8

The Role of Oxidative Stress in Pathogenesis of Diabetic Neuropathy: Erythrocyte Superoxide Dismutase, Catalase and Glutathione Peroxidase Level in Relation to Peripheral Nerve Conduction in Diabetic Neuropathy Patients

Gordana M. Djordjević[1], Stojanka S.Djurić[1], Vidosava B. Djordjević[1], Slobodan Apostolski[2] and Miroslava Živković[1]
[1]*University of Niš, Medical Faculty,*
[2] *University of Belgrade, Medical Faculty,*
Serbia

1. Introduction

Distal symmetrical polyneuropathy is the most common form of neurological complications in diabetes mellitus (DM). It is sensory-motor polyneuropathy dominated by sensitive symptoms and signs, primarily decrease or loss of sensibility in distal parts of extremities, or positive symptoms of prickling, burning pain and tingling. These symptoms can be extremely unpleasant for patients but at the same time they are a huge therapeutic problem as well. Motor symptoms and signs in early phase of DSP are often absent, although by neurophysiologic tests motor fibers damaging signs can be detected and this damage often leads to loss of functions in patients in advanced phase of the disease. The most significant clinical consequence of sensitive and autonomic fibers damage is feet ulceration, what is a leading cause for hospitalization and for lower extremities amputations, if trauma factors are excluded. Thus, diabetic DSP is important not only for clinical reasons but for economic ones as well, especially regarding the fact that DM prevalence is rapidly growing. For many years scientists have done numerous researches for therapeutic solutions to prevent, delay or slow the progression of the disease, aiming to better understanding of ethiopathogenesis of this disease. Special attention has been paid to oxidative stress (OS) role in pathogenesis of DM and diabetic neuropathy. Despite numerous experimental confirmations of OS in diabetic patients, there is still controversy about whether oxidative stress is just a side effect or it is a possible cause of diabetic neuropathy. More than thousand studies about this problem have already been reported and since this problem is not completely solved, further investigations are required. Therefore, a good knowledge of biochemistry of oxidative stress, cell signal transduction and antioxidative protection is necessary.

2. Biochemistry of oxidative stress

Oxidative stress is a consequence of the imbalance between reactive oxygen species (ROS) production and antioxidant capacity. This can occur as a result of either increased ROS

generation, impaired antioxidant system, or a combination of both. Free radicals are defined as an atoms or molecules that contain one or more unpaired electrons, making them unstable and highly reactive **(**Halliwell , 1999**).** The most important free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS)**.** ROS include free radicals such as superoxide (O_2^-), hydroxyl (OH), peroxyl (RO_2), hydroperoxyl (HRO_2^-) as well as nonradical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl) (Turko et al., 2001; Evans et al., 2002; Ziegler, 1994; Soliman & Gellido,2004.). Other non-oxygen species existing as reactive nitrogen species (RNS) include free radicals like nitric oxide (NO) and nitrogen dioxide (NO_2^-), as well as nonradicals such as peroxynitrite ($ONOO^-$), nitrous oxide (HNO_2) and alkyl peroxynitrates (RONOO) (Ziegler, 1994; Soliman and Gellido,2004).

In aerobic cells, free radicals are constantly produced mostly as reactive oxygen species. Once produced, free radicals are removed by antioxidants defenses including enzyme superoxide dismutase, catalase and gluthation peroxidise. Reactive oxygen species, including nitric oxide and related species, commonly exert a series of useful physiological effects. However, imbalance between prooxidant and antioxidant defenses in favour of prooxidants results in oxidative stress associated with the oxidative modification of biomolecules such as lipids, proteins and nucleic acids.

Hyperglycemia is the main cause of increased concentration of free radicals in the plasma of diabetic patients.

Chronic hyperglycemia causes oxidative stress (OS) in a number of ways including enzymatic, non-enzymatic and mitochondrial pathways, thus disrupting the prooxidative/antioxidative balance in cellular systems.

Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate OH radicals. A second consequence of hyperglycemia is nonenzymatic glycation of proteins.

Nonenzymatic glycation of proteins implies the ability of glucose to react in a nonenzymatic process with proteins, reducing molecular oxygen to highly reactive products (superoxide radical, hydrogen peroxide, and hydroxyl radical). The glycation process occurs in two phases: early – reversible, and late – irreversible phase. The early phase is characterized by the development of Amadori products followed by formation of stable adducts - advanced glycation end products (AGEs) in the later phase. The production of intracellular AGE precursors damages target cells via three pathways. First, intracellular proteins, modified by AGE, alter their own function. Second, extracellular matrix components modified by AGEP precursors interact abnormally with other matrix components and with matrix protein receptors on the cells. Third, plasma proteins modified by AGE precursors bind to the AGE receptors on endothelial cells, mesangial cells, and macrophages, causing receptor-mediated production of reactive oxygen species (ROS).

Enzymatic sources of augmented generation of reactive species in diabetes include NOS, NAD(P)H oxidase and xanthine oxidase (Guzik et al.,2000; Guzik et al., 2002; Aliciguzel et al.,2003) , where it is considered that the NAD(P)H oxidase is the major source of O_2^-productionf (Guzik, 2000; Ergul et al., 2004).

The mitochondrial respiratory chain is another source of nonenzymatic generation of reactive species. Hyperglycemia-induced generation of $^{\bullet}O_2^-$ at the mitochondrial level is the initial trigger of vicious cycle of oxidative stress in diabetes (Schultz et al., 2005, Nishikawa et al., 2000, Brownlee, 2001). Iincreased generation of ROS, especially O_2^- precedes the activation of four major pathways involved in the development of diabetic complications.

In hyperglicemia there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in inhanced productions of $O_2^{-\cdot}$ Glucose is a poor substrate for aldose reductase, but at high concentrations this enzyme converts glucose to sorbitole, initiating the polyol pathway of glucose conversion to fructose.

This pathway of glucose metabolism implies the participation of aldosoreductase enzyme, which using the NADPH cofactor, catalyzes the reduction of glucose into sorbitol, which can be further transformed into fructose. Aldosoreductase is widespread in the mammalian tissues such as peripheral nerves, retina, renal glomeruli, eye lenses. Stimulation of this pathway in hyperglycemic conditions is one of the pathogenetic mechanisms responsible for the development of diabetic neuropathy, nephropathy, and cataract. Activation of this pathway leads to structural changes in the tissues via several mechanisms, out of which most significant are increased osmotic pressure in the cell, depletion of myoinositol, and disordered redox potential of the cell due to reduced NADPH concentration, on the account of which the activity of NADPH dependent enzymes (including glutathione reductase and NO synthase) is diminished. Reduced production of NO leads to vasoconstriction, and impossibility of reduced glutathione regeneration in the so called glutathione redox cycle leads to depletion of reduced glutathione, since it is regenerated by the action of NADPH-dependent glutathione reductase (Garlberg & Mannervik, 1975), ultimately producing a permanent oxidative stress. Change of redox status of the cell, as determined by the cellular content of thiol compounds, glutathione above all, is a significant factor in the regulation of signal transduction to appropriate genes. This signal transduction pathway is effectuated via so called redox sensors which react to any change of concentration of intracellular thiols responsible for upkeeping of the cellular redox status. Numerous regulatory transcription factors supress transcription if bound in reduced state to regulatory sequences of certain genes. In the absence of GSH in the cell and in the state of intensified polyol metabolic pathway, the oxidized form of regulatory nuclear transcription factors loses the affinity for regulatory gene sequence, which results in increased transcription and synthesis of appropriate functional and structural proteins responsible for complications in diabetes and dysregulation of glucose metabolism homeostasis. Disturbed redox potential initiates the activation of stress-signaling cascade, resulting in direct activation of other kinases and transcription factors and/or indirect modulation (oxidation) of cysteine-rich redox-sensitive proteins, such as thioredoxin and glutathione S-transferase (Adler et al., 1999).

2.1 Modulation of cell signal transduction

Results of recent clinical and experimental *in vivo* and *in vitro* studies unequivocally suggest that DM is a disease followed by intensified oxidative stress which modulates numerous cell transduction pathways (Pavlović et al.,2002;Tomlinson & Gardiner,2008). This eventually results in tissue damage and the emergence of numerous diabetic complications, including peripheral neuropathy.

Hyperglycemia leading to oxidative stress induces diabetic complications via two signaling pathways: activation of protein kinase C (PKC) (Tomkin, 2001), and activation of mitogen-activated protein kinases (MAPK) (Tomlinson, 1999). PKC represents a family of multifunctional enzymes synthesized on at least three separate genes. They play a fundamental role in signal transduction in various tissues via phosphorylation (up and down regulation) of enzymes, receptors, transcription factors, and other kinases. PKC family consists of at least 11 isoforms, out of which 9 are activated by way of lipid secondary

messenger diacylglycerol (DAG). High glucose concentration increases the level of DAG stimulating de novo synthesis of DAG, which activates PKC. Hyperglycemia can also activate PKC isoforms via AGE receptors (Portilla et al., 2000) and by increased activity of the polyol pathway **(**Keogh, Dunlop & Larkins,1997.), probably by incresing reactive oxygen species (ROS). Intensified DAG-PKC signaling pathway is responsible for the disorders which represent the basic mechanisms in the development of vascular complications in diabetes mellitus.

In the complex process of signal transduction there are interactions of numerous receptor proteins and impulses are being amplified, so that joint, cascade pathways of signal transduction may be talked about. One of these pathways is the PKC-MAPK signal transduction pathway (Tomlinson & Gardiner,2008). Specific isoforms of PKC can activate MAPK, which via phosphorylation of transcription factors lead to altered expression of certain genes, making conditions for the change of cellular phenotype, apoptosis, or survival with a resultant disbalance producing complications in particular organs and systems. Thus MAPK activation represents a key event in the development of diabetic complications, especially diabetic neuropathy. Activation of this signaling pathway induces changes at the level of ion channels or disturbed gene expression, directly leading to nerve conduction disorders and development of axonopathy.

MAP kinases are group of serin/threonine specific kinases which are activated in response to extracellular stimuli through dual phosphorylation at conserved threonine and tyrosine residues. There are three main groups of MAP kinases: extracellular signal-regulated protein kinases (ERK), p38 and c-Jun N.terminal kinases (JNK). Results of recent clinical and experimental *in vivo* and *in vitro* studies show that all three groups of MAP kinases are activated in sensory neurons in hyperglicemia conditions in both diabetic rats and patients. (Purves at al, 2001) . In general the JNK and p38 group mediate responses to osmotic stress and is likely to be involved in the regulation of aldose reductase expression . Activation of p38 MAPK results in generating reduced nerve conduction velocity (NCV). These changes in NCV results from phosphorilation of the sodium channel Na 1.6 (Wittmack et al., 2005), which is the main voltage-activated channel at the node of Ranvier in myelinated fibres. In later stages of DM, other factors prevail: neurofilaments perturbation occurs, as a result of JNK activation and hyperphosphorylation of neurofilament proteins.

2.2 Antioxidant protection

The biological oxidative effects of free radicals on macromolecules are controlled by a spectrum of enzymatic and nonenzymatic antioxidants. Intracellular antioxidative defense is primarily enabled by the antioxidative enzymes, most significant of which are SOD, CAT and GSH-Px (Andrea et al.,2004; Maritim et al., 2003; Szaleczky et al., 1999; Djordjević, 2004). These three antioxidant enzymes differ in their structure, tissue distribution and cofactors required for functioning. SOD works as the first line of enzymatic protection against superoxide radicals. This enzyme catalyses the dismutation of superoxide anion radicals into hydrogen peroxide and oxygen. Hydrogen peroxide is further metabolized by CAT and GSH-Px, and due to lower Km values, GSH-Px is active in lower concentrations of hydrogen peroxide, while CAT activity increases with increased hydrogen peroxide concentration (Djordjević, 2004; Djordjević et al.,2000). Glutathione, together with its related enzymes, comprises a system that maintains the intracellular reducing environment and acts as primary defense against excessive generation of harmful ROS.

During reduced antioxidative protection and/or increased production of free radicals, OS occurs, playing an important role as a mediator of the apoptosis of both neurons and supportive glial cells, which has been confirmed in studies using animal models and tissue cultures (Schmeichel et al.,2003; Russel et al., 1999;Russel et al., 2002; Polydefkis et al., 2003; Person et al., 2003; Chiarelli et al., 2000; Sakaue et al., 2003).

2.2.1 Superoxide dismutase (SOD)

SOD catalyzes the conversion of superoxide anion into hydrogen peroxide and oxygen.

$$O_2^- + O_2^- + 2H+ \rightarrow H_2O_2 + O_2$$

SOD activity was originally described by McCord and Fridovich in 1969 (Szaleczky et al., 1999); they subsequently established that this enzyme is essential in sustaining life in aerobic conditions (McCord et al.,1971). There are several forms of SOD. These are metalloproteins, each containing a metal ion in its center (CuZn SOD, Mn SOD, Fe SOD, and Ni SOD) (Fridovich,1998). Fe SOD can be found in procaryotes and plants, while in humans intra- and extracellular CuZn SOD as well as mitochondrial Mn SOD were identified.

Intracellular CuZn is composed of two identical subunits (Tainer et al.,1982), each containing Cu (II) and Zn (II). Cu (II) is responsible for catalytic activity of SOD, while Zn (II) has a role in the stabilization of enzyme conformation (Djordjević, 2004). Cytosolic CuZn SOD can be inactivated by hydrogen peroxide, leading to the formation of either Cu (II) –OH or the ionized form Cu (II) $–O^-$. This enzyme can further catalyze peroxidation of various substances. The gene coding for CuZn SOD is located on chromosome 21. In connection with that, the results of some studies have demonstrated a significantly elevated activity of this enzyme in the patients with Down syndrome (*Kurobe et al.,1990*).

Extracellular SOD (ECSOD) is structurally similar to intracellular SOD, but it is present in the extracellular space. The gene for ECSOD is located on the chromosome 4. ECSOD is a tetrameric glycoprotein, each subunit of which contains a Cu and Zn atom and has a high affinity for heparin sulphate, enabling its existence in relatively high concentrations in specific regions of extracellular space or on the cell surface. ECSOD expression is principally regulated by cytokines, such as IFNγ, which stimulates the enzyme expression, while TNFα and TGFβ reduce its expression (Marklund,1992). Reduced ECSOD expression lead to reduced mitochondrial GSH and increased oxidative stress (Lebovitz RM et al.,1996). ECSOD is also significant in modulating NO activity. The superoxide can react with NO and form peroxynitrite, which by way of dissociation can form both hydroxil radical and nitric dioxyde, potent oxydants (Fukai T et all, 2000; Oury TD et al., 1996).

Mitochondrial SOD (MnSOD) is located in the mitochondrial matrix. There are two isoforms of MnSOD, dimeric MnSOD and tetrameric MnSOD, each subunit of which contains one Mn (III) ion. It is produced in a constitutive manner, but can also be induced by IL-1, TNF, or an endotoxin (Tang et al.,1994). In addition to cytokines, numerous oxygen metabolites can also induce MnSOD expression in particular cell types, which can be of critical importance in the occurrence of tissue damage in the situation of oxidative stress (Djordjević, 2004; Yoshioka T et al.,1994). It is believed that transcriptional regulation of MnSOD is mediated by the activation of nuclear transcription factor κB (NF- κB), supported by oxidants. Congenital complete inactivity of MnSOD lead to lethal outcome within few days after birth due to renal dysfunction (Lebovitz RM et al.,1996).

2.2.2 Catalase (CAT)

Catalase is a homotetrameric enzyme found in the tissues of almost all mammals, and demonstrating the highest activity in the liver and erythrocytes. Within the cell, catalase is localized primarily in the peroxisomes and mitochondria. The principal role of catalase lies in the degradation of H_2O_2, produced with support of peroxisomal oxidases. In the erythrocytes, catalase constitues the first line of defense against H_2O_2 (Mueller et al., 1997). In the situations of normal H_2O_2 concentration, the reaction goes towards H_2O_2 conversion into water and oxygen:

$$\text{Catalaza} + 2\,H_2O_2 \rightarrow \text{Compaund I} + H_2O$$

$$\text{Compaund I} + H_2O_2 \rightarrow \text{Katalaza} + H_2O + O_2$$

The second H_2O_2 molecule serves as the donor of hydrogen ion.

In the situations of low H_2O_2 concentration and in the presence of small molecular electron donors, catalase may act as peroxydase as well (Ghadermarzi & Moosavi-Movahedi,1996). Catalase forms a firm bond with NADPH, which can prevent the accumulation of inactive forms of the enzyme slowly created when catalase is exposed to hydrogen peroxide. These effects of NADPH are evident in low concentrations in the cell as well (<0.1 μM) (Kirkman et al., 1987).

Numerous diseases can be accompanied with altered catalase activity. Reduced catalase activity has been documented in diabetes mellitus (DM), malignant diseases, Down syndrome, as well as in regenerating tissues and in experimental conditions of nephrotoxicity (Djordjević et al., 2000). Since catalase has a predominant role in the control of H_2O_2 concentration (Mueller et al., 1997; Gaetani et al., 1996), which has been shown to damage pancreatic β cells and inhibits insulin activity (Murata et al.,1998; Tiedge et al., 1998; Jorns et al., 1999), it is believed that catalase in that manner protects β cells from the harmful action of H_2O_2 (Murata et al.,1998).

In the literature, two categories of genetic deficiency of erythrocyte catalase have been described (Eaton and Ma, 1995): acatalasemia (<10% of normal activity) and hypocatalasemia (about 50% of normal activity). In Hungary, a family with acatalasemia and 12 families with hypocatalasemia have been reported (Góth & Eaton, 2000; Góth, 1992; Vitai & Góth, 1997). These families comprised 2 acatalasemic individuals, 61 individuals had hypocatalasemia, while 66 individuals had normal values of catalase. In the group with hereditary catalase deficiency, the incidence of diabetes was 12.7%. DM was diagnosed in 8 persons, in both acatalasemic and 6 persons with hypocatalasemia; all of them had DM type 2. None of those with normal catalase values had DM. These data can indicate the significance of catalase in the pathogenesis of DM.

2.2.3 Glutathione peroxidase (GSH-Px)

GSH-Px is a selenium-dependent enzyme naturally occurring in three isoforms. The so called „classical" GSH-Px is a homotetramer with a molecular mass of 80 kDa, containing four atoms of selenium. GSH-Px demonstrates a special affinity for hydrogen peroxide and glutathione (Djordjević, 2004). It reduces H_2O_2 and organic alkaline hydroperoxides into water and appropriate alcohols, and oxidizes GSH into an appropriate disulfide (GSSG).

$$2GSH + H_2O_2\,(ROOH) \rightarrow GSSG + ROH + H2O$$

GSSG is converted back into GSH by glutathione reductase, which utilizes NADPH for its activity. It is thought that NAD(P)H, by itself, as an antioxidant, can act as a scavenger of toxic free radicals, as well as the radicals originating from repaired biomolecules (Kirsch and De Groot, 2001). The classical, selenium-dependent GSH-Pxs, isolated from various organs (erythrocytes, liver, lungs), demonstrate similar features and catalytic properties regardless of their origin. The maximum rate of reaction of GSH-Px depends on the concentrations of GSH and H_2O_2. In contrast to catalase, GSH-Px demonstrates a higher affinity for the substrate (H_2O_2) at low H_2O_2 concentrations, since the Michaelis constant (K_M) of peroxidase is lower than that of catalase. That is why GSH-Px plays a key role in the tissue detoxication from H_2O_2 at low H_2O_2 concentrations and normal GSH concentrations, while catalase is actively involved at higher H_2O_2 concentrations in the cell. The role of GSH-Px in erythrocytes is predominantly associated with detoxification from organic hydroperoxides and with keeping in reduced state the SH groups of structural and functional erythrocyte proteins, while its role in H_2O_2 degradation is of a lesser significance compared to catalase. The activity of erythrocyte selenium-dependent GSH-Px correlates with the erythrocyte content of selenium, so that the activity of this enzyme can be used as a functional index to assess selenium deficiency.

The second type of peroxidase is extracellular GSH-Px, with a molecular mass of 21.5 kDa. It is a tetramer too, the subunits of which contain an atom of selenium each. It is thought that extracellular GSH-Px is most important regarding the reduction of H_2O_2 in the plasma. GSH-Px in the plasma is a glycoprotein, believed to be most probably a secretory enzyme released from organ cells into the plasma. This enzyme demonstrates significant kinetic differences compared to erythrocyte GSH-Px. In contrast to erythrocyte GSH-Px, GSH-Px in the plasma demonstrates saturation kinetics compared to GSH (Đorđević et all, 2000). The activity of this enzyme declines with increased glutathione via the value of saturation concentration for GSH of 5 mmol. Moreover, GSH-Px in the plasma demonstrates higher affinity for organic hydroperoxides and H_2O_2. It is thought that selenium-dependent plasma GSH-Px is responsible for the process of detoxication from H_2O_2 and organic hydroperoxides created in the process of synthesis of eicosanoids in entothelial cells (Đorđević et all, 2000). Although the concentration of reduced glutathione in the plasma is very low, it is conceivable that the change in glutathione concentration (interorgan distribution, release from the liver into the plasma) can modulate the enzyme activity.

The third type of peroxidase is phospholipid hydroperoxide GSH-Px (PHGpx). It is too a selenium-dependent enzyme which differs from the first two types in view of the substrate and localization. PHGpx is a monomer, with the molecular mass of 23 kDa. This enzyme has a special characteristic – it directly reduces lipid hydroperoxides in the membranes.

Reduced GSH-Px activity can be found in numerous pathologic conditions and diseases, such as in chronic etilism, chronic renal insufficiency, hypertension, endemic nephropathy (Djordjević et all 1998). Altered GSH-Px activity can also be detected in DM (Kaji et al., 1985; Cser et al., 1993; Matkovics et al., 1982).

GSH-Px activity in the nervous system is low (in both peripheral nerves and in the central nervous system). Low values of GSH-Px compared to catalase activity in the peripheral nerves could lead to the conclusion that this enzyme has a minor role in H_2O_2 inactivation. However, GSH-Px function is prominent in the mitochondria, where the enzyme is necessary for the inactivation of H_2O_2 generated during the electron transport (McClain and Crook , 1996) where the catalase activity is insufficient.

2.2.4 Glutathione (GSH)

Glutathione is one of the most important nonenzymatic antioxidant in mammal cells. This ubiquitous tripeptide (γ-Glu-Cys-Gly) accounts for 90% of total non-protein sulphydril compounds in the cell. In the cell, it is most commonly found as a thiol – in a reduced form – and less as a disulfide – in an oxidized form (Toyokuni, 1996; Hann et al., 1990; Natarajan, 1995).

GSH concentration in the cell is determined by the control of enzymes involved in its synthesis, availability of synthesis precursors, intensity of GSH depletion for cellular detoxification processes, interorgan GSH distribution, as well as GSH regeneration in the so called glutathione redox cycle.

Erythrocytes represent a unique transport system for glutathione and its conjugates. In contrast to other cells, GSH appears in erythrocytes in several intermediary metabolic forms. In physiologic conditions, reduced GSH form appears in the highest percentage. The other GSH form is oxidized glutathione (GSSG), occurring in the process of nonenzymatic oxidation or oxidation mediated by GSH-peroxidase. The third erythrocyte form is the disulphide form of glutathione bound to proteins and non-protein sulphydril compounds. Glutathione S-conjugates (occurring via the action of glutathione S-transferase) are the fourth potential intracellular-intermediary form of GSH in the erythrocytes. Erythrocytes take up toxic molecules from the plasma, to be excreted back into plasma after conjugation with GSH. Further detoxification of S-conjugates continues in the liver and kidneys; non-toxic compounds are then excreted from the organism via the bile or urine.

GSH is involved in many cellular functions. Many cells synthesize GSH *de novo* by γ-glutamyl transferase, forming firstly a γ-peptide bond between one cystein and one glutamate residue. The next phase is the addition of glycine, assisted by GSH synthetase. Neurons do not contain γ-glutamyl-cystein synthetase; instead, GSH is synthesized in the glial cells controlling this synthesis via the mechanisms of transcription regulation (Iwata-Ichikawa et al., 1999; Keelan et al., 2001). It is thought that during the evolution, depending on the cellular metabolic needs, glutathione is engaged in the regulation of different processes. Though the role of glutathion has been commonly associated with the protection of the cell from active free radicals, glutathione is involved in many other processes such as detoxification from xenobiotics, synthesis of eicosanoids, synthesis of nucleic acids and proteins, cell signaling, proliferation, and differentiation (Djordjević,2000). However, its essential role lies in the protective antioxidant system. GSH, GSH-Px, GSH-transferase, GSH-reductase, and NAD(P)H constitute an antioxidant system of glutathione, in which GSH-reductase and NAD(P)H are required for the reduction of oxidized glutathione and consequential glutathione recycling in the so called glutathione redox cycle. Depletion of GSH in the cell increases its sensitivity to oxidative damage (Rizzardini et al., 2003). In contrast, accumulation of GSH in the cell, especially in the mitochondria, can prevent neural apoptosis caused by ischemia (Li et al., 2002) and excitotoxicity (Kobayashi et al., 2000). The involvement of GSH in the limitation of prooxidative cell status, has as the ultimate consequence deceleration of aging, atherogenesis, mutagenesis, and cancerogenesis (Anghileri and Thouvenot ,1997; Vostreis et al., 1988)

3. Aim of study

Research in patients with diabetic neuropathy (DN) is mainly based on studying the influence of antioxidative substances on certain biomarkers of oxidative stress and on the

function of peripheral nerves (Ziegler et al., 1995; Reljanović et al., 1999; Ziegler et al., 1999; Ametov et al., 2003; Ziegler and Gries, 1997; Ziegler et al., 1997). However, there are no enough studies, testing the direct correlation between prooxidative/antioxidative parameters and the development of DN, especially in humans. Additional difficulties occur when different kinds of reactive oxygen are directly determined in biological systems due to their short life. For this reason, the measurement of oxidative stress is mainly based on indirect and non-specific measurement of products of their activity.

Considering that antioxidant enzymes are important biomarkers of oxidative stress, the aim of this study was to determine the activities of antioxidant enzymes (SOD, CAT, GSH-Px) and glutathione in the erythrocytes of patients with type 2 diabetes mellitus (DM) in relation to presence or absence of distal symmetrical polyneuropathy (DSP), as well as to analyze the possible connection between the activity of these antioxidant parameters and the function of peripheral nerves. In this way we tried to determine the level of antioxidant defense in erythrocytes of diabetic patients and to establish the potential role of oxidative stress in the development of diabetic neuropathy.

4. Materials and methods

The research took the form of a prospective study, which included 100 patients suffering from type 2 DM and diabetic distal symmetric polyneuropathy (DDSP). Patients suffering from another acute or chronic illness, patients previously subjected to cytotoxic therapy or radiotherapy, and patients who had been treated with antioxidative substances were excluded from the experimental group. The control group gathered 50 healthy individuals who denied ailments and diseases and whose clinical observations and laboratory tests showed no abnormalities. The DDSP was diagnosed after clinical and electrophysiological testing.

The electrophysiological testing checked the conductivity of sensory and motor fibers of upper and lower extremity peripheral nerves. Due to the symmetric nature of the disease, the protocol included the unilateral (right) testing of sural, peroneal, tibial, ulnar and median nerves. We analyzed the latency, amplitude, and conduction velocity of the tested nerves. The minimal criterion for the electrophysiological validation of diabetic neuropathy (DN) was the abnormality of any electroneurographic (ENG) conduction parameter in at least two nerves, one of which had to be sural nerve (England et al., 2005). The values of tested electroneurographic parameters were expressed as the score from 1 to 4, where 1 corresponded to a normal result, while 4 meant that the motor or sensory evoked potential was absent. The ENG testing was carried out on the ENG device (Schwartzer, Mios 2+). During the testing, the surface electrodes were used: the stimulation electrode – for the electrical stimulation of the peripheral nerve and the registration electrode – for the registration of the motor evoked potential (MEP). In order to increase the diagnostic sensitivity of the electrophysiological tests, in addition to the standard ENG, we also tested the conduction sensitivity of the median plantar nerve. Since the sensory evoked potential (SEP) amplitude of medial plantar nerve is distinctly low, a separate technique was used to test this on an evoked potential device (Medelec Sapphire 2). A large number (236) of stimuli were used and the values were subsequently averaged by the computer. The following parameters were analyzed: the peak latency of the sensory neurogram, expressed

in milliseconds, and the amplitude of the sensory neurogram, expressed in μV. The stimulation was carried out with a surface electrode positioned on the plantar part of the thumb. The response was registered via a surface electrode located behind the internal malleolus, proximal to the retinaculum flexorum.

Laboratory analysis determined the level of morning glycemia and glycosylated hemoglobin (HbA1c) by the standard laboratory tests from the venous blood of patients and halthy persons. Erythrocyte SOD activity was determined by a commercial test Ransod provided by Randox (Randox Laboratories, Crumlin, UK), based on the McCord and Fridovich method (Mccord and Fridovich, 1969). Erythrocyte CAT activity was determined by the method of Beutler (*Beutler, 1982*). Erythrocyte GSH-Px activity was determined by commercial test Ransel (Randox Laboratories, Crumlin, UK). Glutathione was measured as total glutathione in the erythrocytes as described by Tietze (1969). Caiman's GSH Assay kit utilizes a carefully optimized enzymatic recycling method, using glutathione reductase for quantification of GSH.

Statistical method: To process results we used widely accepted statistical techniques: means and standard deviation, statistical significance calculation tests, correlation tests. We utilized standard statistics software tools (Origin Pro and MATLAB Statistics Toolbox). The results are presented as mean ± SD.

5. Results

The study encompassed 100 type 2 DM patients who showed signs of distal symmetrical polyneuropathy, whose average age was 58.62±11.62 years. The average duration of the disease was 11.32±7.05 years. The control group included 50 healthy individuals, whose average age was 51.64±12.25 years (Tab. 1). There was significant increase in glycemia and HbA1c values in the patients compared with the control group (*p<0. 0001).

	Number	Sex(M/F)	Age (years) mean ±SD	Duration of DM (years)	Glycemia (mmol/L) mean ±SD	HbA1C (%) mean ±SD
Control	50	22 / 28	51.64±12.25	0	4.81 ±0.63	5.73 ± 0.56
Patients without DSP	40	24 / 16	57.73±11.08	11.23±7.94	8.99±3.11*	9.26 ± 3.33*
Patients with DSP	100	55 / 45	58.62±11.62	11.32±7.05	9.50 ±4.13*	9.09 ±2.13*

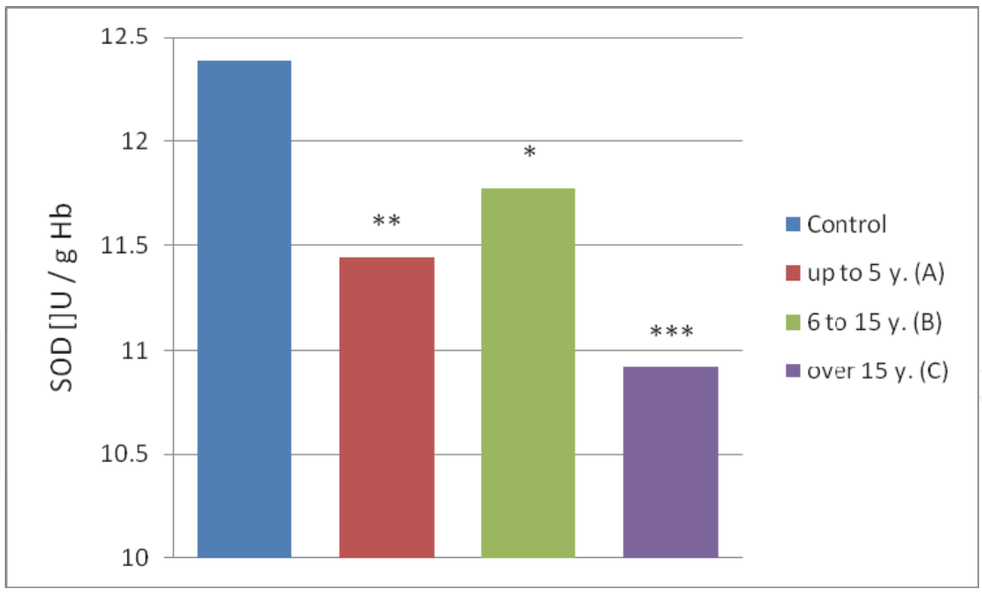
Table 1. Demographic and biochemical characteristics of diabetic neuropathy patients and the control group.

There was a statistically significant decrease SOD and GSH-Px activity and GSH level in erythrocyte of diabetic neuropatrhy patients compared with the control group and patients without DN. The values of erythrocyte CAT were lower in patients compared to the control ones, but this difference did not reach statistical significance (Tab. 2).

	SOD (U/gr Hb) mean ±SD	CAT (U/gr Hb) mean ±SD	GSH-Px(U/gr Hb) mean ±SD	GSH (µmol/grHb) mean ±SD
Control	1238.36 ± 136.86	7.34 ± 1.62	67.35 ± 11.39	0.24 ± 0.05
Patients without DSP	1144.26 ± 103.92**	7.22 ± 2.01	55.63 ±13.07**	0.22 ± 0.05**
Patients with DSP	1101.00 ± 64.36**,*	6.68 ± 1.26	50.25 ± 10.42**,*	0.21 ± 0.04**

Table 2. SOD and CAT in the diabetic neuropathy patients and in the control group. ** p<0,001 vs. control, * p<0,05 vs. patients without DN

Values of antioxidant parameters were variable at different stages of the disease in patients with DDSP (Fig. 1-4).



* p<0.05 vs. control; **p<0.01 vs. control; *** p<0.001 vs. control; A:B p=0.202; A:C p<0.05; B:C p<0.001, y. =year

Fig. 1. SOD values in different periods of illness in patients with DDSP as compared to the control group

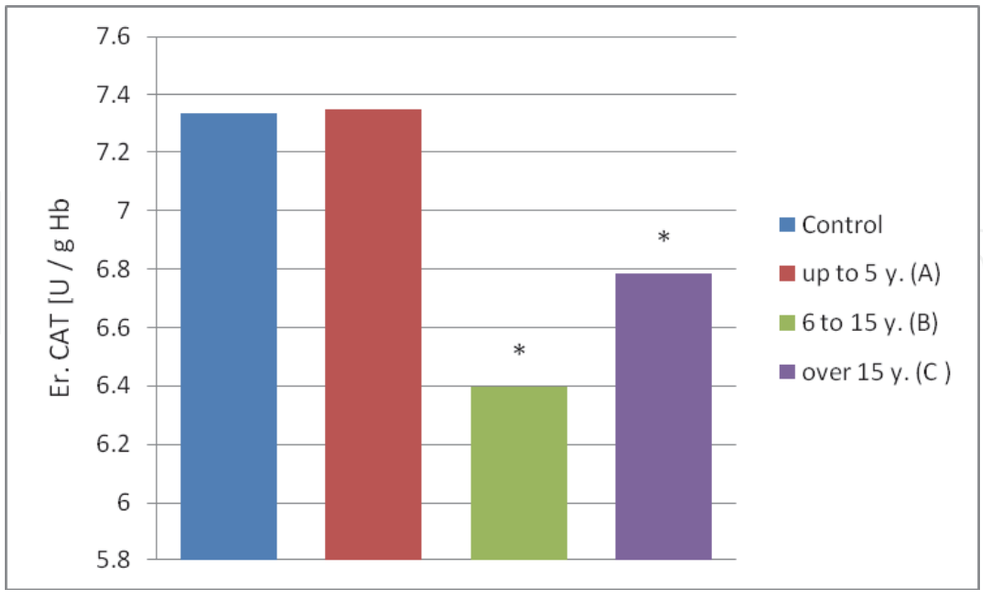
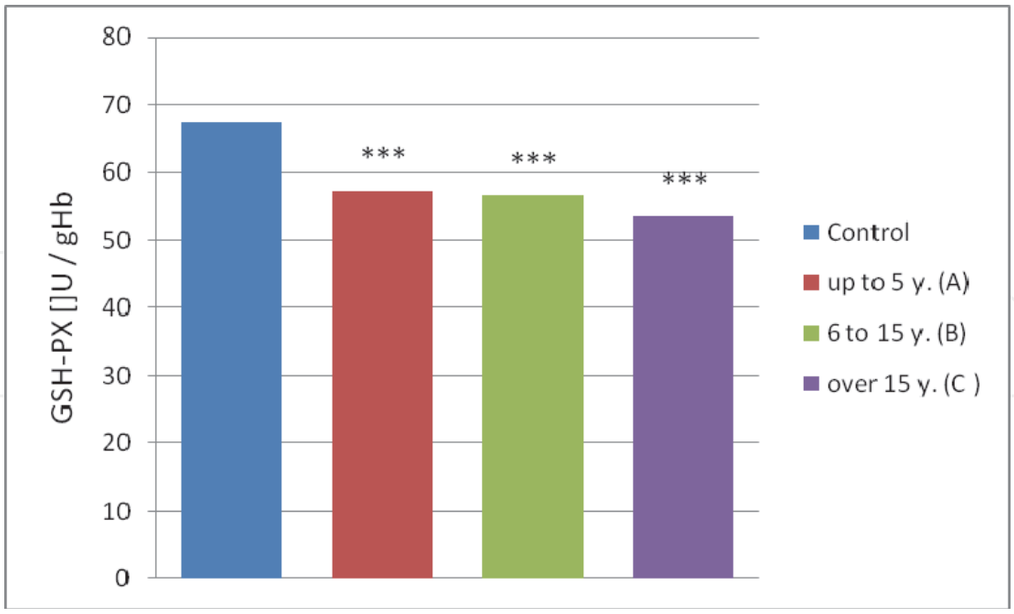


Fig. 2. Erythrocite CAT values in different periods of illness in patients with DDSP as compared to the control group * p<0.05 vs. control, A:B p<0.01; A:C p=0.15; B:C p=0.24, y.= year



*** p<0.001 vs. control A:B p=0.97; A:C p=0.38; B:C p=0.28, y= year

Fig. 3. GSH-PX values in different periods of illness in patients with DDSP as compared to the control group

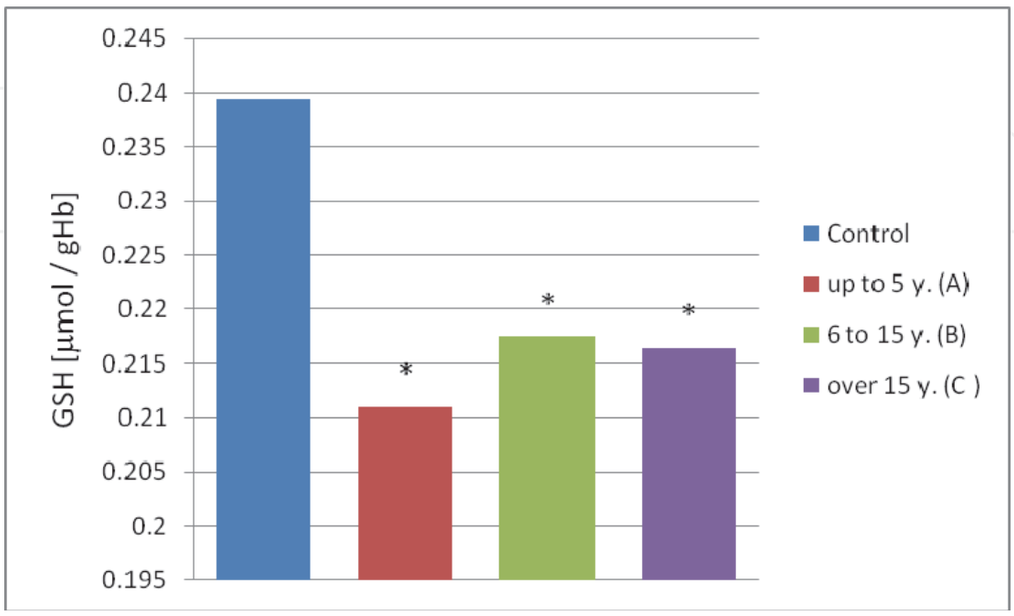


Fig. 4. GSH values in erythrocytes in different periods of illness in patients with DDSP as compared to the control group * p<0.05 vs. control A:B p=0.4 ; A:C p=0.5 ; B:C p=0.9 , y= year

There was statistically significant difference between the latency and amplitude of sensory evoked potentials (SEP) of *medial plantar nerve* in the patients and the controls (Tab. 3).

SEP n.plantaris medialis	Patients mean± SD	Controls mean ± SD	t	p
Latency [ms]	7.50 ± 1.36	5.56 ± 0.89	-8.90	<0. 0001
Amplitude [µV]	0.381± 0.358	0.849 ± 0.606	5.37	<0. 0001

Table 3. Latency and amplitude of the SEP of medial plantar nerve in the diabetic neuropathy patients and in the control

All electrophysiological parameters related to the conduction of motor and sensory fibers of the tested upper and lower extremity nerves showed that there was a statistically significant difference between diabetic neuropathy patients and controls (Tab. 4).

ENG parameters		Controls mean±SD	Patients mean±SD	t	p
CMAP peroneal nerve	Latency (ms)	3.54±0.56	4.66±1.13	-6.64	<0. 0001
	Amplitude (mV)	5.77±2.47	3.46±2.13	5.88	<0. 0001
	NCV (m/s)	51.65±5.79	41.24±6.77	9.20	<0. 0001
CMAP tibial nerve	Latency (ms)	3.56±0.51	4.94±1.32	-7.13	<0. 0001
	Amplitude (mV)	11.19±4.73	4.62±2.98	10.28	<0. 0001
	NCV (m/s)	43.37±3.07	35.72±7.30	7.083	<0. 0001
CMAP median nerve	Latency (ms)	3.45±0.55	4.88±2.24	-4.47	<0. 0001
	Amplitude (mV)	7.00±2.51	4.90±2.35	5.23	<0. 0001
	NCV (m/s)	56.57±7.01	49.52±6.22	6.27	<0. 0001
CMAP ulnar nerve	Latency (ms)	2.90±0.63	3.17±0.78	-2.12	0. 0359
	Amplitude (mV)	7.87±3.35	4.93±7.27	6.36	0. 0025
	NCV (m/s)	57.54±8.57	48.59±8.00	6.30	<0. 001
SEP sural nerve	Latency (ms)	2.93±0.50	4.14±0.90	-8.60	<0. 0001
	Amplitude (mV)	15.61±6.16	8.79±5.77	6.31	<0. 0001
	NCV (m/s)	36.37±4.48	29.49±6.35	6.63	<0. 0001
SEP median nerve	Latency (ms)	3.96±0.49	4.70±0.82	-5.83	<0. 0001
	Amplitude (mV)	43.07±9.33	15.75±9.70	16.47	<0. 0001
	NCV (m/s)	47.36±5.63	38.77±7.85	6.86	<0. 0001
SEP ulnar nerve	Latency (ms)	3.28±0.68	4.12±0.82	-6.23	<0. 0001
	Amplitude (mV)	36.46±11.63	17.28±12.22	9.16	<0. 0001
	NCV (m/s)	46.32±6.10	38.20±7.37	6.69	<0. 0001

Table 4. ENG parameters in the diabetic neuropathy patients and in the control group. CMAP – compound muscle action potential, SEP - sensory evoked potentials, NCV-nerve conduction velocity

A number of scored ENG parameters correlated significantly with erythrocyte SOD and CAT level in the patients studied (Tab. 5 and Tab. 6).

SEP of medial plantar nerve	SOD		CAT		GSH-px		GSH	
	r	p	r	p	r	p	r	p
Latency	-0.1621	0.1071	-0.0488	0.6295	-0.1149	0.2550	-0.0492	0.6265
Amplitude	0.0995	0.3248	0.2059	**0.0399**	0.0580	0.5564	-0.0570	0.5734

Table 5. Correlation between SEP parameters of medial plantar nerve and parameters of OS (SOD and CAT) in diabetic neuropathy patients. Marked values point out a statistically significant correlation.

Nerve	ENG parameters	SOD		CAT		GPx		GSH	
		r	p	r	p	r	p	r	p
CMAP Peroneal nerve	Latency	-0.1096	0.2777	-0.1597	0.1126	-0.0577	0.5683	-0.0605	0.5497
	Amplitude	-0.2526	**0.0112**	-0.2859	**0.0039**	-0.1096	0.2777	-0.0662	0.5129
	MEP NCV	-0.2633	**0.0081**	0.0199	0.8442	-0.1417	0.1598	0.0097	0.9238
CMAP Tibial nerve	Latency	-0.1866	0, 0630	0.0406	0.6883	0.0130	0.8977	-0.0476	0.6383
	Amplitude	-0.1575	0.1175	-0.0568	0.5747	-0.0233	0.8184	0.0148	0.8837
	NCV	-0.2524	**0.0113**	0.0254	0.8016	-0.0910	0.3681	0.0618	0.5416
CMAP Median nerve	Latency	-0.2326	**0.0199**	-0.0959	0.3425	-0.0694	0.4929	0.0140	0.8901
	Amplitude	-0.0663	0.5123	0.0188	0.8529	-0.1198	0.2352	0.1366	0.1753
	NCV	-0.2171	**0.0301**	0.0462	0.6483	-0.0469	0.6431	0.1887	0.0601
CMAP Ulnar nerve	Latency	-0.0442	0.6627	-0.0043	0.9659	-0.1294	0.1996	-0.0209	0.8366
	Amplitude	-0.1531	0.1282	0.0174	0.8639	0.0327	0.7466	0.0224	0.8252
	NCV	-0.1387	0.1687	-0.0629	0.5339	-0.0694	0.4927	-0.0655	0.5174
SEP Median nerve	Latency	-0.2183	**0.0291**	-0.0501	0.6205	-0.0771	0.4456	-0.1923	0.0552
	Amplitude	-0.0881	0.3836	-0.0057	0.9549	0.0904	0.3710	-0.1230	0.2227
	NCV	-0.2079	**0.0379**	-0.0569	0.5741	-0.1253	0.2143	-0.2805	**0.0047**
SEP Ulnar nerve	Latency	-0.1679	0.0949	-0.1659	0.0991	-0.0095	0.9252	-0.1094	0.2786
	Amplitude	-0.0213	0.8336	-0.0997	0.3239	-0.0866	0.3914	-0.1710	0.0889
	NCV	-0.1202	0.2336	-0.0627	0.5357	-0.1009	0.3178	-0.1344	0.1826
SEP Sural nerve	Latency	-0.1129	0.2636	-0.3837	**0.0001**	-0.0245	0.8088	0.0443	0.6613
	Amplitude	-0.2896	**0.0035**	-0.1449	0.1505	-0.0275	0.7861	-0.0194	0.8481
	NCV	-0.1122	0.2662	-0.3840	**0.0001**	0.0035	0.9723	0.0798	0.4298

Table 6. Correlation between ENG parameters and parameters of OS (SOD and CAT) in diabetic neuropathy patients. MEP- Motor Evoked Potential, SEP- Sensory Evoked Potentials, NCV – Nerve Conduction Velocity. Marked values point out a statistically significant correlation.

6. Discussion

Results of experimental studies, conducted both *in vivo* and *in vitro*, suggest that the peripheral nervous system is sensitive to oxidative damage (Schmeichel et al.,2003; Russel et al., 1999;Russel et al., 2002*)*. Neurons take over glucose from the blood by the concentration dependent transport, so that hyperglycemia is always associated with increased glucose values in the neurons, which results in oxidative stress *(*Tomlinson and Gardiner,2008; Andrea et al.,2004*)*. On the other hand, antioxidative defense in peripheral nerves is thought to be limited due to primary lower values of glutathione and glutathione-dependent enzymes (GSH-Px and GSH-r) (Romero et al., 1991; Schmeichel et al.,2003*)*, which further increases the sensitivity of nerves to oxidative damage. SOD could provide efficient antioxidative protection, since, contrary to glutathione-dependent enzymes, it is relatively more active in the peripheral nerves. However, in spite of such a theoretical position, studies carried out on experimental models have not shown any significant changes of the endoneural antioxidative status in the experimentally induced DN, except an increased CAT levels which could not be able to correct by insulin therapy (Van Dam et al., 1996). On the other side, positive effects of antioxidants on the antioxidative capacity of blood, and also on the disturbed function of peripheral nerves in the very same

experimental models, lead one to conclude that systemic OS can have a more significant role compared to endoneural OS in the development of neuropathic changes.

The determination of OS biomarkers is an important step in the understanding of DN pathogenesis. Recent research suggests that there are tissue- and time-dependent changes in the activity of various antioxidative enzymes. The results of our study show that there is a statistically significant reduction of erythrocyte SOD levels in patients with type 2 DM and DDSP in comparison with healthy controls, which corresponds to the data from the literature (Arai et al., 1987; Kawamura et al., 1992; Vijaylingam et al., 1996). Eerythrocyte SOD level was significantly lower in patients with diabetic neuropathy compared with patients without diabetic neuropathy, suggesting the importance of antioxidant protection in the prevention of nerve injury. The main reason for the reduced SOD activity is the glycolization of Cu, Zn-SOD, which has been documented in both *in vitro* and *in vivo* experiments (Arai et al., 1987; Kawamura et al., 1992). However, there are also studies which show no changes in erythrocyte SOD activity (Peuchant et al.,1997; Walter et al.,1991; Faure et al.,1995), or which, quite the contrary, suggest an increased activity of this enzyme (Yaqoob et al., 1994).

In most cases, CAT activity in erythrocytes was not changed in either experimental animals or type 1 and type 2 DM patients (Wohaieb & Godi, 1987; Godin et al., 1988; Matkovich et al., 1982). However, some studies have noted changes in CAT activity, in particular its reduction (Vijaylingam et al., 1996; Alphonsus et al., 2007). In this study, erythrocyte CAT values were reduced in patients as compared with the controls, but this reduction did not reach statistically significant levels, which correlates with the literature data listed above. Catalase values were reduced in patients with DSP, compared with patients without DSP, although these deviations were not statistically significant. Reduced CAT activity could be explained by the accumulation of H_2O_2 in the cells, as a result of glucose autooxidation. As the principal enzymatic role of CAT is to control H_2O_2 concentration, H_2O_2 accumulation in the cells is believed to lead to the exhaustion of this enzyme, which primarily reflects erythrocytes, where CAT is otherwise most active. Studies have shown that in other tissues H_2O_2 accumulation may stimulate CAT synthesis, thus increasing its activity. However, erythrocytes lack the genetic apparatus for such a synthesis, which is a reason that the increased H_2O_2 concentration results in the exhaustion and inactivation of catalase (Alphonsus et al., 2007).

Changes in the activity of glutathione-dependent enzymes were different in experimental models. Most studies have shown tissue and time dependent changes of enzyme activity. However, even if we acknowledge these factors, we cannot precisely match the results of various studies of the association of DM and activity of glutathione-dependent enzymes. The studies dealing with the GSH-Px activity in the erythrocytes have demonstrated variable results. A large number of studies demonstrated that GSH-Px activity in the blood, erythrocytes, and leukocytes was similar in DM patients (types 1 and 2) and in healthy controls (Walter RM et al., 1991; Leonard M.B. et al., 1995; Akkus I et al., 1996). On the other hand, there are studies clearly confirming altered activity of glutathione-dependent enzymes in DM, above all in the form of reduced activity of GSH-Px and increased activity of GSH reductase (Godin et al., 1988; Dohi et al.,1988; Tagami et al., 1992; Langenstroer & Pieper, 1992; Blakytny and Harding,1992). Murakami (1991), studying the erythrocytes in DM, concluded that reduced erythrocyte GSH was caused by reduced activity of gamma glutamyl-cystein synthetase in connection with its glycation, reduced activity of GSH-

reductase and defective glutathione transport. Yoshida (1995) confirmed that in the erythrocytes of diabetics with poorly controlled disease the synthesis of GSH and thiol transport were damaged, rendering the cells more sensitive to oxidative damage. In diabetics with permanently higher values of glucose Uzel et al. (1987) established reduced GSH-Px activity and lower erythrocyte GSH values, with increased products of lipid peroxidation, with changes more apparent in patients with retinopathy. GSH reduction in the erythrocytes in DM was confirmed by Bono et al. (*1987*) as well. Stahlberg and Hietanen (1991) observed a reduced activity of GSH reductase in children with DM but Walter et al. (1991) were not able to demonstrate any difference in GSH-Px and GSH reductase activities in diabetics and nondiabetics. Kaji et al. (1985) failed to find a difference in activity of erythrocyte GSH-Px, but established increased GSH-Px activity in the plasma in women with DM. Osterod et al. (1996) found in their studies a reduced erythrocyte GSH-Px activity in DM type 1. Authors of some studies also described an increased erythrocyte GSH-Px activity in DM (Matkovics et al., 1982). Literature data mostly indicated an unaltered or reduced activity of erythrocyte GSH-Px. The results obtained in this study indicate a statistically significant reduction of GSH-Px activity both in patients with DSP and in patients without DSP, compared to controls. Erythrocyte GSH-Px values were significantly lower in patients with diabetic neuropathy compared with patients without diabetic neuropathy. Reduced GSH-Px could be directly explained by reduced GSH content in studied patients, representing otherwise a substrate and cofactor for GSH-Px enzyme.

The studies of glutathione homeostasis in DM have so far shown tissue differences in the levels of GSH, although in most of the cases reduced GSH contents have been found. The studies of glutathione level in the blood of diabetics demonstrated that those with DM type 2 had reduced erythrocyte level of GSH and increased level of GSSG (De Mattia at all, 1994; Jain & McVie, 1994). The blood level of GSH was significantly reduced in various phases of the type 2 DM, such as glucose intolerance, early hyperglycemia (Vijayalingam et al., 1996) in the first two years and before complications developed (Sundaram et al., 1996), as well as in poorly controlled glycemia (Peuchant et al., 1997). Yoshida et al. (1995) observed reduced GSH in the erythrocytes of type 2 diabetics, with disturbed activity of gamma glutamyl transferase and thiol transport. In contrast to a clear reduction of blood GSH in type 2 DM, in type 1 disease the results were not entirely convincing. The role of GSH in the occurrence of diabetes complications has not been elucidated. Thornalley et al. (1996) found an inverse correlation between the level of erythrocyte GSH and presence of diabetes complications (neuropathy, retinopathy, and nephropathy) in patients with DM type 1 and 2, leaving unanswered the question whether GSH levels were reduced in those without complications, ot these patients have an even more significant reduction GSH levels.

The results of our investigation showed a statistically significant reduction of erythrocite GSH level both in patients with DSP and in patients without DSP compared to controls, which was in accordance with the literature data. In the group of patiens without DSP, erythrocite GSH-Px value did not show larger deviations compared to patients with DSP.

Some studies of the antioxidative protection system in DM, principally in experimental animal models, were related to the study of impact of disease duration on the activity of antioxidative enzymes. Time-dependent changes of the activity of antioxidative enzymes and prooxidative-antioxidative balance were thus observed in DM in various tissues. (Majythija & Jayesh, 2005; Sasvári and Nyakas, 2003**).** Kishi et al.(2005) based on their own study, came to the conclusion that changes in the activity of antioxidative enzymes in

peripheral nerves were not the result of reduced gene expression, but can be linked to the duration of DM or posttranslational modifications.

Bearing these information in mind, this study analyzed the activity of antioxidative enzymes and glutathione in the blood of patients which disease had lasted up to 5 years, 5-15 years, and over 15 years. The activity of SOD enzyme was generally lower in patients in each phase of the disease, though the reduction was variable. The smallest reduction was observed in the first 5 years of DM, while the reduction was most evident in advanced disease phases - in those affected for more than 15 years. More intense inactivation of SOD in more advanced DM could be explained by increased production of H_2O_2 in the processes of non-enzymatic glycation predominating with time, which can further inhibit SOD. In the group of those affected for 5-15 years, a slight increase of SOD activity can be observed related to other two groups, which can be explained by an adaptive increase of antioxidative protection pathways. Increased H_2O_2 production inhibited SOD activity, but, on the other hand, formation of superoxide radicals presents a signal for increased gene expression of SOD (Matsuyama et al. 1993). Available amount and activity of SOD should represent a balance between the enzyme production and its degradation. As shown here, the balance demonstrated time-dependent modulation in different disease phases.

The activity of erythrocyte catalase also demonstrated a declining tendency, with the smallest decline in the first 5 years of DM. The decline of catalase activity was greatest in those affected for 5-15 years. In this group, the values of erythrocyte catalase were significantly different from control ones.

In all groups, a statistically significant reduction of erythrocyte GSH level was observed compared to controls. Among the groups of different disease phases, there was no statistically significant difference in the erythrocyte GSH level.

Changes were observed also with the enzyme associated with glutathione metabolism – GSH-Px. This enzyme demonstrated a statistically significant decline of activity in all disease phases compared to controls. The decline was most evident in advanced DM (>15 years), though there were no statistically significant deviations among the individual groups of patients in different disease phases. Reduced GSH-Px activity can be directly explained by low GSH content in diabetics, in view of the fact that GSH is the substrate and cofactor of GSH-Px. Reduced GSH-Px activity can be caused by the enzyme inactivation as the consequence of increased glycation in the situation of hyperglycemia. Increased glycation and consequential protein reactions can affect amino acids in active enzyme domain or disturb stereochemical configuration and cause structural and functional molecular changes (Bonnefont-Rousselot et al., 2000). Reduced GSH-Px activity causes H_2O_2 accumulation, which also contributes to progressive reduction of activity of SOD in advanced DM.

These changes in the activity of antioxidative enzymes in DM confirm the notion that disturbed carbohydrate metabolism have an impact on the function of antioxidative protection which by itself can affect the development of late complications of DM. In recent years, it has been definitely confirmed that the frequency of late diabetic complications is higher with poorer metabolic control. If oxidative stress has a role in the development of diabetic complications, we should expect adaptive changes in the system of antioxidative protection. These changes would be more or less evident in different situations of metabolic control and largely depend on the duration of unfavorable metabolic status.

Similarly to the literature data, our results suggest that blood of type 2 DM patients has decreased antioxidative protection. However, in spite of strong evidence in the literature that OS is increased in DM, there is still no definite connection between OS levels and the

development of late diabetic complications. Accordingly, in this study, we have looked into the interrelation between the tested antioxidative enzyme levels and the functional damage of peripheral nerves. Our previous study did not show any correlation between plasma total antioxidant capacity (TAC) and the degree of damage of peripheral nerves in type 2 DM and DDSP patients (Djordjević et al., 2008). Having in mind that TAC is not a mere sum of various antioxidant activity, but a dynamical system of interdependent individual serum antioxidant parameters (Koraćević et al., 2001), we have designed this study in such a way as to observe and analyze the influence of individual TAC constituents on the development of peripheral nerve dysfunction in type 2 DM patients.

All electroneurographic parameters of peripheral nerve conduction have showed deviation in the patients as compared with the control group and these deviations have been statistically significant. In the studied patients, we have found a significant negative linear correlation between erythrocyte SOD levels and a number of scored ENG parameters – indicators of DDSP (the lower the SOD values, the higher the ENG score, i.e. the more pronounced the functional damage). The correlation that was found between SOD and ENG indicators of the degree of neuronal damage indicates that there was an important role of toxic effects of superoxide anion radicals in the development of neuronal damage. In vivo, superoxide anion radicals are mostly removed enzymatically, by SOD. When superoxide anion radicals are excessively produced, they react with nitric oxide and form a peroxynitrite, which has numerous cytotoxic effects. A tenfold increase in superoxide anion radicals and nitric oxide has been found to increase peroxynitrite production one hundred times (Djordjević et al.,2000). Excessive production of superoxide anion radicals, nitric oxide and peroxynitrite may, thus, be a significant pathogenetic factor for neuronal damage.

As for catalase, even though the reduced activity of this enzyme did not reach statistical significance in this study, the correlation analysis between CAT blood levels and the electrophysiological conduction parameters of the peroneal, sural, median, and plantar nerves (MEP-amplitude of peroneal nerve, the latency and NCV of the sural nerve, the SEP amplitude of medial plantar nerve) has revealed a statistically significant value. Such results suggest that CAT has a pathogenetic importance, i.e. that hydrogen peroxide has toxic effects on the degree of neuronal damage.

There was no correlation between erythrocite GSH-Px and ENG parameters which could be explained by lower Km and lower GSH-Px activity in erythrocytes, compared to CAT activity. Erythrocite GSH-Px value correlated with censory nerve condaction of median nerve only. This results could be explained by limited activity of GSH and GSH-dependent enzymes in periferal nerves. It should also bear in mind that the values of GSH and GSH-px in our group of patients showed small variation over the course of DM, which could also be the explanation for the lack of correlation with the ENG parameters, given the progressive course of diabetic neuropathy.

7. Conclusion

DM is closely linked to an imbalance in pro/antioxidant status of cells and changes in redox potential. Oxidative stress, as a common denominator, is the biochemical mechanism by which disturbed glucose metabolism and deregulation of cell signaling leads to the development of diabetic complications. Results of our study pointed out a reduced systemic antioxidative defense in the patients with type 2 DM and diabetic distal symmetrical polyneuropathy and indicate that systemic oxidative stress plays a potential role in the

development of diabetic neuropathy. For better understanding of the role of oxidative stress and antioxidative mechanisms, further investigations with standardized methodology, molecular biological technique and better defined experimental models and subjects are required, aiming to prevent, delay or slow the progression of the disease.

8. References

Adler, V.; Yin Z.; Tew K.D.; Ronai Z.(1999). Role of redox potential and reactive oxygen species in stress signalling. *Oncogene*, 18:6104-6111.

Akkus, I.; Kalak, S.; Vural, H.; Caglayan, O.; Menekse, E.; Can, G.; Durmus, B. (1996). Leukocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type II diabetes mellitus. *Clinica Chimica Acta*, 244, 221-227.

Aliciguzel, Y.; Ozen, I.; Aslan, M.; Karayalcin, U. (2003). Activities of xanthine oxidoreductase and antioxidant enzymes in different tissues of diabetic rats. *J Lab Clin Med* , 142(3):172-177

Alphonsus, E.U.; Iya, N.; Okon, E.; Mary, N. (2007). Red cell catalase activity in diabetics. *Pakistan Journal of nutrition* 6 (5): 511-515.

Ametov, A.S;, Barinov, A.; Dyck, P.J.; Hermann, R.; Kozlova, N.; Litchy, W.J.; et al (2003). The sensory symptoms of diabetic polyneuropathy are improved with alpha-lipoic acid: the SYDNEY trial. *Diabetes Care* 26 (3): 770–6.

Anghileri, L.J.; Thouvenot, P. (1997). Non-transferrin-bound iron and tumor cells. Anticancer research, 17(4A):2529-33.

Arai, K.; Iizuka, S.; Tada, Y.; Oikawa, K.; Taniguchi, N. (1987). Increase in the glucosylated form of erythrocyte Cu-Zn-superoxide dismutase in diabetes and close association of the nonenzymatic glucosylation with the enzyme activity. *Biochimica et Biophysica Acta* 924: 292-296.

Beutler, E. (1982). CatalaseIn: Beutler E (ed). Red cell metabolism, a manual and biochemical method. 3rd ed. *Grune and Stratton, New York*, pp 105–6.

Blakytny, R.; Harding, J.J. (1992). Glycation (non-enzymatic glycosylation) inactivates glutathione reductase. *Biochem J,* 288:303-307.

Bonnefont-Rousselot, D.; Bastard, J.P.; Jaudon, M.C.; Delattre, J. (2000). Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes & Metabolism*,26,:163-176

Bono, A.; Caimi, G.; Catania, A.; Sarno, A.; Pandolfo, L.(1987). Red cell peroxide metabolism in diabetes mellitus. Horm Metab Res, 19:264-266

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature* , 414(6865):813-820.

Chiarelli, F.; Santilli, F.; Mohn, A. (2000). Role of growth factors in the development of diabetic complications. *Horm Res* 53:53–67.

Cser, A.; Sziklai, L.I.; Menzel H.; Lombeck, I. (1993). Selenium status and lipoproteins in healthy and diabetic children. *Trace Elem Electroly*, 7:205-210.

De Mattia, G.; Laurenti, O.; Bravi, C.; Ghiselli, A.; Iuliano, L.; and Balsano, F. (1994). Effect of aldose reductase inhibition on glutathione redox status in erythrocytes of diabetic patients. *Metabolism*, 43, 965-968.

Djordjević, B. V.; Ćosić, V.; Pavlović, D.; Vlahović, P.; Jevtović T.; Kocić G.; Savić, V. (2000). Does captopril change oxidative stress in puromycin aminonucleoside nepropathy? *Renal. Fail.*, 22, 535-544.

Djordjević, B. V. (2004). Free Radicals in Cell Biology. *International Review of Cytology*, Vol.237: 57-89.

Djordjević B.V.; Grubor-Lajšić,G.; Jovanović-Galović, A.; Pavlović, D.; Cvetković, T.; Pejović, M.; Lečić, N. (1998). Selenium-dependent GSH-Px in erythrocites of patients with hypertension treated with ACE inhibitors. *J. Environ. Pathol. Toxicol. Oncol.,* 17, 277-280.

Djordjević, G.; Đurić, S.; Apostolski, S.; Djordjević, V.B.; Živković, M. (2008). Total antioxidant blood capacity in patients with type 2 diabetes mellitus and distal symmetrical polyneuropathy. *VSP*, 65 (9):1-7.

Djordjević, V.B.; (2004). Free radicals in cell biology. *International review of cytology* 237:57-89.

Djordjević, V.B.; Pavlović, D.D.; Kocić, G.M. (2000). Biochemistry of free radicals. Medical Faculty Niš.

Dohi T, Kawamura K, Morita K, Okamoto H, Tsujimoto A. (1988). Alterations of plasma selenium concentrations and the activities of tissue peroxide metabolism enzymes in streptozotocin induced diabetic rats. *Horm Metab Res*, 20:671-675.

Đorđević, V.B.; Pavlović, D.D.; Kocić, G.M.(2000). Biohemija slobodnih radikala, *Medicinski fakultet*, *Niš. (*Byochemistry of free radicals*, Medical faculty, Nis, Serbia)*

Eaton, J.W., Ma, M. (1995). Acatalasemia. In The Metabolic Bases of Inherited Disease. 7th ed. Scriver C, Beudet A, Sly W, Valle DL, Eds. New York, McGraw-Hill, , p. 2371–2383.

England, J.D.; Gronseth G.S.; Franklin, G.; Miller, R.G.; Asbury, A.K.; Carter, G.T.; Cohen,J.A.; Fisher, M.A.; Howard, J.F.; Kinsella, L.J.; Latov,N.; Lewis, A.; Low, P.A.; Sumner, A.J. (2005). Distal symmetric polyneuropathy: A definition for clinical research. *Neurology* 64:199-207.

Ergul, A.; Schultz Johansen J.; Stromhaug C.; Harris, A.K.; Hutchinson J.; Tawfik,A.; Rahimi, A.; Rhim, E.; Wells, B.; Caldwell, R.W., et al. (2004). Vascular dysfunction of venous bypass conduits is mediated by reactive oxygen species in diabetes: Role of endothelin-1. *J Pharmacol Exp Ther*, 313:70–77.

Evans, J.L.; Goldfine, I.D.; Maddux, B.A.; Grodsky, G.M. (2002). Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev*, 23(5):599-622.

Faure,P.; Benhamou, P.Y.; Perard,A.; Halimi, S.; Roussel, A.M- (1995). Lipid peroxidation in insulindependent diabetic patients with early retina degenerative lesions: effects of an oral zinc supplementation. *Eur. J. Clin. Nutr*. 49: 282-288.

Faure, P.; Benhamou, P.Y.; Perard, A.; Halimi, S.; Roussel, A.M. (1995). Lipid peroxidation in insulindependent diabetic patients with early retina degenerative lesions: effects of an oral zinc supplementation. *Eur. J. Clin. Nutr*. 49: 282-288.

Fridovich, I. (1998). Oxygen toxicity: A radical explanation. *J. Exp. Biol*. 201, 1203-1209.

Gaetani, G.F;, Ferraris, A.M.; Rolfo, M.; Mangerini, R.; Arena, S.; Kirkman, H.N. (1996). Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood*, 87:1569–1599.

Garlberg, I.; Mannervik, B. (1975). Purification and characterization of the flavoenzime glutathione reductase from rat liver. *J Biol Chem*, 250:5475-5480

Ghadermarzi, M.; Moosavi-Movahedi A.A.(1996). Determination of the kinetic parameters for the suicide substrate inactivation of bovine liver catalase by hidrogen peroxide. *J. Enzime Inhibition*, 10, 167-175.

Godin, D.V.; Wohaieb, S.A.; Garnett, M.E.; Goumeniouk, A.D. (1988). Antioxidant enzyme alterations in experimental and clinical diabetes. *Mol Cell Biochem* 84:223-231

Góth, L.; Eaton, J. (2000). Hereditary catalase deficiencies and increased risk of diabetes. *Lancet*, 356:1820–1821.

Góth, L. (1992). Two cases of acatalasemia in Hungary. *Clin Chim Acta*,207:155–158.

Guzik, T.J.; Mussa, S.; Gastaldi, D.; Sadowski, J.; Ratnatunga, C.; Pillai, R.; Channon, K.M. (2002). Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation*, 105(14):1656-1662.

Guzik, T.J.; West, N.E.; Black, E.; McDonald, D.; Ratnatunga, C.; Pillai, R.; Channon, K.M. (2000). Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. Circ Res, 86(9):E85-90.

Halliwell, B. (1999). Antioxidant defense mechanisms:from the beginning to the end (of the beginning). *Free Rradic. Res.*, 31:261-272

Hann, H.L.; Stahlhut, M.W.; Hann, C.L. (1990). Effect of iron and deferoxamine on cell growth and in vitro ferritin synthesis in human hepatoma cell lines. *Hepatology*, 11:566-569.

Iwata-Ichikawa, E.; Kondo, Y.; Miyazaki, I.; Asanuma, M.; Ogawa, N. (1999). Glial cells protect neurons against oxidative stress via transcriptional up-regulation of the glutathione synthesis. *J Neurochem*, 72:2334–2344.

Jain, S.K. and McVie, R. (1994). Effect of glycemic control, race (white versus black), and duration of diabetes on reduced glutathione content in erythrocytes of diabetic patients. *Metabolism* 43, 306-309.

Jörns, A.; Munday, R.; Tiedge, M., Lenzen, S. (1999). Effect of superoxide dismutase, catalase, chelating agents, and free radical scavengers on the toxicity of alloxan to isolated pancreatic islets in vitro. *Free Rad Biol Med*, 26:1300–1304.

Kaji, H.; Kurasaki, M.; Ito, K. et al. (1985). Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type Í diabetic women. *Klin Wochenschr*, 63:76576-76578.

Kaneto, H.; Fujii, J.; Myint, T.; Miyazawa, N.; Islam,K.N.; Kawasaki, Y.; Suzuki, K.; Nakamura, M.; Tatsumi, H.; Yamasaki, Y.; Taniguchi, N. (1996). Reducing sugars trigger oxidative modification and apoptosis in pancreatic beta-cells by provoking oxidative stress through the glycation reaction. *Biochemical Journal* 320: 855-863

Kawamura, N.; Okawara, T.; Suzuki, K.; Konishi, K.; Mino, M.; Taniguchi, N. (1992). Increased glycated cu, Zn.SOD levels in erythrocites of patients with IDDM. *J. Clin. Endocrinol. Metab.* 74: 1352-1354.

Keelan, J.; Allen, N.J.; Antcliffe, D.; Pal, S.; Duchen, M.R. (2001). Quantitative imaging of glutathione in hippocampal neurons and glia in culture using monochlorobimane. *J Neurosci Res*; 66:873–884.

Keogh, R.J.; Dunlop, M.E. & Larkins R.G. (1997). Effect of inhibition of aldose reductase on glucose flux, diacylglycerol formation, protein kinase C, and phospholipase A2 activation. *Metabolism*; 46, 41–47.

Kirkman, H.N.; Galiano, S.; Gaetani, G.F. (1987). The function of catalase-bound NADPH. *J. Biol. Chem.*, Vol. 262, Issue 2, 660-666.

Kirsch, M. & De Groot,, H. (2001). NAD (P) H, a directly operating antioxidant? *The FASEB Journal.*, 15:1569-1574.

Kishi, Y.; Nickander, K.K.; Schmelzer, J.D.; Low, P.A. (2000). Gene expression of antioxidant enzymes in experimental diabetic neuropathy. *Journal of the Peripheral Nervous System*, Volume 5, Issue 1: 11-18,

Kobayashi, M.S.; Han, D.; Packer, L. (2000). Antioxidants and herbal extracts protect HT-4 neuronal cells against glutamate-induced cytotoxicity. *Free Radic Res* , 32:115–124.

Koraćević, D;, Koraćević, G.; Djordjević, V.; Andrejević, S.; Ćosić, V. (2001). Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol*. 54 (5): 356–61.

Kurobe, N.; Suzuki, F.; Okajima, K.; Kato, K. (1990). Sensitive enzyme immunoassay for human Cu/Zn superoxide dismutase. *Clin Chim Acta*. 31;187 (1):11-20.

Langenstroer, P., Pieper, G.M. (1992). Regulation of spontaneous EDRF release in diabetic rat aorta by oxygen free radicals. *Am J Physiol*, 63:H257-H265.

Lebovitz, R.M.; Zhang, H.; Vogel, H.; Cartwright, J.; Dionne, L.; Lu, N.; Huang, S.; Matzuk, M.M. (1996). Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc Natl Acad Sci*, 93:9782–9787.

Leonard, M.B.; Lawton, K.; Watson, I.D.; Patrick, A.; Walker, A.; MacFarlane I. (1995). Cigarette smoking and free radical activity in young adults with insulin- dependent diabetes. *Diabetic Medicine*, 12, 46-50.

Li, L.; Shen, Y.M.; Yang, X.S.; Wu, W.L.; Wang, B.G.; Chen, Z.H.; Hao, X.J. (2002). Effects of spiramine T on antioxidant enzymatic activities and nitric oxide production in cerebral ischemia-reperfusion gerbils. Brain Res, 944:205–209.

Majythija, J.B.; Balaraman, R.(2005). Time depemdent changes in antioxidant enzyme and vascular reactivity of aorta in streptozotocin-induced diabetic rats treated with Curcumin. *Journal of Cardiovascular farmacology Novembar,* 46(5):697-705.

Maritim, A.C.; Sanders, R.A.; Watkins, III J.B. (2003). Diabetes, oxidative stress and antioxidants: A Review. *J.Biochem Molecular Toxicology* 17(1): 24-38.

Matkovics, B.; Varga, S.I.; Szabo, L.; Witas, H. (1982). The effect of diabetes on the activities of the peroxide metabolism enzymes. *Horm Metab* Res14:77-79.

Matsuyama, T., Michishita, H., Nakamure, H., Tsuchiyama, M., Shimizu, S., Watanabe, K., Sugita, M. (1993). Induction of copper-zinc superoxide dismutase in gerbil hippocampus after ischemia. J Cereb Blood Flow Metab, 13:135-144.

McCord, J.M., Fridovich, I. (1969). Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 244: 6049–6055

McCord, J.M.; Keele, B.; Fridovich, I. (1971). An enzyme based theory of obligate anaerobiosis: the physiological function of SOD. *Proc Natl Acad Sci*, 68:1024–7.

McClain, D.A. & Crook, E.D. (1996). Perspectives in diabetes. Hexosamines in insulin resistance. *Diabetes*, 45: 1003–1009.

Mueller, S.; Riedel, H.D.; Stremmel, W. (1997). Direct evidence for catalase as the predominant H2 O2– removing enzyme in human erythrocites. *Blood* 90, 4973-4978.

Mukherjee, B., Mukherjee, J.R., Chatterjee, M. (1994). Lipid peroxidation, glutathione levels and changes in glutathione related enzyme activities in streptozotocin induced diabetic rats. *Immun Cell Biol*, 72:109-114

Murakami, K. (1991). Glutathione metabolism in erythrocytes from patients with diabetes mellitus. *Hokkaido-Igaku-Zasshi*, 66:29-40.

Murata, M.; Imada, M.; Inoue, S.; Kawanishi, S. (1998). Metal mediated DNA damage by diabetogenic alloxan in the presence of NADH. *Free Radic Biol Med*, 25:586–595.

Natarajan, V. (1995). Oxidants and signal transduction in vascular endothelium [Review]. *J Lab Clin Med*, 125: 26-37.

Nishikawa, T.; Edelstein, D.; Du, X.L.; Yamagishi, S.; Matsumura, T.; Kaneda, Y.; Yorek, M.A.; Beebe, D.; Oates, P.J. Hammes HP, *et al*. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*, 404(6779):787-790.

Osterode, W.; Holler, C.; Ulberth, F. (1996). Nutritional antioxidants, red cell membrane fluidity and blood viscosity in type 1 (insulin dependent) diabetes mellitus, *Diabet Med*., 13, 1044–1050.

Oury, T.D.; Day, B.J.; Crapo, J.D. (1996). Extracellular superoxide dismutase: a regulator of nitric oxide bioavailability. *Lab. Invest*., 75:617-636.

Pavlović, D.; Djordjević, V.; Kocić, G., (2002). Ćelijska signalna transdukcija-modulacija slobodnim radikalima, *Jugoslov. Med. Biohem.* 21:69-84

Peuchant, E.; Delmas Beauvieux, M.C.; Couchouron, A.; Dubourg, L.; Thomas, M.J.; Perromat, A.; Clerc, M.; Gin, H. (1997). Short-term insulin therapy and normoglycemia. Effects on erythrocyte lipid peroxidation in NIDDM patients. *Diabetes Care* 20: 202-207.

Pierson, C.R.; Zhang, W.; Sima, A.A. (2003). Proinsulin C-peptide replacement in type 1 diabetic BB/Wor-rats prevents deficits in nerve fiber regeneration. *J Neuropathol Exp Neurol* 62:765–779.

Polydefkis, M.; Griffin, J.W.; McArthur, J. (2003). New insights into diabetic polyneuropathy. JAMA 290:1371–1376.

Portilla, D. et al. Etomoxir -induced PPARalpha-modulated enzymes protect during acute renal failure. *Am. J. Physiol. Renal Physiol*. 2000; 278, F667–F675 .

Purves, T.; Middlemas, A.; Agthong, S.; Jude, E.B.; Boulton, A.J.; Fernyhough, P.;Tomlinson, D.R. (2001). A role for mitogen-activated protein kinases in the etiology of diabetic neuropathy. *Fed Am Soc Exp Biol J*, 15:2508–14.

Reljanović, M.; Reichel, G.; Rett, K.; Lobisch, M.; Schuette, K.; Möller, W.; et al. (1999). Treatment of diabetic polyneuropathy with the antioxidant thioctic acid (alpha-lipoic acid): a two year multicenter randomized double-blind placebo-controlled trial (ALADIN II). Alpha Lipoic Acid in Diabetic Neuropathy. *Free Radic Res* 31 (3): 171–9.

Rizzardini, M., Lupi, M.; Bernasconi, S.; Mangolini, A.; Cantoni, L. (2003). Mitochondrial dysfunction and death in motor neurons exposed to the glutathione-depleting agent ethacrynic acid. *J Neurol Sci*, 207:51–58.

Romero, F.J.; Monsalve, E.; Hermenegildo, C.; Puertas, F.J.; Higueras, V., Nies, E.; Segura-Aguilar, J.; Roma, J. (1991). Oxygen toxicity in the nervous tissue: comparison of the antioxidant defense of rat brain and sciatic nerve. *Neurochem Res*, 16:157–161.

Russell, J.W.; Golovoy, D.; Vincent, A.M.; Mahendru, P.; Olzmann, J.A.; Mentzer, A.; Feldman, E.L. (2002). High glucose induced oxidative stress and mitochondrial dysfunction in neurons. *FASEB J* 16:1738–1748.

Russell, J.W.; Sullivan, K.A.; Windebank, A.J.; Herrmann, D.N.; Feldman, E.L. (1999). Neurons undergo apoptosis in animal and cell culture models of diabetes. *Neurobiol Dis* 6:347–363

Sakaue, Y.; Sanada, M.; Sasaki, T.; Kashiwagi, A.; Yasuda, H. (2003). Amelioration of retarded neurite Zutgrowth of dorsal root ganglion neurons by overexpression of PKC in diabetic rats. *Neuroreport* 14:431–436.

Sasvári, M.; Nyakas, C.(2003). Time dependent changes in oxidative metabolism during chronic diabetes in rats. *Acta Biologica Szegediensis,* Volume 47(1-4):153-158.

Schmeichel, A.M.; Schmelzer, J.D.; Low, P.A. (2003). Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. *Diabetes* 52:165–171.

Soliman, E.; Gellido, C. Diabetic Neuropathy. *In eMedicine Septembar, 2004. Available from URL: http://www.emedicine.com/NEURO.*

Schultz, J.J.; Harris A.K.; Richly, D.J.; Ergul,A. (2005). Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovascular diabetology*.4:5.

Stahlberg, M.R.; Hietanen, E. (1991). Glutathione and glutathione metabolizing enzymes in the erythrocytes of healthy children and children with insulin dependent diabetes mellitus, juvenile rheumatoid arthritis, coeliac disease and acute lymphoblastic leukemia. *Scand J Clin Lab Invest*, 51:125-130

Sundaram, R.K.; Bhaskar, A.; Vijayalingam, S.; Viswanathan, M.; Mohan, R.; and Shanmugasundaram, K.R. (1996). Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clinical Science*,90, 255-260.

Szaleczky, E.; Prechl, J.; Feher, J.; Somogyi, A. (1999). Alterations in enzymatic antioxidant defense in diabetes mellitus − a rational approach. *Postgrad Med J*, 75 (879): 13–17.

Tagami, S.; Kondo, T.; Yoshida, K.; Hirokawa, J.; Ohtsuka, Y.; Kawakami, Y. (1992). Effect of insulin on impaired antioxidant activities in aortic endothelial cells from diabetic rabbits. Metabolism, 41:1053-1058

Tainer, J.A., Getzoff, E.D., Beem, K.M., Richardson, J.S., Richardson, D.C. (1982). Determination and analysis of the 2 Å structure of copper, zinc superoxide dismutase. *J Mol Biol*, 160: 181–217.

Tang, L.; Ou, X.; Henkle-Dührsen, K.; Selkirk, M. E. (1994). Extracellular and cytoplasmic CuZn superoxide dismutases from Brugia lymphatic filarial nematode parasites. *Infect Immun*, 62 (3): 961–967.

Thornalley, P.J.; McLellan, A.C.; Lo, T.W., Benn, J.; Sonksen, P.H. (1996). Negative association between erythrocyte reduced glutathione concentration and diabetic complications. *Clinical Science*, 91, 575-582.

Tiedge, M.; Lortz, S.; Drinkgern, J.; Lenzen, S. (1997). Relation between antioxidant enzymes gene expression and antioxidative defense status of insulin producing cells. *Diabetes*, 46:1733–1742.

Tiedge, M.; Lortz, S.; Munday, R.; Lenzen, S. (1998). Complementary action of anti-oxidant enzymes in the protection of bioengineered insulin-producing RINm5F cells against toxicity of reactive oxygen species. *Diabetes*, 47:1578–1585.

Tietze, F. (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. *Anal Biochem*, 27: 502-522.

Tomkin, G.H. (2001). Diabetic vascular disease and the rising star of protein kinase C. Diabetologia, 44:657-658.

Tomlinson, D,R.; Gardiner, N.J. (2008). Diabetic neuropathies: components of etiology; *Journal of the peripheral Nervous* System 13:112-121.

Tomlinson, D.R. (1999). Mitogen-activated protein kinases as glucose trasducers. *Diabetologia*, 42:1271-1281.

Toyokuni, S. (1996). Iron-induced carcinogenesis: the role of redox regulation. *Free Radic Biol Med*, 20:553-566

Turko, I,V.; Marcondes, S.; Murad, F.(2001). Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. *Am J Physiol Heart Circ Physiol*, 281(6):H2289-2294.

Uzel, N.; Sivas, A.; Uysal, M.; Oz, H. (1987). Erythrocyte lipid peroxidation and glutathione peroxidase activities in patients with diabetes mellitus. *Horm Metab Res*, 19:89-90

Van Dam, P.S.; Bravenboer, B.; Van Asbeck, B.S.; Van Oirschot, J.F.L.M.; Marx, j. J,M.; Gispen, W.H. (1996). Effects of insulin treatment on endoneurial and systemic

oxidative stress in relation to nerve conduction in streptozotocin-diabetic rats. *European Journal of Clinical Investigation* 26 (12): 1143-1149 (7).

Vijayalingam, S.; Parthiban, A.; Shanmugasundaram, K.R.; Mohan, V. (1996). Abnormal antioxidant status in impaired glucose tolerance and non-insulindependent diabetes mellitus. *Diabetic Medicine*, 13, 715-719.

Vincent, A.M.; Russell, J.W.; Low, P.; Feldman, E.L. (2004). Oxidative Stress in the Pathogenesis of Diabetic Neuropathy. *Endocrine Reviews* 25 (4): 612-628.

Vitai, M.; Góth, L.(1997). Reference ranges of normal blood catalase activity and levels in familial hypocatalasemia in Hungary. *Clin Chim Acta*, 261:35–42.

Vostreis, M.; Moran, P.L.; Seligman, P.A. (1988). Transferrin synthesis by small cell lung cancer cells acts as an autocrine regulator of cellular proliferation.; *J Clin Invest* 82:331.

Walter, R.M. Jr.; Uriu Hare J.Y.; Olin, K.L.; Oster, M.H.; Anawalt, B.D.; Critchfield, J.W.; Keen, C.L. (1991). Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. *Diabetes Care* 14: 1050-1056.

Wittmack, E.K.; Rush, A.M.; Hudmon, A.; Waxman, S.G.; Dib-Hajj, S.D. (2005). Voltage-Gated Sodium Channel Nav1.6 Is Modulated by p38 Mitogen-Activated Protein Kinase *J. Neurosci.*, 13, 25 (28): 6621-6630.

Wohaieb, S.A., Godin, D.V. (1987). Alterations in free radical tissue defense mechanisms in streptozotocin induced diabetes in rat. *Diabetes* 36:1014-1018.

Yaqoob, M.; McClelland, P.; Patrick, A.W.; Stevenson, A.; Mason, H.; White, M.C.; Bell, G.M. (1994). Evidence of oxidant injury and tubular damage in early diabetic nephropathy. *Q. J. Med*. 87: 601-607.

Yoshida, K.; Hirokawa, J.; Tagami, S.; Kawakami, Y.; Urata, Y.; Kondo, T. (1995). Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. *Diabetologia*, 38, 201-210.

Yoshioka, T.; Homma, T.; Meyrick, B.; Takeda, M.; Moore-Jarrett, T.; Kon, V.; Ichikawa, I. (1994). Oxidants induce transcriptional activation of manganese superoxide dismutase in glomerular cells. Kidney Int., 46 (2):405-13.

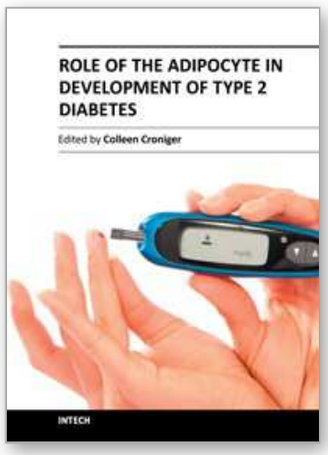
Ziegler, D.; Gries, F.A. (1997). Alpha-lipoic acid in the treatment of diabetic peripheral and cardiac autonomic neuropathy. *Diabetes* 46 (Suppl 2): 62–6.

Ziegler, D.; Hanefeld, M.; Ruhnau, K.J.; Hasche, H.; Lobisch, M.; Schütte, K., et al. (1999). Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a 7-month multicenter randomized controlled trial (ALADIN III Study). ALADIN III Study Group. Alpha-Lipoic Acid in Diabetic Neuropathy. *Diabetes Care* 22 (8): 1296–301.

Ziegler, D.; Hanefeld, M.; Ruhnau, K.J.; Meissner, H.P.; Lobisch, M.; Schütte, K., et al. (1995). Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant alpha-lipoic acid. A 3-week multicentre randomized controlled trial (ALADIN Study). *Diabetologia* 38 (12): 1425–33.

Ziegler, D.; Schatz, H.; Conrad, F.; Gries, F.A.; Ulrich, H.; Reichel, G. (1997). Effects of treatment with the antioxidant alpha-lipoic acid on cardiac autonomic neuropathy in NIDDM patients. A 4-month randomized controlled multicenter trial (DEKAN Study). Deutsche Kardiale Autonome Neuropathie. *Diabetes Care* 20 (3): 369–73.

Ziegler, D. (1994). Diagnosis, staging and epidemiology of diabetic peripheral neuropathy. *Diabetes Nutrition and Metabolism*, 7:342-348.



Role of the Adipocyte in Development of Type 2 Diabetes

Edited by Dr. Colleen Croniger

ISBN 978-953-307-598-3
Hard cover, 372 pages
Publisher InTech
Published online 22, September, 2011
Published in print edition September, 2011

Adipocytes are important in the body for maintaining proper energy balance by storing excess energy as triglycerides. However, efforts of the last decade have identified several molecules that are secreted from adipocytes, such as leptin, which are involved in signaling between tissues and organs. These adipokines are important in overall regulation of energy metabolism and can regulate body composition as well as glucose homeostasis. Excess lipid storage in tissues other than adipose can result in development of diabetes and nonalcoholic fatty liver disease (NAFLD). In this book we review the role of adipocytes in development of insulin resistance, type 2 diabetes and NAFLD. Because type 2 diabetes has been suggested to be a disease of inflammation we included several chapters on the mechanism of inflammation modulating organ injury. Finally, we conclude with a review on exercise and nutrient regulation for the treatment of type 2 diabetes and its co-morbidities.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Gordana M. Djordjević, Stojanka S.Djurić, Vidosava B. Djordjević, Slobodan Apostolski and Miroslava Živković (2011). The Role of Oxidative Stress in Pathogenesis of Diabetic Neuropathy: Erythrocyte Superoxide Dismutase, Catalase and Glutathione Peroxidase Level in Relation to Peripheral Nerve Conduction in Diabetic Neuropathy Patients, Role of the Adipocyte in Development of Type 2 Diabetes, Dr. Colleen Croniger (Ed.), ISBN: 978-953-307-598-3, InTech, Available from: http://www.intechopen.com/books/role-of-the-adipocyte-in-development-of-type-2-diabetes/the-role-of-oxidative-stress-in-pathogenesis-of-diabetic-neuropathy-erythrocyte-superoxide-dismutase

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen