The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease

Jakus V

Úloha voľných radikálov, oxidačného stresu a antioxidačných systémov pri diabetickej vaskulárnej chorobe

Abstract

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Bratisl Lek Listy 2000; 101 (10): 541–551

Recent experimental findings suggest that overproduction of reactive oxygen and nitrogen species (ROS/RNS), lowered antioxidant defense and alterations of enzymatic pathways in humans with poorly controlled diabetes mellitus can contribute to endothelial, vascular and neurovascular dysfunction. Over the past decade, there has been substantial interest in oxidative stress and its potential role in diabetogenesis, development of diabetic complications, atherosclerosis and associated cardiovascular disease. Consequences of oxidative stress are damage to DNA, lipids, proteins, disruption in cellular homeostasis and accumulation of damaged molecules.
This review summarizes recent knowledge on the pathomechanism of ROS/RNS in vascular oxidative stress and Maillard reactions. Evidence suggests that Maillard reactions act as amplifier of oxidative damage in aging and diabetes. Furthermore, results of experimental observations with antioxidant systems and antioxidant pharmacotherapy in the treatment of diabetes mellitus are discussed. These data indicate that the targeting therapy to specific macromolecules, tissues and organs of diabetics by specific antioxidants or combined drug preparates could become a relevant adjuvant pharmacotherapy with improved glycaemic control, blood pressure control and management of dyslipidemia for the treatment or prevention of progression of micro- and macrovascular diabetic complications. Supplementation with antioxidants as a promising complementary treatment can exert beneficial effects in diabetes. Some antidiabetic drugs may have antioxidant properties independently of their main role on glycaemia control. Therapeutic potential of inhibitors of AGEs for-

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Abstrakt

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Bratisl. lek. Listy, 101, 2000, č. 10, s. 541–551

Súčasné experimentálne výsledky naznačujú, že nadprodukcia reaktívnych foriem kyslíka a dusíka, znížená antioxidačná ochrana a alterácia v enzýmových reakciách u ľudí so zle kontrolovaným diabetom môže prispievať k endotelovej, vaskulárnej, a neurovaskulárnej dysfunkcii. Za posledné desaťročie sa venovala značná pozornosť oxidačnému stresu a jeho potenciálnej úlohe v diabetogenéze, vývoji diabetických komplikácií, ateroskleróze a s tým spojeným kardiovaskulárnym ochorením. Následky oxidačného stresu sú poškodenie DNA, lipidov, proteínov, porušenie bunkovej rovnováhy a akumulácia poškodených molekúl.
Tento prehľad sumarizuje súčasné znalosti o patomechanizme voľných radikálov pri vaskulárnom oxidačnom strese a Maillardových reakciách.
Možno predpokladať, že Maillardove reakcie účinkujú ako zosilňovač oxidačného poškodenia pri starnutí a diabete. Ďalej sa tu diskutuje o výsledkoch experimentálnych pozorovaní s antioxidačnými systémami a antioxidačná farmakoterapia pri liečbe diabetes mellitus. Tieto údaje ukazujú, že cielená terapia k špecifickým makromolekulám, tkanivám a orgánom diabetikov špecifickými antioxidantmi alebo kombinovanými liekovými prípravkami by sa mohla stať relevantnou adjuvantnou farmakoterapiou spolu s vylepšenou kontrolou glykémie, kontrolou krvného tlaku a manažmentom dyslipidémie pre liečbu alebo prevenciu vývoja mikro- a makrovaskulárnych diabetických komplikácií.
Suplementácia antioxidantmi ako sľubná komplementárna liečba môže vykazovať blahodarné účinky pri diabete. Niektoré antidiabetiká majú antioxidačné vlastnosti nezávisle od ich hlavnej úlohy kontroly glykémie.

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mation for delaying of diabetic complications is now intensively studied in several laboratories.
Furthermore, for functional outcomes of the intervention with antioxidants is also important development of accurate and sensitive methods for early detection of oxidative damage in diabetes. ***(Tab. 6, Fig. 3, Ref. 117.)***
Key words: free radicals, diabetic complications, hyperglycaemia, advanced glycation, glycoxidation, lipoxidation, oxidative stress, antioxidant.

Terapeutický potenciál inhibítorov tvorby AGE-produktov na spomalenie vývoja diabetických komplikácií sa intenzívne skúma vo viacerých laboratóriách.
Okrem toho, pre úspešnosť antioxidačnej terapie je dôležitý aj vývoj presných citlivých metód pre skorú detekciu oxidačného poškodenia pri diabete. *(Tab. 6, obr. 3, lit. 117.)*
Kľúčové slová: voľné radikály, diabetické komplikácie, hyperglykémia, pokročilá glykácia, glykooxidácia, lipooxidácia, oxidačný stres, antioxidant.

The recent growth in knowledge of free radicals and reactive oxygen species (ROS) in biological systems is producing a medical revolution that promises a new age of health. ROS plays an important role in living systems through their beneficial and detrimental effects (1, 2). ROS play role not only in acute conditions, such as trauma, stroke and infection, but also in physical exercise and mental stress. Oxidative stress has been implicated in the etiology of a chronic diseases and aging. If oxygen free radicals are involved in all of these clinical conditions, then antioxidants could be effective in preventing their occurrence. Thus, research of ROS lead to a new paradigm of human health, with a shift toward a greater emphasis on disease prevention.

ROS can also participate in diabetogenesis (3) and development of late diabetic complications (4). For effective pharmacological interventions with antioxidants, it is important to understand the basic molecular mechanisms by which ROS cause disease. Furthermore, the development of accurate and sensitive methods for early detection of oxidative damage, is also important for the treatment of diabetes.

Diabetes of type 1 and 2

Diabetes is associated with a variety of metabolic abnormalities, principle among them is hyperglycaemia. The so-called metabolic syndrome includes dyslipidemia characterized hypertriglyceridemia, reduced HDL cholesterol and abnormal postprandial lipemia (5), atherosclerosis and pro-coagulant state. The metabolic syndrome represents a cycle whereby insulin resistance leads to compensatory hyperinsulinemia which maintain normal plasma glucose and may exacerbate insulin resistance.

Type 1 diabetes or insulin-dependent diabetes mellitus (IDDM) is a complex, multifactorial disease involving severe destruction of the insulin-producing pancreatic ß-cells. Type 1 diabetes is generally associated with a young or juvenile onset (6). Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) typically occurs with older age and obesity (7). Although glycaemic control, insulin treatment and other chemical therapies can control many aspects of diabetes, numerous complications are common and diverse. Diabetic patients have a increased risk of developing of various clinical complications that are largely irreversible and due to microvascular or macrovascular disease (Tab. 1).

Tab. 1. Vascular complications of diabetes mellitus.

Microvascular	Macrovascular
Nephropathy	Ischaemic heart disease
Retinopathy	Stroke
Neuropathy	Peripheral vascular disease

The impact of microvascular disease in diabetes include nephropathy, retinopathy and neuropathy. Macrovascular disease is associated with the 2—4 fold increased risk for atherosclerosis and ischaemic heart disease that can occur in diabetic individuals. The complications of macrovascular disease are important causes of morbidity, mortality and disability in people with Type 2 diabetes mellitus. Although the increased death rate is mainly due to cardiovascular disease, deaths from non-cardiovascular causes are also increased. In the pathogenesis of diabetic complications important risk factors include not only duration of diabetes, but also dyslipidemia, hypertension and cigarette smoking.

The results of the Diabetes Control and Complications Trials clearly established hyperglycaemia as the major causal factor for the development of diabetic microvascular complications (8). The role of hyperglycaemia as an independent risk factor (not the major) for the development of cardiovascular disease is supported by the United Kingdom Prospective Diabetes Study (9). Improving glycaemic control delays the onset and reduce the severity of diabetic complications (10). However, even with intensive use of current antidiabetic agents more then 50 % of diabetic patients type 2 suffer poor glycaemic control and 18 % develop serious complications within six years of diagnosis. There is need for new antidiabetic agents.

Several theories have emerged to explain the adverse effects of hyperglycaemia on vascular tissues (Tab. 2). Other risk factors that could be operating at cellular level initiate and promote progression of diabetic vascular disease include insulin resistance and hyperinsulinemia (21), altered fatty acid metabolism — dyslipidaemia (22—23), hypertension, ketoacidosis, osmotic effects, vasoactive hormones and dysfunction in sympathetic regulation of glucose and fat metabolism (24).

Insulin resistance is a primary risk factor for Type 2 diabetes mellitus. It is also associated with cardiovascular disease, hyper-

Tab. 2. Proposed mechanisms of the adverse effect of hyperglycaemia.

Mechanism	References
Glycation and advanced glycation-AGE concept	11
Activation of the polyol pathway	12
Activation of diacylglycerol and protein kinase C	13
Redox imbalance – “pseudohypoxia” concept	14
Oxidative stress concept	15-17
Carbonyl stress concept	18
Growth factors alteration	19
Altered gene expression	20

tension and certain forms of cancer. Patients with type 2 diabetes often have elevated serum levels of fatty acids. It appears that control of nonesterified fatty acids (NEFA) in the blood could be an important approach to reduce insulin resistance. It was hypothesized (25) that the primary route of which insulin maintains control over glucose production is indirect and is mediated by regulation of NEFA release from the adipocyte.

In one recent study, a Yale research group showed that elevated plasma levels of fatty acids suppress glucose uptake by interfering with the IRS-1 signaling pathway (26). It may be that any perturbation that results in accumulation of intracellular fatty acids or their metabolites in muscle and liver sets off a cascade that leads to reduced IRS-1 and IRS-2 activity (located inside the cell membrane), leading to insulin resistance in these tissues.

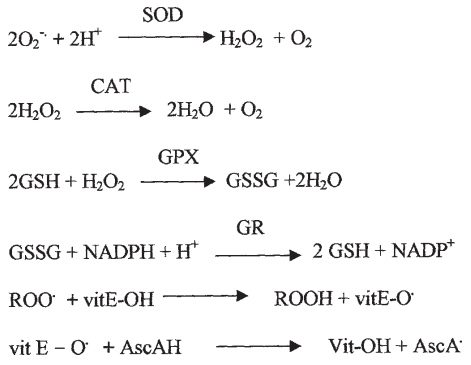


Fig. 1. ROS scavenging system.

Free radicals, oxidative stress and antioxidant systems

Free radicals are defined as an atoms or molecules that contain one or more unpaired electrons, making them unstable and highly reactive. The most important ROS are the superoxide anion radical $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2), alkoxyl ($RO^{\cdot}$), peroxyl ($ROO^{\cdot}$), hydroxyl radicals ($^{\cdot}OH$), and hypochlorous acid (HOCl). Other non-oxygen species existing as reactive nitrogen species (RNS), such as nitric oxide ($NO^{\cdot}$) and peroxynitrite have also important bioactivity. ROS is continuously generated in physiological conditions and effectively eliminated of several intracellular and extracellular antioxidant systems (27).

Uncontrolled production of ROS often leads to damage of cellular macromolecules (DNA, lipids and protein) and other small antioxidant molecules. A number of major cellular antioxidant defense mechanisms exist to neutralize the damaging effects of free radicals. Enzymatic antioxidant system (Cu,Zn- and Mn-superoxide dismutase (SOD), catalase, glutathione (GSH) peroxidase (GPX), and GSH reductase (GR)) function by direct or sequential removal of ROS, thereby terminating their activities (Fig. 1). To minimize transition metal-induced catalysis of Fenton and Haber-Weiss reactions that generate the most reactive hydroxyl radical:

Haber Weiss $H_2O_2 + O_2^{\cdot-} \rightarrow O_2 + OH^- + {}^{\cdot}OH$

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

Several specific metal-binding proteins such as ceruloplasmin, ferritin, transferrin, haptoglobin, lactoferrin and albumin ensure that these metals (copper and iron) are cryptic. Nonenzymatic antioxidant systems consist of scavenging molecules that are endogeneously produced (GSH, ubichinol, uric acid) or those derived from the diet (vitamins C and E, carotenoids, α-lipoic acid, selenium, etc) (Tab. 3). Functioning as scavengers, these molecules donate electrons, and themselves become free radicals that can either initiate chain reactions, or conversely be regenerated.

In health, balance between production of ROS/RNS and antioxidant defenses lies slightly in favour of ROS/RNS production. Oxidative stress occurs when there is an imbalance between free radical reactions and the scavenging capacity of antioxidative defense mechanism of the organism (28). Oxidative stress is a severe disruption of balance in favour of ROS/RNS. In principle, oxidative stress can result from increased production of ROS/RNS, excessive activation of phagocytic cells in chronic inflammatory diseases, diminished antioxidants e.g. mutations affecting antioxidant defence systems and depletions of dietary antioxidants and micronutrients.

Consequences of oxidative stress are adaptation or cell injury, i.e. damage to DNA, proteins and lipids; disruption in cellular homeostasis and accumulation of damaged molecules (Fig. 1).

The tissue level of antioxidants critically influences the susceptibility of various tissues to oxidative stress. Enhanced oxidative stress and oxidative damage to tissues are general features of most chronic diseases such as Alzheimer's disease, cancer, atherosclerosis and rheumatoid arthritis.

Oxidative stress in diabetes mellitus

Many studies have shown that increased lipid peroxides and/or oxidative stress are present in diabetic subjects (29—31). Oxidative stress can be increased before clinical signs of diabetic complications. However, the role of oxidative stress in the initiation and progression of diabetes remains uncertain. It is debatable whether oxidative stress precedes the appearance of diabetic complica-

Tab. 3. Micronutrients and endogeneous antioxidants involved in free radical defense.

Nutrients	Functional role
Carotenoids	Hydrophobic antioxidant
Vitamin E	Hydrophobic antioxidant
Niacin, tryptophan	Precursors to NADH/NADPH
Riboflavin	Cofactor for GSH reductase
α–lipoic acid	Cofactor for oxid. decarboxylation of pyruvate to acetyl-coenzyme
Selenium	Integral part of GSH peroxidase
Zinc/copper	Integral part of SOD
Manganese	Integral part of SOD
Bioflavonoids	Hydrophobic antioxidant
Plant phenolics	Hydrophobic antioxidant
GSH	Endogeneous hydrophilic antioxidant
Ubiquinol (coenzyme Q)	Endogeneous hydrophobic antioxidant
NADH/NADPH	Endogeneous hydrophilic antioxidant

tions or whether it merely reflects the presence of complications or consequence of complications.

In diabetes, oxidative stress seems caused by both increased production of ROS, sharp reduction in antioxidant defenses and altered cellular redox status (32).

Hyperglycaemia may lead to an increased generation of free radicals via multiple mechanisms. Patients with diabetes may be especially prone to acute and chronic oxidative stress which enhances the development of late diabetic complications (33).

Although the source of this oxidative stress remains unclear, it has been suggested that the chronic hyperglycaemia in diabetes enhances the production of ROS from glucose autoxidation, protein glycation and glycoxidation, which leads to tissue damage (34). Also, cumulative episodes of acute hyperglycaemia (fasting or postprandial hyperglycaemia) can be source of acute oxidative stress (35). A number of studies have summarized the relation between glycation and oxidation (36—37).

Enhanced oxidative stress in diabetes Type 2 has further a variety of important effects in atherogenesis, including lipoprotein oxidation, particulary LDL oxidation. Lipid peroxidation of polyunsaturated fatty acids (PUFA), one of the radical reaction in vivo, can adequately reflect increased oxidative stress in diabetes (38).

Further possible sources of oxidative stress are decreased antioxidant defenses, alterations in enzymatic pathways and other mechanisms (Tab. 4) such as ischaemic-reperfusion injury (38). ROS are implicated also in the pathogenesis of the inflammatory response to ischaemic-reperfusion which is exacerbated in diabetes. Oxidative stress during reperfusion is markedly balanced in diabetes and this appears to results from increased leukocyte recruitment and a higher capacity of diabetic leukocytes to generate ROS in response to stimulation (39). Another proposed mechanism is hydrolysis of NAD(P)H into ADP-ribose(P) and nicotinamide induced by ROS in postischemic tissues (40).

Tab. 4. Possible sources of oxidative stress in diabetes mellitus (modified by ref. 17).

Increased generation of ROS
Autoxidation of carbohydrates, autoxidation of fatty acids in triglycerides, phospholipids and cholesteryl esters
Acute and chronic hyperglycaemia
Glycation, advanced glycation and glycoxidation
Decreased antioxidant defense
Alterations in glutathione concentration or metabolism
Decreases in antioxidant systems, such as catalase, SOD or GPX
Alterations in vitamin E and ascorbate homeostasis
Alterations in concentrations of other antioxidants, such as ubiquinol, carotene, taurine and uric acid
Alterations in enzymatic pathways
Increased polyol pathway activity
Decreased glyoxalase pathway activity
Alteration in mitochondrial oxidative metabolism
Altered prostaglandin and leukotriene metabolism
Other mechanisms
Ischaemia-reperfusion injury, hypoxia and pseudohypoxia

Hyperglycaemia, insulin, insulin resistance and oxidative stress

Several studies show that acute hyperglycaemia can impair the physiological homeostasis of many systems in living organisms. Excessive hyperglycaemia may impair insulin activity and sensitivity by the mechanism of "glucose toxicity" (41). Insulin stimulates the uptake and utilisation of glucose in muscle and adipose tissue, inhibits glycogenolysis and gluconeogenesis in the liver and inhibit lipolysis in adipose tissue. Deficient action of insulin reverses the metabolism in the opposite direction.

Thus, with increased lipolysis is enhanced level of free fatty acids and their oxidation in liver. In animal models, hyperglycaemia increases fatty acid availability in muscle. Thus, both "glucotoxicity" and "lipotoxicity" could lead to insulin resistance and hyperinsulinemia (42). It appears that insulin resistance must occur in both muscle and liver for type 2 diabetes.

Both hyperglycaemia and insulin resistance are accompanied by reduced insulin action (43—44). Hyperglycaemia and insulin resistance may also be accompanied by oxidative stress (45—46). Ceriello (47) hypothesized a model that oxidative stress represents the common pathway through which hyperglycaemia and insulin induce a depressed insulin action. This point of view is supported by studies with antioxidants, which are able to improve the action of insulin (48—50).

Free radicals, hyperglycaemia, insulin resistance, dyslipidemia and endothelial dysfunction

The chronic hyperglycaemia, insulin resistance and abnormal lipoprotein profiles found in diabetes may contribute to a decrease of bioavability of vascular nitric oxide (NO), impairing endothelium-dependent vasodilatation documented in animal models and in humans with diabetes.

NO is a normal product of arginine metabolism and that it reacts rapidly with superoxide to form peroxynitrite:

$$O_2^{\bullet -} + {}^{\bullet}NO \rightarrow ONOO^-$$

$$ONOO^- + H^+ \leftrightarrow ONOOH \rightarrow {}^{\bullet}OH + {}^{\bullet}NO_3 \rightarrow NO_3^- + H^+$$

NO possesses a variety of antiatherogenic properties, and loss of these protective mechanisms may lead to an increase in susceptibility to vascular disease. NO may be an important antioxidant in the vascular system.

NO is synthesized by at least three distinct isoforms of NO synthase (NOS) (51, 52). All three isoforms have implications (physiological or pathophysiological) in the cardiovascular system. Endothelial NOS III is physiologically important for vascular homeostasis, keeping the vasculature dilated, protecting the intima from platelet aggregates and leukocyte adhesion and preventing smooth muscle proliferation. Central and peripheral neuronal NOS I may also contribute to blood pressure regulation.

Enhanced oxidative stress may directly induce endothelial dysfunction by decreased synthesis or release of NO by endothelial cells and by inactivating NO with superoxide in subendothelial space (53). Furthermore, the alkoxyl radicals detected by electron paramagnetic resonance (EPR) spectroscopy have been shown

to directly interact with NO (54). NO is very good scavenger of other free radicals and therefore may be an important antioxidant in vascular system.

The mechanisms leading to the decrease in biological NO activity in diabetes are not well understood. The biological activity of NO, as measured by endothelium-dependent vasodilatation, has been studied extensively in animal models of Type 1 diabetes induced by either alloxan or streptotozotocin. Extensive evidence exists for endothelial dysfunction in diabetes. Hyperglycaemia has been shown to directly inhibit NO synthase activity. Decreased NO release or inactivation of NO by ROS may be responsible for these impaired endothelium-dependent vasodilator responses. The majority of human studies suggest abnormal endothelium dependent vasodilatation in Type 1 diabetes. Furthermore, NO activity in diabetes Type 2 appears also to be abnormal. Oxidative stress related to hyperglycaemia may reduce the amount of intracellular thiol present and thus decrease the vascular effects of nitrates, leading to an abnormality in endothelium-independent as well endothelium-dependent vasodilatation. This mechanism is supported by evidence that vitamin E supplementation may normalise tolerance to the rheological effects of nitrates in diabetic patients. Furthermore, ascorbate may acutely reverse the microvascular endothelial dysfunction in Type 2 diabetic subjects (55).

Acute hyperglycaemia can lead to abnormal vasodilator response to acetylcholine (in vitro and in vivo). This evidence supports the hypothesis that ROS formed during hyperglycaemia interferes with the interaction with NO at the vascular smooth muscle level.

The inactivation of NO by advanced glycation end-products (AGEs), formed in subendothelial space, may play also a role in defective vasodilatory responses that occur in diabetes. Finally, some animal models of diabetes have demonstrated decreased levels of endogeneous antioxidant systems such as SOD and catalase, which may in turn lead to an increased breakdown of NO by free radicals. A number of investigators have reported that scavengers of ROS (vitamin E, SOD, parenteral vitamin C) improve endothelium-dependent relaxation in diabetic animals in vitro and in vivo (56—58).

What is the effect of the insulin resistance and hyperinsulinemia on the balance of insulin action in vasculature? Insulin promotes vasodilatation by stimulating NO production from endothelial cells and has antiatherogenic activity. However, increased insulin action associated with hyperinsulinemia is thought to contribute to atherogenesis (59). Multiple signalling pathways mediate the actions of activated insulin receptor and insulin resistance in the vasculature may selectively inhibit some of the antiatherogenic actions of insulin.

Free radicals, vascular oxidative stress and antioxidant systems in vascular wall

Experimental observations, experiments with antioxidants and antioxidant pharmacotherapy have shown that oxidative stress may be one important factor in the genesis of atherosclerotic vascular disease (60—61). For example, oxidized LDL has been isolated from atherosclerotic lesions. Chemical and immunohistochemical studies detect oxidized lipids in human and animal atherosclerotic lesions. Experimentally, antioxidants have been shown to retard lesion formation in hypercholestrolemic animals. Finally, vitamin E dramatically reduces the risk for acurate coronary events in patients with established coronary artery disease (62—63).

The possible sources of vascular oxidative stress in vascular smooth muscle cells and endothelial cells are shown in Table 5.

Tab. 5. Sources of vascular oxidative stress.

Acute and chronic hyperglycaemia
Glycation, advanced glycation, glycoxidation
Lipoxidation
Extracellular metal ions
Superoxide and HOCl
NADPH/NADH oxidase
Nitric oxide synthase
Cyclooxygenase
Lipoxygenase
Myeloperoxidase
P450 monooxygenases
Enzymes of mitochondrial oxidative phosphorylation

The antioxidant systems in the extracelullar fluid present in the vascular wall have not been evaluated systematically since this fluid is difficult to obtain for experimentation. However, it is reasonable to assume that the antioxidant composition of the fluid surrounding cells, extracellular matrix and lipoprotein particles in intima is comparable to that of human plasma (64) and suction blister fluid (65).

In the artery wall proteinaceous antioxidants are present. Compared to cells, extracellular fluids are devoid of the antioxidants systems such as Cu,Zn-SOD, Mn-SOD, GSH peroxidase, GSH reductase, GSH transferase and catalase. GSH peroxidase is effective against H_2O_2 and fatty acid hydroperoxides. However, GSH peroxidase does not act on hydroperoxides of phospholipids, cholesteryl ester and triglycerides. Possibly, methionine residues in apolipoproteins A-I and A-II reduce lipid hydroperoxides in lipoproteins while becoming oxidized to methionine sulfoxide (66):

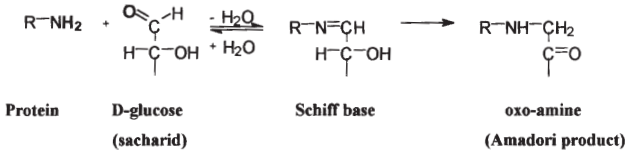
$$Met_{apo} + LOOH_{lip} \rightarrow \text{Methionine Sulfoxide}_{apo} + LOH_{lip}$$

Intersticial fluids contain large amounts of extracellular SOD (EC-SOD) within the arterial wall. In contrast, the levels of extracellular Cu,Zn-SOD and Mn-SOD are low compared to other tissues. In the human aorta, EC-SOD is produced by and surrounds smooth muscle cells beneath the endothelium, and is localized throughout the connective tissue matrix of the vessel. The role of EC-SOD and Cu,Zn-SOD is likely to prevent inactivation of NO by superoxide and hence to protect endothelium-dependent arterial relaxation.

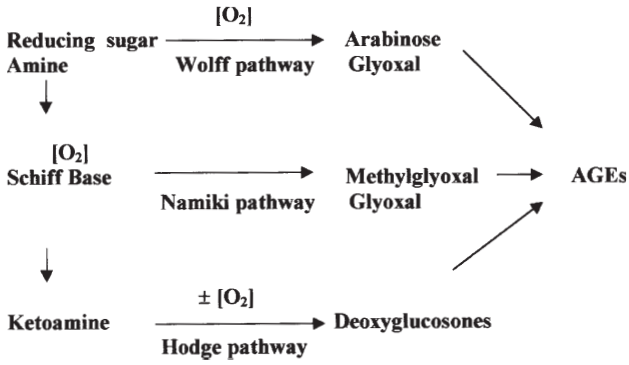
Interstitial fluid and lymph contain ascorbate and urate at concentrations comparable to those in human blood plasma. It is now generally accepted that the reduced form of vitamin C represents a first line antioxidant defense against lipid peroxidation induced by free radicals. Ascorbate is a highly efficient scavenger of various radicals including α-tocopherol radical and its prevents tocopherol-mediated peroxidation. Among the lipid soluble antioxidants, α-tocopherol plays a central role as it controls radical-induced lipoprotein lipid peroxidation (67—68).

Glycation, advanced glycation, glycoxidation, lipoxidation and vascular disease

In hyperglycaemia, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars, such as glucose, to yield a Schiff-base intermediate (Maillard reaction). These Schiff base intermediates undergo Amadori rearrangement to stable ketoamine derivative (fructosamine)



Through the Wolff pathway glucose may undergo metal-catalyzed autoxidation to produce reactive carbonyl precursors of AGEs, such as arabinose and glyoxal:

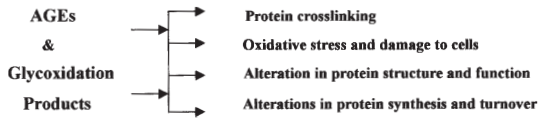


Schiff bases through Namiki pathways can also be fragmented to glyoxal or methylglyoxal. These compounds are more reactive then the parent sugars with respect to their ability to react with amino groups of proteins.

Amadori products degrade through rearrangement and autoxidation (Hodge pathway) into α-dicarbonyl compounds. Thus, the α-dicarbonyl compounds or α-ketoaldehydes are mainly responsible for forming inter- and intramolecular crosslinks of proteins known as AGEs and glycoxidation products (69—72). These products in tissue proteins involve pyrraline (lysine pyrrole carboxyladehyde adduct), 3-DG-hydroimidazolone (arginine-3DG imidazolone adduct), pentosidine (arginine-lysine crosslink), crosslines and vesperlysines (dilysine crosslinks). First two adducts are formed under non-oxidative conditions. Advanced glycoxidation products such as the crosslines pentosidine and vesperlysines are produced by secondary modifications of proteins by products of carbohydrate oxidation.

The most important product of glycoxidation is carboxymethyllysine (CML) and was proposed as marker of local oxidative stress in tissues (73). AGEs and glycoxidation products accumulate with aging, atherosclerosis and diabetes; especially associated with long-lived proteins such as collagens, lens crystallins and nerve proteins. The AGE concept proposes that chemical modification and crosslinking of tissue proteins, lipids and DNA affect their structure, function and turnover, contributing to gradual decline in tissue function and the pathogenesis of diabetic complications.

AGEs have been shown to induce cellular lipid peroxidation through interacting with RAGE receptor and this effect can be attenuated by vitamin E.



So called advanced lipoxidation products (ALEs) (see Tab 6) including malondialdehyde (MDA) and 4-hydroxynonenal (HNE) adducts to lysine (MDA-Lys, HNE-Lys) and pyrroles are formed during lipid peroxidation reactions, whereas other compounds (EAGLEs), such as CML and N-carboxyethyllysine, GOLD, MOLD, are formed during glycoxidation and lipoxidation reactions (74, 75).

Hyperglycaemia and Maillard reactions in relation to free radicals generation

There are a number of various putative mechanisms that link hyperglycaemia to oxidative stress. Among the most direct is autoxidation of glucose. Monosaccharides with an α-hydroxyaldehyde structure, like glucose, are subject to enediol rearrangement that results in the formation of an enediol radical ion (76). The formation of this radical anion has two important implications. First, this species is capable of reduced molecular oxygen to form superoxide anion which under certain circumstances, may contribute to the lipoxidation or the activation of platelets. Second, the dicarbonyl products are reactive and modify lysine and arginine groups to form AGEs, such as CML and N-carboxymethylarginine (CMA). These reactions are dependent on transition metal ions. The source of extracellular transition metal ions can be ferritin, ceruloplasmin or SOD that release transition metal ion after conformation changes induced by glycation.

Formation of free radicals is associated with Maillard reactions (77—80). Baynes (81) proposed that Maillard reaction is amplifier of oxidative stress. Glycated proteins produce nearly 50 fold more free radicals than non glycated proteins (82). In diabetes, process of production of superoxide radicals by the transition metal-catalyses autoxidation, followed by the dismutation of superoxide to hydrogen peroxide; and the generation of hydroxyl radicals by Fenton reaction results in a site specific attack on proteins, with consequent protein damage and lipoxidation and damage to other cell components, such as DNA (Fig. 2).

Alternative of oxidative stress — carbonyl stress

Carbonyl stress explain increassed modification of proteins in diabetes, uremia and other diseases by a generalized increase in the concentration of reactive carbonyl precursors of AGEs, glycoxidation and lipoxidation products (18). So, these carbonyls may damage not only proteins but also phospholipids and nucleotide base DNA.

Carbonyl stress may result from an increase in substrate stress and/or a decrease in the efficiency of detoxification of carbonyl compounds. It seems that generalized oxidative stress in diabetes not occur (Fig. 3). Possibly, exist a local oxidative stress, which may be an important modulator for the development of diabetic

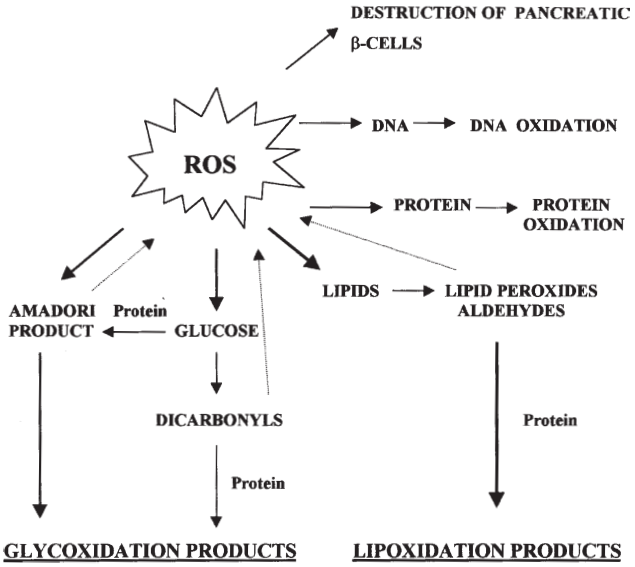


Fig. 2. ROS attack glucose, Amadori product, lipids, protein, DNA and also pancreatic ß-cells. The forming dicarbonyl products mediate glycoxidation and lipoxidation. In the process, more ROS are generated (....>), establishing further vicious and interactive cycles of molecule damage.

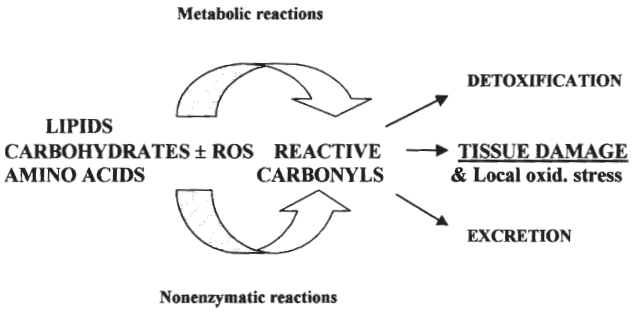


Fig. 3. Factors contributing to carbonyl stress.

complications. Oxidative stress may increase vascular damage and may be further amplified or subsequently damaged (83).

Biomarkers of oxidative stress

Several biomarkers of DNA oxidation, lipid peroxidation, amino acids oxidation, glycoxidation and lipoxidation reactions have been identified and can be measured in short-lived intracellular proteins, plasma proteins, long-lived extracellular proteins and in urine by chemical methods (Table 6). These markers are measured by sensitive high-performance liquid chromatography or gas chromatography-mass spectrometry, requiring both complex analytical instrumentation and derivatization procedures. Also sensitive immunohistochemical and ELISA assays are now available for many of these biomarkers (85). Immunochemical techniques are becoming an increasingly important part of the methodology for detection and measurement of oxidation products in tissues.

The only technique that can detect free radicals directly is the spectroscopic technique of electron spin resonance (ESR). However, this method often is too insensitive to directly detect superoxide and hydroxyl radicals in living systems. Another method is trapping (for example spin trapping), in which a radical is allowed to react with a trap molecule to give one or more stable products, which are then measured. An alternative to trapping is fingerprinting. The detection relies on the formation of secondary products and appropriate chemical manipulation.

Many indirect methods have been proposed and are generally used to assess in vivo oxidative stress. These methods evaluate not only levels of damaged biological products, but also antioxidant status (33). In many studies, measures of total trapping antioxidant parameter (TRAP) were reduced in diabetes. However, no consistent correlation has been found between total antioxidant capacity and diabetic metabolic control. Furthermore, there is no definite link between changes in these oxidative stress levels in diabetes and the development of late diabetic complications.

Lipid peroxidation

Malondialdehyde (MDA) is a highly toxic product formed in part by lipid oxidation derived free radicals. Many studies have shown that its concentration is considerably in diabetes mellitus, correlating with poor glycaemic control. However, an increase in lipid peroxidation products in well controlled diabetics with no evident vascular complications was found.

4-HNE reacts with lysine from proteins and forms pyrroles. Very promising are F2-isoprostanes as in vivo markers of lipid peroxidation (86-87). Davi et al (87) have found an increased plasma urinary level of F2-isoprostanes in diabetics of both types. It seems that increased lipid peroxidation in NIDDM has important implications for late vascular disease in diabetes. Improved metabolic control of NIDDM significantly reduced level of F2-isoprostanes. These data suggest that extent of metabolic control has a profound influence on the degree of oxidative stress in diabetic patients.

Amino acid and protein oxidation

Modification of amino acids and proteins is initiated mainly by $OH^{\bullet}$. However, the course of the oxidation process is determined by the availability of O_2 and $O_2^{-\bullet}$ or its protonated form (HO_2). ROS can lead to oxidation of amino acid residue side chains, formation of the protein-protein cross-linkages, and the oxidation of the protein backbone resulting in protein fragmentation (88—89). Other forms of ROS may also yield similar products and that transition metal ions can substitute for OH and $O_2^{-\bullet}$ in some of the reactions.

Primary products of oxidation of proteins results from direct reaction of proteins with ROS, and their concentration in tissue proteins should provide a direct index of oxidative stress. These compounds are isolable by acid hydrolysis of proteins, such as o-tyrosine, dityrosine and methionine sulfoxide. Other products such as protein carbonyls, may be derived from several sources, are unstable to acid hydrolysis.

Secondary products of oxidation of proteins result from reaction of proteins with products of oxidation of small molecules, including lipids, carbohydrates and amino acids. Carbonyl groups may be introduced into proteins by reactions with MDA, 4-HNE during lipid pe-

roxidation. These lipoxidation products are MDA-Lysine or 4-HNE-Lysine adducts which are labile. Another secondary product of oxidation results from reaction of proteins with reactive carbonyl derivatives (ketoamines, ketoaldehydes, deoxyosones) generated as a consequence of the reaction of reducing sugars or other oxidation products with lysine residues of proteins (glycation and glycoxidation). These glycoxidation products such as carboxymethyllysine (CML), carboxyethyllysine (CEL), pentosidine and vesperlysines are stable to hydrolysis. Another AGEs such as crosslines are labile to hydrolysis.

CML, CEL, pentosidine, GOLD and MOLD accumulate with age and in long lived-proteins, such as collagens and crystallines. The level of secondary oxidation products is determined from level of oxidative stress and the ambient concentration of oxidizable substrates.

Conversely, some AGEs, such as pyrralines and imidazolones formed by reaction of 3-deoxyglucosone with lysine and arginine residues in protein do not require oxidation for their formation from reducing sugars. These AGEs are useful as markers of nonoxidative chemical modification of proteins (Tab. 6).

Free radicals, activation of NF-kappa-B and diabetogenesis

The development of IDDM can trigger risk factors such as genetic, developmental, environmental or dietary. However, it is likely that ROS/RNS play central role in pancreatic ß-cell death and disease progression. It was suggested that pancreas-specific ROS/RNS production play a critical role in signalling the cellular autoimmune-inflammatory responses by activating the transcription factor, NF-kappa B (90). Some antioxidants such as lipoic acid, N-acetylcysteine and selenoproteins can suppress NF-kappa B activation. Therefore the specificity of antioxidants to inhibit NF-kappa B activation and the hyperglycaemic response emphasizes the importance of selectivity in antioxidant therapy. The key to successful antioxidant therapy in IDDM will rely both on effective targeting to the islet cells and on pharmacological dose.

Tab. 6. Biomarkers of oxidative stress (modified by ref. 84).

ROS and RNS		
Superoxide radical, hydrogen peroxide, nitric oxide, hypochlorous acid, peroxides, peroxyl radical, peroxynitrite, metal-oxo complexes, semiquinone radical, heme proteins, singlet oxygen		
Products of lipid peroxidation		
MDA, 4-HNE, hydroperoxides, conjugated dienes, F2-isoprostanes dicarboxylic acids		
Products of DNA oxidation		
modified bases, 8-oxo-2'-deoxyguanosine, strand breaks		
Primary products of protein oxidation		
o-tyrosine, o,o'-dityrosine, 3-chlorotyrosine,3-nitrotyrosine, dihydroxyphenylalanine, protein disulfides, methionine sulfoxide hydroperoxides of isoleucine, leucine, valine protein carbonyls – adipic semialdehyde, 2-oxohistidine		
Secondary products of protein oxidation		
AGEs	ALEs	EAGLEs
Pentosidine	MDA-Lys, MDA-LDL	CML, CMA, CEL
Crosslines	HNE-(Lys, His, Cys)	Argpyrimidine
Vesperlysines	Pyrroles	GOLD, MOLD
Antioxidant defence systems and total antioxidant status		
Levels of enzymes and antioxidants		

Diabetic nephropathy, oxidative stress and antioxidant systems

About 30 % of diabetics have nephropathy. Diabetic nephropathy is characterized by proteinuria >300 mg/24 h, increased blood pressure, and progressive decline in renal function. At its most severe, diabetic nephropathy results in end stage renal disease (ESRD) requiring dialysis or transplantation. In early stages of diabetic nephropathy is microalbuminuria defined as albumin excretion rate (EAR) of 20—300 mg/24 h. The development of proteinuria is a marker of widespread vascular damage and signifies and increased risk of subsequent ESRD and macrovascular disease, especially coronary heart disease (CHD).

The pathological hallmark of diabetic nephropathy in the renal glomerulus is the expansion of the mesangial matrix and thickening of the capillary basement membrane.

Hyperglycaemia can generate not only more ROS but can also attenuate antioxidative mechanism through glycation of the scavenging enzymes. In the pathogenesis of diabetic nephropathy have a possible role oxidative stress and protein kinase C. A causal relationship between oxidative stress and diabetic nephropathy has been established by observations that (1) lipid peroxides and 8-hydroxydeoxyguanosine were increased in the kidneys of diabetic rats with albuminuria, (2) high glucose directly increases oxidative stress in glomerular mesangial cells, a target cell of diabetic nephropathy, (3) oxidative stress induces mRNA expression of TGF-ß1 and fibronectin which are the genes implicated in diabetic glomerular injury and (4) inhibition of oxidative stress ameliorates all the manifestations associated with diabetic nephropathy (91). Proposed mechanisms involved in oxidative stress are glucose autoxidation, formation of both, AGEs and lipoxidation products — ALEs and EAGLEs (92—94).

Immunohistochemical studies demonstrated the accumulation of CML and pentosidine in mesangial matrix and nodular lesions of diabetes. CML is thought to be an important epitope for many of currently available AGE antibodies. It seems that CML is a potential marker for diabetic nephropathy. In model study with neonatal mesangial cells AGEs induced intracellular oxidative stress, increase in intracellular calcium and translocation of PKC from cytosol to membrane (95). The activation of PKC under diabetic conditions may also have a modulatory role in oxidative stress-induced renal injury in diabetes mellitus.

Furthermore, a pathofysiological role of Amadori albumin in nephropathy in diabetic patients of Type 1 has been proposed (96).

The protective effect of antioxidants on renal injury in diabetic animals has been recently reported (92). Taurine and vitamin C effectively reduced glomerular hypertrophy, albuminuria, glomerular collagen and TGF-ß1 accumulation in rats with streptozotocin induced diabetes. Administration of vitamin E prevented glomerular hyperfiltration, albuminuria and renal PKC activity.

Diabetic retinopathy, oxidative stress and antioxidant systems

Diabetic retinopathy is a progressive disorder classified according to the presence of various clinical abnormalities. It is the

most common cause of blindness in people aged 30—69 years. After 15 years almost all patient with diabetes type 1 and two thirds of those with Type 2 diabetes have background retinopathy. In diabetic retinopathy is hypothesized the role of increase ROS species or to be associated with ischaemia-reperfusion injury at the boundaries of perfused and non perfused retina.

Age-modified proteins may play an important role in pathogenesis of diabetic retinopathy (97). Studies in humans suggested that antioxidant therapy with vitamin E might normalize diabetic retinal hemodynamics (98). Many epidemiological studies showed that supplementation with antioxidants did not influenced retinopathy (99). Aminoguanidine, the best prototype inhibitor of AGEs formation in experimental models, inhibits the development of retinopathy, nephropathy and neuropathy (100). Aminoguanidin has also antioxidative properties, inhibit lipid peroxidation (101). However, all clinical studies with aminoguanidine (Pimagidine) in Europe and then in USA were stopped because its toxicity.

Diabetic neuropathy, oxidative stress and antioxidant systems

The diabetic neuropathies present in several ways. The most common form is diffusive progressive polyneuropathy affecting mainly feet. It is predominantly sensory, often asymptomatic, and affect 40—50 % of all patients with diabetes.

In the genesis of the neuropathy can play hyperglycaemia-induced polyol pathway hyperactivity associated with nerve sorbitol accumulation and myo-inositol depletion (102). Sugimoto et al. (103) demonstrated that CML is accumulated in diabetic peripheral nerve.

Treatment using a variety of antioxidant compounds such as vitamin E, α-lipoic acid, adduct of lipoic and gamma-linolenic acid, glutathione, probucol, N-acetylcysteine, and transition metal chelators results in a significant reduction in neuropathic symptoms in human and experimental diabetes (104—106).

Macrovascular disease, oxidative stress and antioxidant systems

Atherosclerotic disease accounts for most of the excess mortality in patients with diabetes. Hyperlipidemia is no more common in patients with well controlled type 1 diabetes. Diabetes type 2 is associated with a more atherogenic lipid profile. Hypertension affect at least half of patients with diabetes.

The oxidative modification hypothesis of atherogenesis states that oxidation of lipids and lipoproteins, such as LDL, is important, if not obligatory for the atherogenic process. There is evidence that oxidation of LDL takes place in vivo: presence of epitopes of oxLDL in atherosclerotic lesions, oxLDL can be isolated from atherosclerotic lesions, lipid peroxidation is specific for diseased tissue, small quantities of modified LDL have been detected in the circulation, oxLDL epitopes in vivo can be quantified and animal studies suggest reducing LDL oxidation limits atherogenesis (61, 107). In animal studies a variety of antioxidants (Probucol, vitamin E, dietary antioxidants, BHT, DPPD) inhibit atherosclerosis providing strong support that oxidative modification of LDL is an important, if not obligatory, event in atherogenesis. Although epidemiologic data in humans support a role of antioxidants in the prevention of clinical events, intervention trials thus far have given mixed results. This may be due to technique of measurement of specific biomarkers of lipid peroxidation.

Ceruloplasmin, 15-lipoxygenase, myeloperoxidase and the enzymes essential for peroxynitrite production (nitric oxide synthase and NDD(P)H oxidase) are present in both animal and human lesions and could contribute to LDL oxidation.

Given the oxidative theory of atherosclerosis, the question of an increased in vitro oxidizability of low density lipoproteins is adressed. Glycaemic control is very important in order to avoid an increased susceptibility of LDLs to oxidation in diabetic patients (108, 109). Several studies showed that glycation of apolipoproteins, especially apolipoprotein B, was enhanced in diabetic subjects.

The existing evidence (110) supports the view that oxidative stress may play a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases and that antioxidant therapy may prove beneficial in combating these problems. So, antioxidant therapy can have a beneficial effects in hypertension, atherosclerosis, ischaemic heart disease, cardiomyopathies and congestive heart failure.

The future of antioxidant therapy in diabetes mellitus

Generally, the possibilities of antioxidant pharmacotherapy can be divided on using of antioxidant systems — antioxidant enzymes and substrates, biogenic elements, combined drugs preparates, syntetic antioxidants and drugs with antioxidant activity.

An adequate antioxidant therapy may represent a strategy to protect pancreatic ß-cells against destruction during the development of autoimmune diabetes. There is evidence for role of inflammatory mediators in cytokine induced pancreatic ß-cell dysfunction. Interestingly, recent studies have shown supporting evidence that links predisposition to diabetes with inflammation, which in turn is related to oxidative damage (111).

Furthermore, oral antidiabetic compounds such as sulfonylureas (gliclazide) (112) and thiazolidinediones (113) can have a potential antioxidant activity. Metformin treatment can reduces level of methylglyoxal and so inhibit AGEs formation (114). Improvement of glycaemic control seems to be a beneficial factor to decrease oxidative stress in diabetes.

In the case of macrovascular complications is antioxidant therapy evident beneficial together with blood pressure control, management of dyslipidemia (115) and optimal glucose control. Apart of classical antioxidants such as vitamin C and E, lipoic acid, probucol, N-acetylcysteine to decrease of oxidative stress, AGE inhibitors, for example "amadorins" such as pyridoxamin (116, 117) may help to delay the development of diabetic complications. These novel drugs are able to inhibit conversion of Amadori compounds to AGEs and also lipoxidative modification of proteins. Their therapeutic potential is now being investigated in several laboratories.

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Received August 10, 2000.
Accepted October 20, 2000.

PREDSTAVUJEME NOVÉ KNIHY

Szelepcsényi J.: Nedýchajte, prosím! Rozhovory s akademikom Niederlandom. Bratislava, Tankred 2000, 286 strán, ISBN 80-968180-1-5.

Dostala sa mi do rúk výnimočná kniha od Jána Szelepcsényiho Nedýchajte, prosím! Ide o monografiu, ktorá bola spracovaná po podklade rozhovorov s akademikom Niederlandom. So záujmom a so zatajeným dychom som knihu prečítal. Je jednoducho výnimočná a úžasná, pretože formou spomienok opisuje život a dielo akademika Niederlanda. Kniha je doplnená významnou obrázkovou dokumentáciou z archívu prof. Niederlanda. Pred očami čitateľa sa predstaví životné dielo akademika Niederlanda v širokom diapazóne od klinickej biochémie, klinickej farmakológie, klinického skúšania liečiv i kontrolnej činnosti a etických prístupov klinickej farmakológie. Túto časť diela vysoko pozitívne zhodnotili vynikajúce osobnosti našej medicíny a ja sa môžem k nim len skromne pripojiť. Čo ma osobitne zaujalo z historického hľadiska. Krásny a láskavý prístup akademika Niederlanda k svojim rodičom, súrodencom, manželke, kolegom a žiakom. Každému, kto ho požiadal o radu, vždy ochotne pomohol a poradil. Toto sa stalo aj mne dvakrát na križovatke môjho života a boli to rady veľmi múdre, na ktoré stále spomínam a nimi sa riadim. Je to rada vnímať iných a pochopiť ich v bolesti a strasti a vedieť uniesť ťažkosti života.

Z reumatologického hľadiska ma zaujala stať o prof. Bywatersovi, ktorý predsedal sympóziu o salicylanoch, na ktorom vystúpil aktívne akademik Niederland. Prof. Bywaters bol osobným priateľom prof. Siťaja, môjho učiteľa a mal veľmi vrelý vzťah k slovenskej reumatológii. Patrí k popredným velikánom britskej a svetovej reumatológie. Som rád, že autor knihy dr. Szelepcsényi aj takýto detail uviedol vo svojej monografii. Pochopiteľne fascinujúca je stať o manželoch Carla a Grety Coriových a účasti prof. Niederlanda na biochemických projektoch týchto nositeľov Nobelovej ceny v Saint Louis.

Záverom dovoľte, aby som zaprial pánu akademikovi T.R. Niederlandovi dobré zdravie do ďalších rokov a autorovi diela dr. Szelepcsényimu úprimnú gratuláciu k skvelej monografii.

J. Rovenský