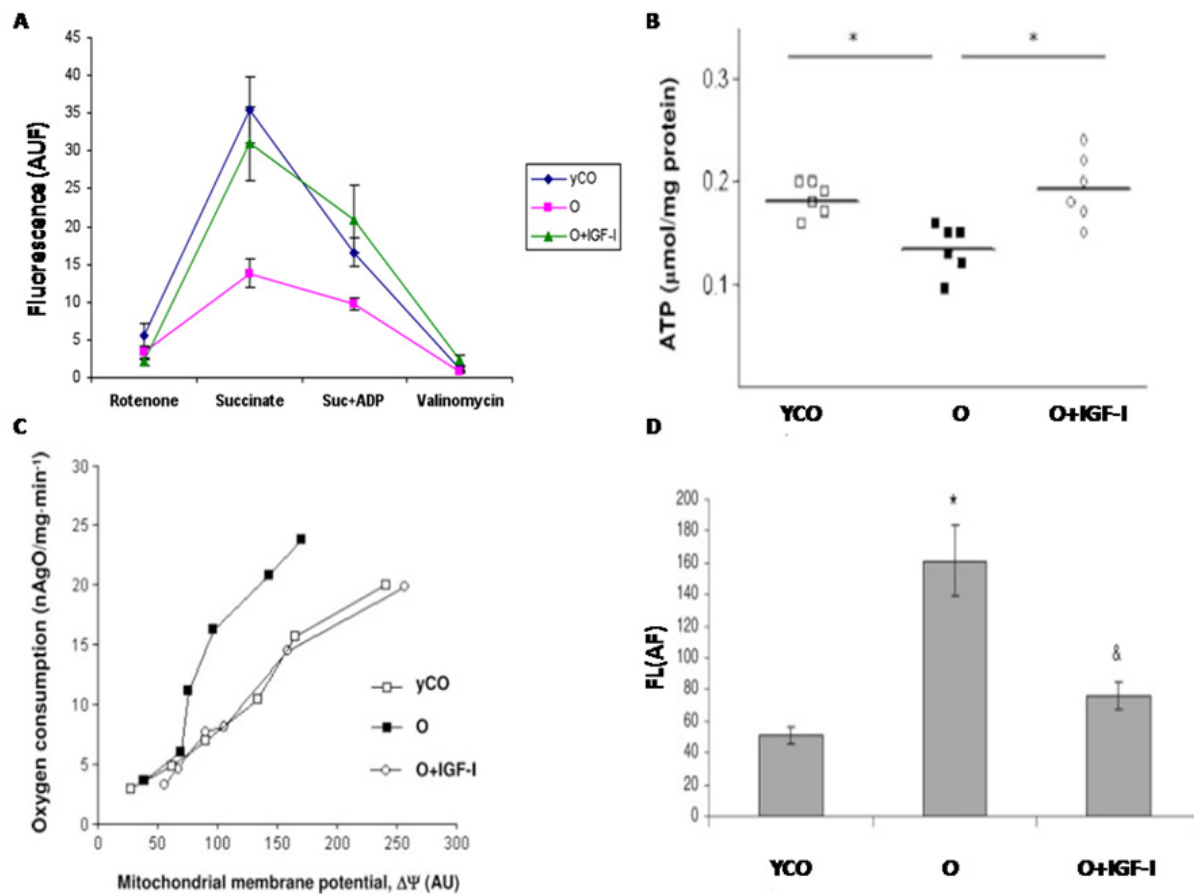


Figure 6. Different mitochondrial parameters were measured by cytometry in isolated liver mitochondria from healthy young control animals, old rats and old rats treated with low doses of IGF-I: A) MMP is considered a good marker of mitochondrial function, B) ATP synthesis, C) Proton leak and D) ROS intramitochondrial production.

4. Conclusions

Our results show that the cytoprotective effect of IGFs is closely related to a mitochondrial protection, leading to the reduction of intramitochondrial free radical production, oxidative damage, and apoptosis, increased ATP production and a normalization of antioxidant enzyme activities. Further studies are necessary to elucidate all mechanisms involved in the IGFs mitochondrial protection, including the effects of IGF-I on autophagy of dysfunctional mitochondria and apoptosis. In agreement with these results, it has been reported that IGF-I differentially regulates Bcl-xL and Bax. Previously, we reported that low doses of IGF-I restored the expression of several protease inhibitors such as the serine protease inhibitor 2 in cirrhotic rats [137], which could contribute to the described mitochondrial protection. Our work provides new evidence of beneficial effect of IGF-I replacement therapy in degenerative diseases including aging.

Abbreviations

Adenine Nucleotide Translocator (ANT)

Amyotrophic Lateral Sclerosis (ALS)

Antioxidants response elements (ARE)

Catalase (CAT)

Coenzyme Q10 (CoQ10)

Copper and Zinc SOD (Cu-Zn SODs)

Cytochrome c (cyt c)

Deoxyribose nucleoside triphosphate (dNTP)

Electron Transport Chain (ETC)

Glutathione (GSH)

Glutathione Peroxidase (GSP)

Glutathione Ttransferases (GST).

Glutation Reductases (GSR)

Growth factor hormone (GH),

IGF-I receptor (IGF-1R)

Inner mitochondrial membrane (IM),

Insulin-like growth factor I (IGF-I)

Manganese SOD (MnSOD)

Mitochondrial DNA (mtDNA).

Mitochondrial Membrane Permeability Transition (MMPT)

Nerve Growth Factor (NGF)

Nuclear Factor κ B (NF κ B)

Pentose phosphate pathway (PPP)

Phosphatidylinositol 3, 4, 5 triphosphate (PIP3)

Phosphatidylinositol 4, 5 bisphosphate (PIP2)

Platelet-Derived Growth Factor (PDGF)

Protein Carboxyl Content (PCC)

Reactive Nitrogen Species (RNS)

Reactive oxygen species (ROS)

Rhodamine 123 (Rh123)

Superoxide Dismutase (SOD)

Szeto-Schiller (SS)

Thioredoxin (Trx)

Triphenylphosphonium ion (TPP+)

Tyrosine Kinases (RTKs)

Vascular Endothelial Growth Factor (VEGF)

Voltage-Dependent Anion Channel (VDAC)

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