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Glutathione and glutathione–dependent enzymes in various pathophysiological states

Glutathion a glutathion-dependentní enzymy za různých patofyziologických stavů

Doctoral Thesis

Supervisor: RNDr. Eva Tvrzická, CSc.

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Abstract

Backround: Oxidative stress (OS) has been implicated in pathogenesis of human disorders such as depressive disorder, sepsis, cardiovascular disease, acute and chronic pancreatitis, and cancer. Increased OS is result of imbalance between increased reactive oxygen and nitrogen species (RONS) production and / or insufficient activity of antioxidant defence system. Antioxidant system, which is composed of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidases (GPX), glutathione reductase (GR) and non-enzymatic antioxidant reduced glutathione (GSH) plays an important role in the protection of cells against enhanced OS. The aim of this study was to assess the OS markers and antioxidant enzymes in different pathophysiological states.

Materials and methods: Activities of erythrocyte glutathione peroxidase (GPX1), GR and concentration of GSH as well as levels of OS markers were analysed in six different pathophysiologic states. These parameters were measured in 35 women with depressive disorder (DD), 40 patients with metabolic syndrome (MetS), 30 septic patients (S) followed up in the course of sepsis; 15 non-septic critically ill patients (NC), 13 patients with acute pancreatitis (AP), 50 with chronic pancreatitis (CP) and 50 patients with pancreatic cancer (PC), compared to age- and sex-matched controls (CON). Activities of GPX1 and GR and levels of GSH were determined spectrophotometrically in erythrocytes.

Results: The erythrocyte activities of GPX1 has been found to be decreased in DD patients, AP, S as well as in CP and PC patients, whereas no significant differences in GPX1 activities were observed in MetS patients compared with CON. Moreover, in the course of AP GPX1 activities did not differ among individual samplings. In the contrast to GPX1 activity, higher GR activity has been observed in DD, MetS and S compared to CON and S in comparison with NC. Whereas GR activity was found unaffected in the course of sepsis and AP, the decrease in GR activity has been observed in CP and PC patients compared to CON. In all aforementioned pathophysiologic states the levels of GSH were decreased.

Conclusion: It has been shown that there are alterations in antioxidant enzymes and antioxidants in different pathophysiologic states. Deficiency of antioxidant defence system results in increased OS, which is implicated in the pathogenesis all above mentioned diseases.

Key words: oxidative stress, antioxidant enzymes, depressive disorder, metabolic syndrome, sepsis, acute and chronic pancreatitis, pancreatic cancer

Abstrakt

Úvod: Oxidační stres (OS) hraje významnou úlohu v patogenezi neurodegenerativních onemocnění (depresivní porucha), kardiovaskulárních onemocnění, sepse, akutní a chronické pankreatitidy a rakoviny. Zvýšený OS je výsledkem nerovnováhy mezi produkcí reaktivních forem kyslíku a dusíku (RONS) a / nebo nedostatečnou kapacitou antioxidačního systému. V ochraně buněk proti zvýšenému OS a volným radikálům hraje důležitou roli glutathionový systém, tvořený antioxidačními enzymy glutathionreduktasou (GR) a glutathionperoxidasou (GPX) a redukovaným glutathionem (GSH), hlavním intracelulárním neenzymovým antioxidantem. Cílem této disertační práce bylo změřit aktivity antioxidačních enzymů GPX1, GR a hladiny GSH za různých patofyziologických stavů.

Materiál a metody: Aktivity GPX1, GR a hladina GSH, stejně jako koncentrace markerů OS byly měřeny u šesti různých onemocnění. Tyto parametry byly sledovány u 35 žen s depresivní poruchou (DD), 40 pacientů s metabolickým syndromem (MetS), 30 septických pacientů (S) sledovaných v průběhu sepse; 15 kriticky nemocných neseptických pacientů (NC), 13 pacientů s akutní pankreatitidou (AP), 50 s chronickou pankreatitidou (CP) a 50 pacientů s rakovinou slinivky břišní a porovnány se zdravými kontrolami párovanými podle věku a pohlaví. Aktivity GPX1 a GR a koncentrace GSH v erytrocytech byly stanoveny spektrofotometricky.

Výsledky: U pacientů s DD, AP, S v průběhu sepse, stejně jako u CP a PC pacientů byly pozorovány snížené aktivity GPX1 v erythrocytech v porovnání s kontrolami. Nepozorovali jsme žádné významné rozdíly v aktivitě GPX1 mezi pacienty s MetS a kontrolním souborem. Aktivity GPX1 se také významně nelišily v průběhu AP mezi jednotlivými odběry. Naopak aktivita GR byla zvýšená u pacientek s DD a pacientů s MetS ve srovnání se zdravými lidmi. Vyšší aktivita GR byla pozorována také u S oproti NC, zatímco v průběhu S a AP se aktivita GR významně nelišila. Pacienti s CP a PC měli statisticky významně snížené aktivity GR oproti kontrolám. Snížené hladiny GSH byly zjištěny u všech výše uvedených patofyziologických stavů.

Závěr: Prokázali jsme oslabený antioxidační systém u pacientů s různými onemocněními, v jejichž rozvoji hraje významnou roli oxidační stres.

Klíčová slova: oxidační stres, antioxidační systém, sepse, deprese, metabolický syndrom, akutní a chronická pankretitida, karcinom pankreatu

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Abbreviations

ANOVA	Analysis of Variance
AP	acute pancreatitis
AP1	1 st sampling within 24 hours after onset of acute pancreatitis
AP3	sampling after 72 hours
AP5	5 days after onset of signs
AP10	10 days after admission
APACHE II	Acute Physiology and Chronic Health Evaluation II score
CAT	catalase
CD/LDL	conjugated dienes in precipitated low density cholesterol
CON	controls
CP	chronic pancreatitis
CRP	C- reactive protein
cGSH-Px	cytosolic or cellular glutathione peroxidase
cTRAP	calculated total peroxyl radical trapping
Cys	cysteine
Da	dalton
DD	depressive disorder
DSM	Diagnostic and Statistical Manual of Mental Disorders
EDTA	ethylen diamnine tetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
FAD	flavin adenine dinucleotide
FCH	familial combined hyperlipidemia
FH	familial hypercholesterolemia
Gln	glutamin
GI-GPx	gastro-intestinal glutathione peroxidase
GPX1	glutathione peroxidase 1
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
HAM-D	Hamilton Depression Rating Scale
Hb	hemoglobin
HDL	high density lipoprotein

HSP25, HSP27	heat-shock proteins
HT	hypertension
IL	interleukin
LDL	low density lipoprotein
MetS	metabolic syndrome
MDD	major depressive disorder
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate
NC	non-septic critically ill patients
NF-κB	nuclear factor-kappaB
OS	oxidative stress
ox-LDL	oxidized low density lipoproteins
PAP	patients after acute pancreatitis (post-acute pancreatitis)
PC	pancreatic cancer
PCT	procalcitonin
pGPX	plasma glutathione peroxidase
PHGPX	phospoholipid hydroperoxide glutathione peroxidase
Prx or PRDX	peroxiredoxin
R7	3 rd sampling of sepsis (7 days after recovery from sepsis)
RBC	red blood cells
RONS	reactive oxygen and nitrogen species
ROS	reactive oxygen species
S	septic patients
S1	1 st sampling of sepsis
S7	2 nd sampling of sepsis (7 days after onset of sepsis)
SD	standard deviation
SeCys	selenocysteine
SIRS	systemic inflammatory response syndrome
SOD	superoxide dismutase
TC	total cholesterol
TG	triglycerides
TNF- α	tumor necrosis factor alpha
Trp	tryptophan
Tyr	tyrosine

1. Introduction

Reactive oxygen and nitrogen species (RONS) may play a dual role in biological systems because their effect can be beneficial or deleterious (Valko *et al.,* 2004, Valko *et al., 2006*). Beneficial effects involve their physiologic role in defence against infectious agents or cancer cells and in the number of cellular signalling and regulating pathways (Poli *et al*., 2004; Valko *et al.*, 2006; Bindolli *et al*., 2008). Reactive oxygen and nitrogen species include molecules like superoxide, hydrogen peroxide, hydroxyl and peroxyl radical, hydroperoxyl radical, hypochlorous acid, nitric oxide and peroxynitrite (Sies, 1991; Valko *et al.*, 2006; Goetz and Luch, 2008; Macášek *et al.*, 2011). At high concentrations, RONS can be mediators of damage to cell structures especially membranes and lipids, proteins and nucleic acids (Bergendi *et al.,* 1999). They are very transient due to their high chemical reactivity that leads to lipid peroxidation (peroxidation of polyunsaturated fatty acids in membranes), proteins (oxidation of sulfhydryl groups, hydroxylation and nitrosylation of aromatic amino acids) and nucleic acid (hydroxylation of basis, strand breaks, mutation) oxidative modification (Matés, 2000).

Reactive oxygen and nitrogen species play a role in a variety of cellular processes. ROS react with proteins, mainly with cysteine and methionine residues that lead to their inhibition or modification. Furthermore, they react with DNA and chromatin to cause mutations or double stranded breaks, important steps in carcinogenesis (Hawkes and Alkan, 2010). Enhanced levels of RONS and /or insufficient activity of antioxidant defence system result in imbalance and increased oxidative stress (OS) which has been implicated in pathogenesis of many diseases, including cancer, sepsis, inflammation, neurodegenerative diseases such as Alzheimer and Parkinson´s disease, diabetes mellitus and cardiovascular disease (Wu *et al.,* 2004). Redox balance is accomplished by various enzymes e.g. antioxidant enzymes that metabolize toxic oxidants, such as RONS.

In the protection of cells against RONS play an important role antioxidant defence system, which is composed of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPX) and glutathione reductase (GR), peroxiredoxins, thioredixin reductase and nitric oxide synthase. Antioxidant enzymes are present in all cells of eukaryotic organism. Antioxidant function also includes several biologically important non - enzymatic molecules such as reduced glutathione (GSH), vitamin C (ascorbic acid), vitamin E (α- tocopherol), vitamin A, bilirubin, uric acid, β - carotene, polyphenols and flavonoids (Valko *et al.,* 2004, Valko *et al.*, 2006).

Glutathione peroxidase catalyses the reduction of hydrogen peroxide and polyunsaturated fatty acids hydroperoxides to water and related alcohols, respectively. Together with the SOD and CAT is the most important antioxidant enzyme in cells. Additionally, it was shown that GPX is the most efficient enzyme protecting cells from OS (Michiels, *et al*.1994). The enzyme with indirect antioxidant function is the flavoprotein GR which regenerates reduced glutathione from oxidized glutathione disulfide (GSSG) which is formed in the reaction catalysed by GPX (Sies, 1993). In addition to these above mentioned antioxidant enzymes, antioxidant defence system includes also non-enzymatic antioxidants e.g. GSH, which is the major low-molecular-weight thiol that maintains redox homeostasis in cells. Glutathione together with its oxidized form GSSG, especially ratio GSH/GSSG play a key role in the regulation of the redox potential of the cell (Mittl and Schulz, 1994; Filomeni *et al.*, 2002; Wouters *et al*., 2010).

1.1 Glutathione peroxidase

1.1.1 Glutathione peroxidases

There are eight human GPX family members (GPX8 is putative), that are found in different cell fractions and tissues. Moreover, they have similar amino acid sequence and the presence of conserved redox site (selenocysteine or cysteine) at the active site whereas they differ in substrate specificity (Brigelius-Flohé, 1999; Burk and Hill, 2010). Five of them contain selenocysteine at the active site (GPX1, GPX2, GPX3, GPX4, and depend on species GPX6) and in two or three isozymes selenocysteine is replaced by cysteine. Glutathione peroxidases 1,2,3,5,6 are homotetramers, while GPX4 and GPX7 are monomers. In mammalian tissues, there are four major selenium containing GPX isozymes: classical GPX (GPX1); GPX1 is described in detail below; gastrointestinal (GPX2) - expression is not specific for gastrointestinal tract and mRNA has been found in epithelial cells, lung, skin, and breast (Hawkes and Alkan, 2010). Cellular localisation of GPX2 has been identified in cytosol and nucleus (Chu *et al.*, 1993). Plasma or extracellular GPX (pGPX; GPX3) is glycoprotein, which has been found in lung, kidney, epididymus, placenta, seminal vesicle, heart muscles and milk (Takahashi *et al.*, 1987).

Fourth of GPX isozymes is phospholipid hydroperoxide GPX (PHGPx or GPX4), it is a monomer in contrast to the other GPXs, which may allow it to bind with wider range of substrates than the tetrameric GPXs (Arthur, 2000). It has been supposed that GPX4 is able to reduce as substrate fatty acid hydroperoxides in phospholipids. Glutathione

peroxidase 4 is present in cytosol and as membrane bound enzyme in nucleus and mitochondria. This enzyme is important for embryonal development, spermatogenesis and as defence against OS (Margis *et al.*, 2008). Epididymis GPX like protein has also been called GPX5 and differs from other GPXs by having cysteine at the active site of enzyme. It has been predominantly secreted in epididymis and this association thus protects sperm against organic hydroperoxides (Ghyselinck *et al.*, 1991; Burk and Hill, 2010). Olfactory metabolizing protein GPX also called GPX6 (found in the Bowman´s gland of the olfactory system) contains selenocysteine at the active site in humans but cysteine in mice, respectively (Dear *et al.*, 1991; Burk and Hill, 2010). GPX7 is novel protein, monomer and non-selenocysteine, containing phospholipid hydroperoxidase, whereas about its function is little known. It is known that oesophageal epithelial cells may express high levels of GPX7 (Utomo *et al.*, 2004; Toppo *et al.*, 2009; Peng *et al.*, 2012). Finally, presence of GPX8 known as probable glutathione peroxidase is assumed. The role of the eight known GPX is not fully clear in the present time, nevertheless all are able to detoxify hydroperoxides, therefore their function could be related to the removal and metabolism of hydroperoxides (Brigelius-Flohé *et al.*, 2009). All of above mentioned members of GPX family are summarized in Table 1-1.

Table 1-1. Members of glutathione peroxidase family

Systematic designation	General designation	Presence of SeCys	Expression	Cellular localization	Species	References
GPX1 (cGSH-Px)	Cytosolic, cellular, classical	Yes	Lung, kidney, red cells, liver, any tissue	Cytosol, mitochondria, endoplasmic reticulum, nuclei	Human, rat, mouse, rabbit, bovine	Ganther *et al.*, 1976; Flohé, 1988; Sunde, 1994
GPX2 (GI-GPX)	Gastro-intestinal	Yes	Stomach, intestine	Cytosol	Human, rat, mouse	Chu *et al.*, 1993
GPX3 (pGSH-PX)	Plasma, extracellular	Yes	Kidney, lung, epididymis, vas deferens, seminal vesicle, placenta, heart, muscle	Secreted/cytosol	Human, rat, mouse, bovine	Takahashi and Cohen *et al.*, 1987
GPX4 (PH-GSH-PX)	Phospholipid hydrroperoxide	Yes	Testis, spermatozoa, heart, brain	Membrane-bound (mitochondria), nuclei	Human, rat, mouse, pig, dog, monkey	Ursini *et al.*, 1985; Brigelius-Flohé *et al.*, 1994; Roveri *et al.*, 1994
GPX5 (ep-GSH-Px-MEP24)	Epididymal	No	Epididymis, spermatozoa, liver, kidney	Secreted, membrane bound	Human, rat, mouse, pig	Glyselinck *et al.*, 1993
GPX6	Olfactory	Yes in humans No in mouse	Bowman´s gland (olfactory system)	Secreted	Human, rat, mouse	Dear *et al.* 1991
GPX7	Nonselenocysteine phospholipid hydroperoxide	No	Testis, lung, kidney, adipose tissue, mammary gland	Cytosol	Human, mouse,	Utomo *et al.*, 2004
GPX8	Probable glutathione peroxidase	No	Not known	Membrane-bound	Human, mouse	http://www.uniprot.org/uniprot/Q8TED1

1.1.2 Glutathione peroxidase 1

Glutathione peroxidase 1 (glutathione: H_2O_2 oxidoreductase, E.C. 1.11.1.9) was the first identified GPX and now is also called as cytosolic or cellular as well as classical GPX. Glutathione peroxidase was first described by Mills in 1957 as enzyme which protects red blood cells against oxidative damage by hydrogen peroxide (Mills, 1957). Presence of GPX1 in other tissues and its ability to catalyse reduction of hydroperoxides of unsaturated fatty acids in addition to hydrogen peroxide was determined by Little and O´Brien (Little

and O´Brien, 1968). Further studies have characterized its kinetic and physiologic function, that GPX1 reduces low molecular hydroperoxides or lipid hydroperoxides and thus prevent lipid peroxidation (Flohé *et al.*, 1971). In 1973 Rotruck *et al.* and independently Flohé *et al.* explained essential role of selenium as a structural component of the active site of red blood cell GPX1 in rats. Since it was known that glutathione peroxidase does not contain heme or flavin or any other functional prosthetic groups (Flohé *et al.*, 1973; Rotruck *et al.*, 1973). Flohé and co-workers found one atom of selenium per subunit of enzyme (4 g of selenium/mole of enzyme) purified from bovine blood thus identifiing GPX1 as a selenoenzyme. GPX was discovered as one of the first selenoproteins (Flohé *et al.*, 1973). Further research confirmed selenocysteine (an unusual amino acid) as the form of selenium in GPX1 structure (Forstrom *et al.*, 1978).

1.1.2.1 Structure

Three dimensional structure of GPX1 shows that it is homotetramer of total molecular weight about 83-95 kDa consisting of four identical asymmetric subunits of ~ 22-23 kDa (Awasthi *et al.*, 1975; Miwa *et al.*, 1983). Each subunit contains one selenocysteine (SeCys) residue at the active site, which is involved in catalytic mechanism. Sequence data from cDNA analysis shows that the polypeptide chain of human GPX1 monomer contains between 202-204 amino acid residues with a selenocysteine at position 47 (Sunde, 1994; Drevet, 2006; Lubos *et al.*, 2011). Only two crystal structures have been known, human plasma GPX3 and the other is bovine erythrocyte GPX1 (Epp *et al.*, 1983; Ren *et al.*, 1997). Refined structure of GPX1 at 0.2 nm resolution and schematic drawing of the folding pattern of a GPX1 subunit have been shown in Figure 1-1 and Figure 1-2A (http://www.rcsb.org; Epp *et al.*, 1983). Glutathione peroxidase subunit consists of central β-strands surrounded by α-helices (Epp *et al.*, 1983). One helix connects an antiparallel β-strand to adjacent β-strand forming βαβ substructure which might constitute the substrate binding region (Epp *et al.*, 1983; Lu and Holmgren, 2009).

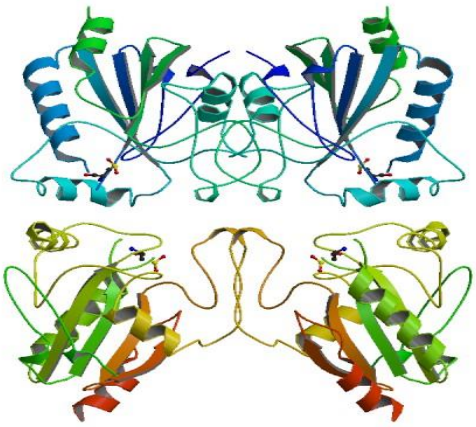

Figure 1-1. Refined structure of GPX1 at 0.2 nm resolution (http://www.rcsb.org)

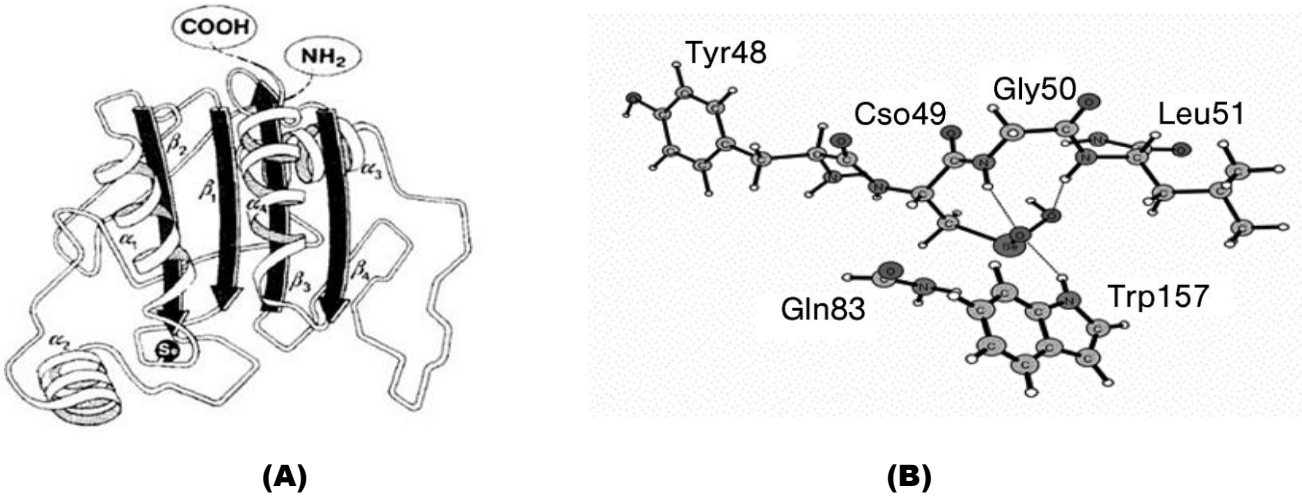

Figure 1-2. (A) Schematic drawing of the folding pattern of a GPX1 subunit (Epp *et al.*, 1983), (B) X ray structure of GPX1 active site (Prabhakar *et al*., 2008)

The active site is localized in depression on the molecular surface. Catalytically active selenocysteine residue 35 is located at the N-terminal end of the helix α_1 (Epp *et al.*, 1983). Important arrangement of active site for catalysis and substrate binding is localized near the SeCys-35, where the carboxy ends of two parallel β strands and N-terminal end of helix α_1 meet one another. Exposure of selenocysteine residues at the molecular surface leads to easy access of substrates and thus high reaction GPX rate (Epp *et al.*, 1983). The active site includes except SeCys, also Tyr48, Gly50, Leu51, Gln83 and Trp157 residues Figure 1-2B (Prabhakar *et al.*, 2008). Amino acid SeCys form with Gln83 and Trp157 catalytic triad that is important for enzyme-substrate interactions (Epp *et al*., 1983). It is known that only two molecules of GSH bind to tetramer (Ren *et al.*, 1997). Further it was showed that replacement of selenium from active site of enzyme causes a large decrease in

GPX1 activity moreover the selenium deficiency causes a rapid loss of GPX1 activity (Arthur, 2000; Lu and Holmgren, 2009).

1.1.2.2 Catalytic mechanism

Mammalian GPX1 catalyses the reduction of wide range organic hydroperoxides, organic peroxides and peroxonitrite (or peroxynitrous acid) using reduced glutathione as reducing substrate. Glutathione peroxidase can metabolize also lipid hydroperoxides produced within membranes by exposure to lipooxygenases pre-treated with phospholipase A2 (Grossmann and Wendel, 1983; Sevanian *et al.*, 1983; Sies *et al.*, 1997; Klotz and Sies, 2003; Toppo *et al.*, 2009).

$$ROOH + 2GSH \rightarrow GSSG + ROH + H_2O$$

$$H_2O_2 + 2GSH \rightarrow GSSG + 2\,H_2O$$

The reaction includes two independent steps; an organic hydroperoxide (ROOH) oxidation of enzyme reduced form and then reduction of oxidized form of GPX1 is provided by GSH. The basic reaction has tert-uni ping-pong or enzyme substitution mechanism. The reaction rate increases linearly with the concentration of substrate and GPX1 is unsaturable by GSH (Zhao and Holmgren, 2002). GPX1 is specific for the H donor (GSH), whereas for substrate is much less specific (Michiels *et al.*, 1994). The catalysis does not comply with the Michaelis-Menten hypothesis, where degradation of enzyme-substrate complex is the rate-limiting step and leads to saturation kinetics (Toppo *et al.*, 2009). Glutathione peroxidase catalysis is a complex of three-substrate reaction involving of 6 forward and 5 reverse steps, schema of catalytic mechanism is shown in Figure 1-4.

Enzymatic reduction of peroxides involves the formation of intermediate stable modifications to the SeCys active site of enzyme (Lubos *et al.*, 2011). Active state of the SeCys residue could be either selenolate anion- E-Se^- or selenol – E-SeH (E) In the first step, the active form of the enzyme (reduced form of the enzyme) (E) is oxidized by hydroperoxide substrate to form the corresponding alcohol or in case of hydrogen peroxide water and selenenic acid (E-SeOH) (F) (Prabhakar *et al.*, 2008; Burk and Hill, 2010). This reaction is one of the reverse steps, but quite hardly reversed. Rate constants for this step represents this reaction as one of the fastest ever measured for bimolecular enzymatic reactions ($5x10^7$ $M^{-1}s^{-1}$), only rate constants of enzyme superoxide dismutase are higher,

reaching value 2.3 x 10^9 $M^{-1}s^{-1}$ (Bindolli *et al.*, 2008; Brigelius-Flohé and Kipp, 2009; Toppo *et al.*,2009). This extremely fast reaction thus provides fast removal of H_2O_2.

In the second step, the selenenic acid formed reacts with first molecule of GSH to form glutathionylated enzyme (a weak selenenic acid - GSH complex (E-SeSG) (G) at the active site. Reduced glutathione is covalently bound to enzyme subunit via selenosulfide linkage (Epp *et al.*, 1983). The rate of this reaction is dependent on GSH concentration.

In the third step, selenyl-sulphide bridge of Se-glutathionylated intermediate is reduced by a second GSH molecule to form stable end product oxidized glutathione GSSG and lead to regeneration of the active site to selenol (E-SeH). The active form of the enzyme is regenerated and prepared for a next cycle. It has been suggested that in the reductive part of cycle are the rate-limiting steps the complex formations of GSH with F or G (Prabhakar *et al.*, 2008; Toppo *et al.*, 2009). The end product is immediately released from the complex (E) under physiological conditions thus inhibition of enzyme by GSSG is not observed. In the contrast high GSSG levels may cause inhibition of GPX1 (Epp *et al.*, 1983).

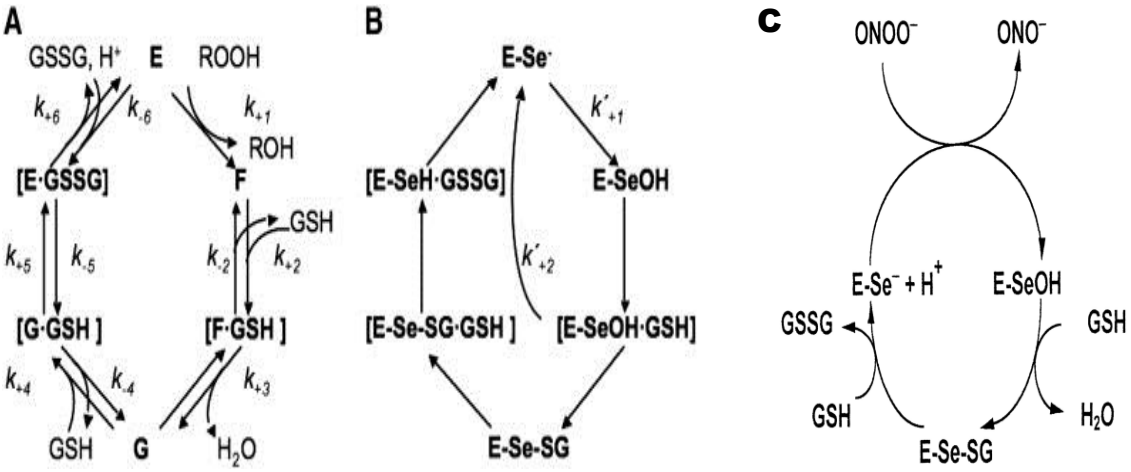

Figure 1-3. Catalytic cycle of GPX1

A) Schematic presentation of individual reactions. B) Oxidation states of selenocysteine corresponding to the enzyme forms. (Toppo *et al.*, 2009), C) Reduction of peroxynitrite to nitrite (or peroxynitrous acid to nitrous acid) (Sies *et al.*, 1997).GSH- reduced glutathione, GSSG – oxidized glutathione, $ONOO^-$ - peroxynitrite, ONO^- - nitrite; E- ground state, F – first oxidized intermediate, G – half reduced intermediate; $E\text{-}Se^-$ selenol, E-SeOH – selenenic acid, E-SeOH-GSH – selenenic acid derivate, E-SeSG-GSH – glutathionylated enzyme

In summary:

1) (E-SeH) + H_2O_2 → (E-SeOH) + H_2O
2) (E-SeOH) + GSH → (E-Se-SG) + H_2O
3) (E-Se-SG) + GSH →(E-SeH) + GSSG

1.1.2.3 Function

Glutathione peroxidase is one of the main antioxidant enzymes of the organism, which protects erythrocytes against oxidative stress, where catalyses reduction of hydrogen peroxides formed by dissociation of oxyhemoglobin (Hawkes and Alkan, 2010). As mentioned above it is selenoenzyme that detoxifies only soluble hydroperoxides (ROOH) such as hydrogen peroxide and a wide range of organic hydroperoxides (ROOR´) like hydroperoxy long chain fatty acids, cumene hydroperoxide, t-butyl hydroperoxide and also may reduce phospholipid monoacylglycerol hydroperoxides but not di- or triacylglycerol hydroperoxides (Brigelius Flohé., 1999; Marinho *et al.,* 1997). Furthermore, degradation of long chain fatty acids and cholesterol peroxides is dependent on fatty acids release from peroxidized membranes by phospholipase A_2 (Arthur, 2000). It protects cell membranes and other cellular components against oxidative damage. Moreover, it was shown that GPX1 decompose also peroxynitrite / peroxynitrous acid yields nitrite, nitrous respectively and thus acts as peroxynitrite reductase under physiological condition (Padmaja *et al*., 1998; Sies *et al.*, 1997). It was shown that GPX1 cannot be replaced by any other selenoprotein in defence against generalized OS (Brigelius-Flohé and Kipp, 2009). High levels of GPX1 are found especially in tissues with high rate of H_2O_2 production, like erythrocytes, kidney, lung, liver, and pancreas (Brigelius–Flohé, 1999; Cullen *et al.*, 2003; Drevet, 2006; Robertson and Harmon, 2007) shown in Table 1-1.

The stability of GPX1 mRNA in the Se deficiency is low not only in the hierarchy of GPXs but also among all selenoproteins (Brigelis Flohé, 1999; Wingler *et al.*, 1999). Some proteins decrease fast in the Se deficiency (GPX1 and GPX3), while others are synthesized despite a large selenium deficiency (GPX2 and GPX4) belonging among the most stable GPXs (Brigelius-Flohé, 1999; 2009). Activities of GPX1 as well as the GPX1 protein synthesis are down-regulated during progressive Se deficiency thus demonstrating a regulation of GPX1 protein level and activity by selenium level (Takahashi and Cohen, 1986; Takahashi *et al.*, 1987). Selenium deficiency may also result in the down-regulation of the GPX1 mRNA (Brigelius- Flohé, 1999). Moderate Se deficiency leads to a decrease

of GPX1 mRNA levels, due to loss of stability (enhanced degradation of the mRNA) and also depletion of SeCys that may lead to inability to translate the message for GPX1 (Takahashi and Cohen, 1986). It is known that Se is incorporated into selenoproteins by recognition of the stop codon UGA as a codon for SeCys and deficiency of Se leads to terrmination of translation (Bermano *et al.*, 1996; Driscoll and Copeland, 2003). The loss of corresponding protein and activity are more pronounced than the decrease in mRNA (Wingler *et al.*, 1999, Takahashi *et al.*, 1987; Brigelius-Flohé 1999, 2009). Since erythrocytes are unable synthetize proteins, it is possible that inactive GPX1 protein is made in erythroid precursors (Cohen *et al.*, 1985). It is also evident that Se is incorporated into the active site of enzyme only during erythropoiesis (Zachara *et al.*, 2006). Furthermore, abnormal protein may be rapidly degraded and thus is not present in circulating erythrocytes (Takahashi and Cohen, 1986).

Glutathione peroxidase, CAT and peroxiredoxins are all able to detoxified H_2O_2, but it depends on hydrogen peroxide concentrations. It has been suggested that at low physiological H_2O_2 levels peroxiredoxins reduce the most H_2O_2 produced inside cells, whereas they are inactivated by increased H_2O_2 concentration (Halliwell and Gutteridge, 2007; Burk and Hill, 2010). Moreover, peroxiredoxins has by one to three orders of magnitude lower catalytic efficiency ($\sim 10^5\ M^{-1}s^{-1}$) than that of GPX1 ($\sim 10^8\ M^{-1}s^{-1}$) or CAT ($\sim 10^6\ M^{-1}s^{-1}$), but this fact may be compensated by their abundance in the cytosol (0.1 to 0.8 % of soluble proteins) and higher affinity toward H_2O_2 (Wood *et al.*, 2003; Rhee *et al.*, 2005; Halliwell and Gutteridge, 2007). Catalase is localized predominantly in peroxisomes, so it may be the key enzyme for H_2O_2 removal in these organelles (peroxiredoxin (Prx) V may contribute), this reduction requires its diffusion into peroxisomes (Halliwell and Gutteridge, 2007). Early papers suggested that CAT plays an important role in removing of higher H_2O_2 intracellular concentrations, whereas it has been shown that the bacterial Prx alkyl hydroperoxide reductase C22 is responsible for detoxification of endogenously generated H_2O_2 (Jones *et al.*, 1981; Gaetani *et al.*, 1989; Spolarics and Wu, 1997, Wood *et al.*, 2003). Glutathione peroxidase is probably more important in the elimination of H_2O_2 than CAT due to its localization in the same intracellular organelles as SOD (Halliwell, 1989).

Combination of GPX, CAT and SOD better maintain integrity of cells against oxidative damage. Glutathione peroxidase also catalyzes reduction of lipid peroxides, thus indirectly protecting the hydrophobic part of cell membrane, whereas CAT and SOD act in hydrophilic part of the cell membrane (Michiels *et al.*, 1994). Hydroperoxides that are not

longer detoxified will accumulate causing lipid peroxidation and cell death. Similarly, decrease in GSH concentration induces a comparable effect (Michiels *et al.*, 1994). Cells compensate enhanced generation of hydrogen peroxide by increasing GPX or CAT activity (Michiels *et al.*, 1994). Expression of GPX1 parallels metabolic activity, which complies with an antioxidant function of GPX1 (Brigelius-Flohé, 1999).

Glutathione peroxidase 1 may play a role in modulating nuclear factor-kappaB (NF-κB) activation. Overexpression of GPX1 inhibits NF-κB activation induced by hydrogen peroxide or tumor necrosis factor alpha (TNF-α) (Kretz-Remy and Arrigo, 2001). GPX1-mediated decrease of ROS leads to inhibition of hydrogen peroxide-mediated 1κB-α phosphorylation (an inhibitory component of the inactive cytosolic NF-κB complex) followed by subsequent degradation (Kretz-Remy and Arrigo, 2001). Since degradation of 1κB-α precedes NF-κB activation (Kretz-Remy *et al.*, 1996). It was suggested that GPX1 could play a role in NF-κB activation by the following factors such as inhibition of lipooxygenase and cyclooxygenase activities which may prevent NF-κB activation (Brigelius Flohé, 1999). Glutathione peroxidase 1 was identified as endogenous inhibitor of 5-lipooxygenase and 15-lipooxygenase activity in vitro, the key enzyme in biosynthesis of leukotrienes where catalyzes conversion of arachidonic acid into leukotrienes (inflammatory mediators) (Brigelius-Flohé and Flohé, 2003; Werz and Steinhilber, 2005), thus contributes to generation of ROS, leading to activation of NF-κB (Bonizzi *et al.*, 1999; Jatana *et al.*, 2006; Chung *et al.*, 2008). It has been found that GPX1 is very effective suppressor of 5-lipooxygenase activity in monocytic cells (Straif *et al.*, 2000). Glutathione peroxidase may suppress activity of cyclooxygenase, key enzyme in prostaglandin synthesis, which is dependent on level of hydroperoxides regulated by GPX1 (Kulmacz, 2005).

Glutathione peroxidase has been suggested as a storage form for selenium and thus maintains physiological levels of Se for metabolic functions (Cheng *et al.*, 1998). Furthermore selenium deficiency facilitated activation of NF-κB, whereas in Se-supplemented cells NF-κB activation by H_2O_2 was decreased (Sappey *et al.*, 1994), probably via the oxidative activation of NF-κB is GPX1 upregulated by estrogens (Borrás *et al.*, 2005). Selenium stimulation of GPX1 activity also induces a protective effect against cell activation by TNF-α (Brigelius-Flohé, 1999; Sappey *et al.*, 1994).

Glutathione peroxidase can regulate the cellular redox state by modulating the concentrations of the H_2O_2, GSH and NADPH molecules that are critical players in the

cellular redox system, thus GPX1 plays an important role in the regulation of cellular redox state (Li *et al.*, 2000).

1.1.3 Peroxiredoxins

Peroxiredoxins (Prx or PRDX) are a family of thiol-specific antioxidant proteins, also called thioredoxin peroxidases and alkyl-hydroperoxide-reductase – C22 proteins that reduce and detoxify H_2O_2, peroxynitrite and also wide range of organic hydroperoxides using thioredoxin, glutathione or tryparedoxin as the reducing substrate (Wood *et. al*, 2003). Peroxiredoxins control cytokine-induced H_2O_2 levels which have been shown to mediate signalling cascades (Wood *et. al*, 2003). They contain cysteine at the active site but no prosthetic group (Georgiou and Masip, 2003; Halliwell and Gutteridge, 2007). All Prx have conserved cysteine residue in the N-terminal region that is site of oxidation by H_2O_2 (Rhee *et al.*, 2005). The first Prx was identified in yeast. In mammalian cells was found six Prx isoforms (Prx I-VI) (Flohé *et al.*, 2003; Rhee *et al.*, 2005; Halliwell and Gutteridge, 2007). Peroxiredoxins use redox active cysteines to reduce peroxides and can be divided according to the number and location of catalytic cysteines into three classes: 2-Cys Prx (Prx I-IV) are the typical and the most common, which contain two conserved cysteine residues; one is in the N-terminal region and the other in C-terminal region and both of them are important for catalytic function; atypical 2-Cys Prx (Prx V) contains only N-terminal cysteine residue but for catalytic activity needs non-conserved additional cysteine residue and the 1 Cys Prx (Prx VI), which contains only N-terminal conserved cysteine residue and requires the conserved cysteine for catalytic function (Seo *et al.*, 2000; Wood *et. al*, 2003; Rhee *et al.*, 2005; Burk and Hill, 2010). Peroxiredoxins I, II and VI are cytosolic, whereas Prx III occurs in mitochondria, and Prx IV is extracellular. Peroxiredoxin V is found in cytosol, mitochondria and peroxisomes (Halliwell and Gutteridge, 2007; Burk and Hill, 2010). Peroxiredoxins are the second or third the most abundant protein in erythrocytes (Wood *et. al*, 2003). In Prx1, II and III has been shown to be thioredoxin peroxidases, whereas in Prx VI has been described to have glutathione peroxidase activity (Fisher *et. al*, 1999).

Peroxiredoxins reduce low levels of hydroperoxides and peroxinitrites that are formed by normal cellular metabolism in the cytosol. However, increased H_2O_2 production results in inactivation of Prx by hyperoxidation (Georgiou and Masip, 2003; Burk and Hill, 2010).

1.2 Glutathione reductase

Glutathione reductase (NADPH: oxidized glutathione oxidoreductase, E.C. 1.6.4.2) is ubiquitous enzyme that catalyzes reduction of oxidized form of glutathione (GSSG) to two molecules of reduced glutathione using nicotineamide adenine dinucleotide phosphate (NADPH) generated by hexose monophosphate cycle (Yan *et al.*, 2012). This reaction is supposed to be essential for red blood cell (RBC) stability and integrity (Tandoğan and Ulusu, 2006). Glutathione reductase maintains a high ratio of GSH/GSSG and thus reducing environment within the cells (Mittl and Schulz, 1994). It plays an important role in regulation of hexose monophosphate pathway in erythrocytes (Beutler, 1969) and particularly in biosynthesis of deoxyribonucleotides (Rescigno and Perham, 1994). Mammalian GR is localized in the cytosol and in the mitochondria. Glutathione reductase has been considered one of the thermostable enzymes (Tandoğan and Ulusu, 2006).

1.2.1 Structure

Human GR is a homodimeric flavoprotein with two 55 kDa subunits with one FAD molecule per subunit (Figure 1-4). In the absence of thiols GR tends to form tetramers and larger aggregates (Worthington and Rosemeyer, 1975). The polypeptide chain of human GR contains 478 amino acid residues with a blocked alanine which is N-terminus (Untucht-Grau, 1981). The GR molecule consists of two identical polypeptide chains which are covalently connected by a disulfide bond. Each subunit contains three domains named according their function; from the N-terminus: a flavin adenine dinucleotide (FAD)-binding domain (residues 1-140; 265-336), an NADPH-binding domain (residues 158-293) and a dimerization (interface) domain (365-478) follow at the COOH terminal side (Untucht-Grau, 1981; Krauth-Siegel *et al.*, 1982; Pai and Schulz, 1983). The two active sites are at the dimer interface with the substrate glutathione bond by residues in both subunits (Bashir *et al.*, 1995). Glutathione reductase has a central five-stranded parallel beta sheet (β1, β2, β3, β7 and β8), which is surrounded by α-helices 1 and 2 with a crossover connection of a three-stranded antiparallel β-sheet (β4-6) (Dym and Eisenberg, 2001).

The FAD and NADPH domains are at the active site, in which both monomers participate (Berkholz *et al.*, 2008). The FAD-binding domain is formed by N-terminal part of amino acid residues and contains the redox-active dithiol which represents the centre of the enzyme's catalytic site (Untucht-Grau *et al.*, 1981). The NADPH is bound in an extended conformation in a cleft and most of contacts being made with the NADPH-

binding domain (Rescigno and Perham, 1994). Residues from the FAD-binding domain and the interface domain contribute to the binding of the reduced nicotinamide. This orientation is important for the transfer of electrons from reduced nicotinamide to the isoalloxazine ring of the prosthetic group FAD (Rescigno and Perham, 1994). It was shown that the isoalloxazine ring of the prosthetic group of FAD separates the binding sites for the two substrates in GR (Krauth-Siegel *et al.*, 1998). Active site of GR is distributed over both domains (Mittl and Schulz, 1994). Dimeric form of enzyme is important for its catalytic function, because each subunit has essential residues, which contributes to the constitution of the active site. Enzyme is inactive in the monomer form (Nordhoff *et al.*, 1997). In the active site of GR are highly conserved tyrosine residues Tyr114 (in the GSSG site) and Tyr197 (in the NADPH site) that play role in catalysis by human GR, moreover Tyr 197 also plays a stabilizing role in NADPH-EH_2 complex. Reduced nicotinamide ring is positioned between isoalloxazine ring of FAD and the phenol ring of Tyr197 (Pai *et al.*, 1988). Mutations of both tyrosine residues may negatively affect the catalysis. Mutation of Tyr114 leads to a decrease in Km for the substrate in opposite substrate binding site (NADPH) as well as mutation of Tyr197 leads to a decrease in Km for GSSG (Krauth-Siegel *et al.*, 1998). Tyrosine residue Tyr114 represents the catalytic site of GR since it contributes to interaction between the GSSG and redox active dithiol Cys58/Cys63 by making direct hydrogen bond with GSSG (Schönleben-Janas *et al.*, 1996).

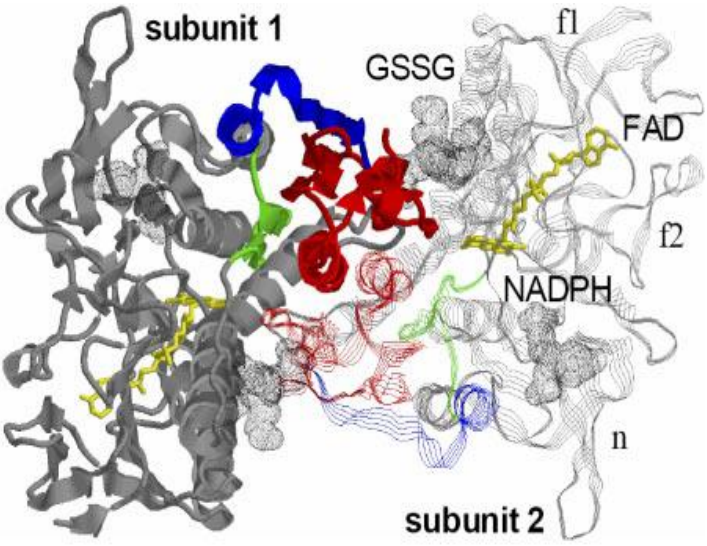

Figure 1-4. The overall structure of human glutathione reductase dimer. f1 and f2 indicate FAD domains 1 and 2,; n is NADPH domain; the interface domain is highlighted in colour. (Kamerbeek *et al.*, 2007)

1.2.2 Catalytic mechanism

The catalytic centre of GR is divided into two parts; one part binds GSSG and the other one NADPH (Pai and Schulz, 1983). The catalytic cycle has two stages involving a reductive and an oxidative reaction, where oxidative half-reaction is 2-3 times slower than the reductive one (Sweet and Blanchard, 1991). The maximum rate of GR occurs at pH 8.6. Glutathione reductase exists in two stable forms EH_2 and E. E state of enzyme is characterized by disulphide bridge between Cys58 and Cys63 at the active site, whereas stable two-electron reduced form of the enzyme EH_2 is characterized by characteristic charge-transfer complex with the prosthetic group FAD (Pai and Schulz, 1983). Glutathione reductase has high substrate specificity. It has been suggested that GR is acting according two mechanisms (ping-pong and sequential) and moreover it depends on substrate concentration (Worthington and Rosemeyer, 1976). In low GSSG concentration the ping-pong mechanism prevails. Dinucleotides FAD and NADPH as well as the substrate GSSG and amino acid residues are important for catalysis. The active site of GR is not easily accessible for NADPH; the binding cleft is blocked by Tyr 197 residue, therefore catalytic mechanism includes movement of this tyrosine away from flavin (Karplus and Schulz, 1987). First NADPH binds and reduces the flavin and reducing equivalents are transferred to redox-active disulphide (Pai and Schulz, 1983). In the second stage, resulting dithiol reacts with GSSG at the active site. The catalytic centre is surrounded by protein so that the NADPH and GSSG binding sites are in deep pockets. The catalytic cycle consists of six steps; each of its two substrates - NADPH and GSSG react in the absence of the other (Figure 1-5)

Stage 1: Reductive half reaction

In the first step oxidized enzyme, which contains a redox active disulphide bond between Cys63 and Cys58, binds NADPH as the enzyme's first substrate forming an enzyme – substrate complex, E-$NADP^+$. NADPH immediately reduces flavin. Reduced flavin anion ($FADH^-$) has only transient existence and quickly breaks a disulphide bond (Cys58-Cys63) resulting in thiol groups forming of both cysteines (Voet and Voetová, 1995; Voet and Voet, 2011). An electron pair is rapidly transferred from S_{63} to the flavin ring through the transient formation of a covalent bond from S_{63} (the S atom of Cys63) to flavin atom 4a. Cys 58 is thus released and form thiolate anion. Short-lived covalent intermediate Cys63-flavin quickly breaks followed by formation a charge-transfer complex between the flavin and Cys63 thiolate (Berkholz *et al.,* 2008; Voet and Voet, 2011). S_{63} contacts the flavin

ring near its 4a position. A charge-transfer complex provides a non-covalent interaction in which an electron pair is partially transferred from a donor, in this case S_{63}, to an acceptor, in this case the oxidized flavin ring. The $NADP^+$ is then released yielding reduced form of enzyme (EH_2), which contains an oxidized flavin and a reduced disulfide (open Cys58-Cys63) disulphide bridge (Pai and Schulz, 1983). The phenol side chain of Tyr 197 continues to block access of solvent to the flavin ring of FAD, so as to prevent the enzyme's oxidation by oxygen (Voet and Voet, 2011).

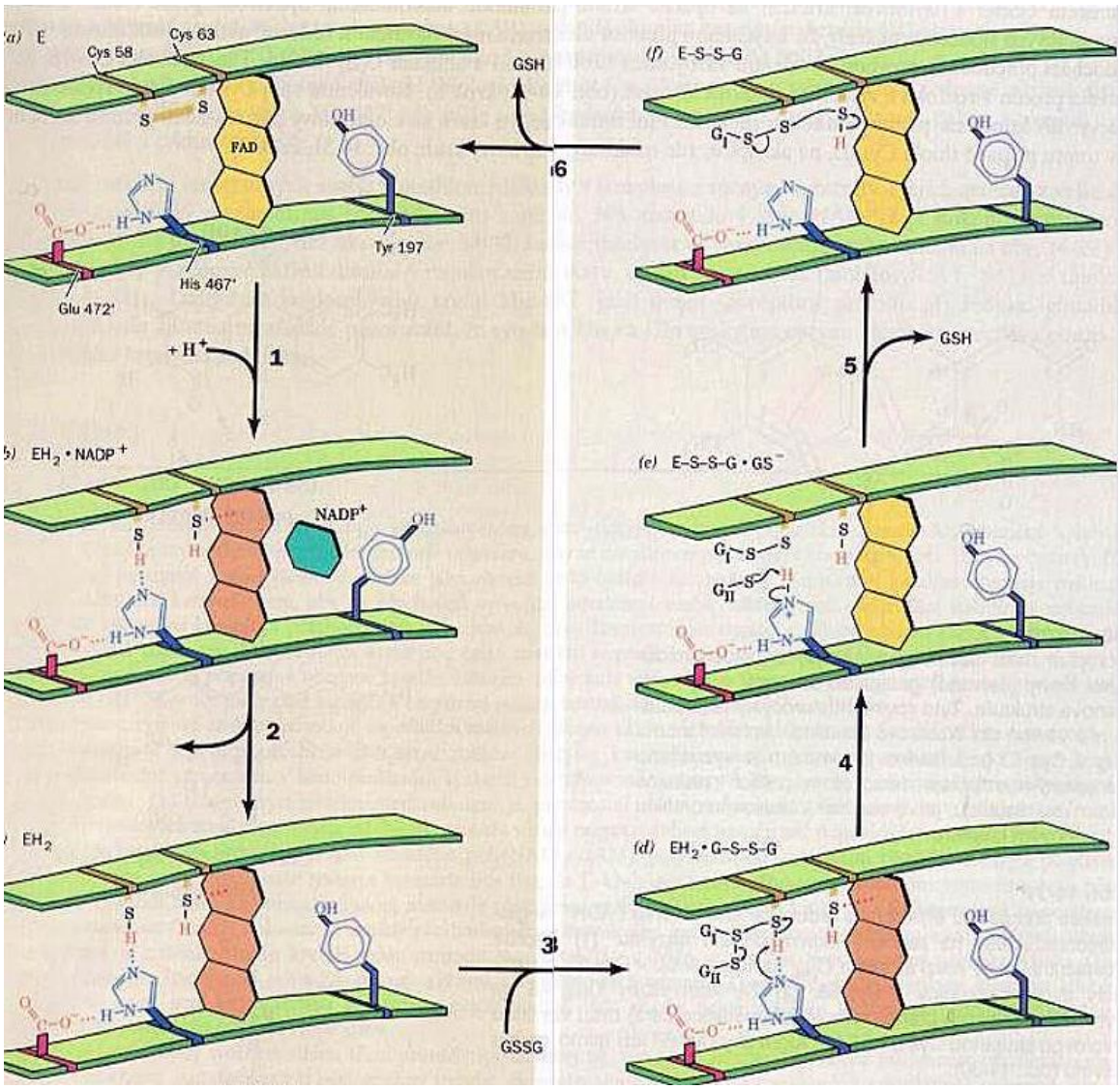

Figure 1-5. Catalytic mechanism of glutathione reductase (Voet and Voet, 2011)

Stage 2: Oxidative half reaction

The enzyme's second substrate, GSSG binds to EH_2 to form EH_2-GSSG. In the next step S_{58} (thiol Cys58) nucleophically attacks the nearest sulphide (S_1) in the GSSG molecule to form mixed disulphide (GS_1-Cys58) and GS_{II}^- anion. His_{467} then protonates the first glutathione thiolate anion and enzyme release the first GSH molecule. Glutathione I is

tightly bound to protein to form mixed disulphide with the protein (E-SG), therefore glutathione II leaves the enzyme first (Pai and Schulz, 1983). It has been shown that this step to be rate limiting in the oxidative half-reaction (Wong *et al.*, 1988). Finally Cys63 nucleophilically attacks S58 to reform the redox-active disulphide and breaks mixed disulphide. Releasing GS^- anion which picks up a second proton from His467 and yields GSH as the reaction's second product thereby is completed the catalytic cycle (Voet and Voetová, 1995).

It has been shown inactivation of GR by NADPH via an intermolecular association, a possible mechanism is that NADPH reduces a site on one molecule and interacts with this site, thus blocks a site on a second molecule, which may lead to an enzyme aggregation (Worthington and Rosemeyer, 1976). The high concentration of reduced glutathione protects GR against inactivation in erythrocytes (Worthington and Rosemeyer, 1976). Inhibition of GR by substrate has been shown for GSSG concentration about 1mM (Janes and Schulz, 1990).

In the mammalian glutathione system is usually not glutathione reductase a rate limiting enzyme. Regeneration of GSH from GSSG is dependent on supply of NADPH, which in most tissues predominantly depends on pentose-phosphate cycle, thus formation of NADPH determines detoxification rate of hydrogen peroxide by GPX (Toppo *et al.*, 2009). There is evidence that a low activity of GR could still be sufficient in recycling of GSH from GSSG for the GPX activity (Michiels *et al.*, 1994).

1.2.3 Function

Glutathione reductase maintains the GSH/GSSG ratio to facilitate the transfer of electrons from glucose to H_2O_2 within the cytosol thus prevents oxidative damage and plays the role in the regulation of phagocytic oxidative burst (Yan *et al.*, 2012). It is known that GR plays an important role in the innate immune system against massive bacterial infection (Yan *et al.*, 2012). It has been shown in GR deficient mice that GR deficient neutrophils produce less ROS with weakened oxidative burst (Yan *et al.*, 2012). Exact mechanism is unclear; the authors assumed that GR deficient neutrophils are less resistant to the oxidative damage. In the cytosol is H_2O_2 detoxified by GPX therefore regeneration of GSH from GSSG by GR facilitates detoxification of H_2O_2 in the cytosol and thus protects cells against oxidative damage. Glutathione reductase deficiency is characterized

by hemolysis due to increased sensitivity of erythrocyte membrane to H_2O_2 exposure (Tandoğan and Ulusu, 2006).

1.3 Reduced glutathione

In 1888 de Rey Pailhade discovered the substance "hydrogénant le soufre" which proved to be GSH, as unravelled by Sir Frederick Gowland Hopkins in the 1929 and Harrington and Maed (Harrington and Maed, 1935; Sies, 1999; Cotgreave and Gerdes, 1998). Glutathione is the tripeptide L-γ-glutamyl-L-cysteinyl-glycine synthesized from the amino acid precursors (L-glutamate, L-cysteine and glycine) that contains an unusual γ amide bond between glutamate and cysteine (Figure 1-6) (Anderson, 1998). Glutathione exists in thiol (reduced) and disulphide (oxidized) form and its molecular weight is 307.32; 612.63, respectively. It is found in all mammalian tissues, especially high concentration is in the liver (Jefferies *et al.*, 2003; Lu and Holmgren, 2009). Reduced glutathione is present in the cytosol (almost 90 % of cellular GSH), 10 % in the mitochondria and small amount is found in the endoplasmic reticulum and nucleus. The synthesis of GSH takes place only in the cytoplasm (Wu et al., 2004; Anderson, 1998). Under normal conditions is intracellular concentration of GSH (1-10 mM) whereas in plasma, its concentration is relatively low (~ 0.01 mM) due to rapid break down in the circulation (Hammond *et al.*, 2001, Balendiran *et al.*, 2004, Lu and Holmgren, 2009). In the cytoplasm, the GSH/GSSG ratio 30-100:1 is the major redox couple that determines the antioxidative capacity of cells and leads to redox potential approximately – 230 mV (Wouters *et al.*, 2010). After synthesis, GSH is transported to mitochondria, endoplasmic reticulum and nucleus. Glutathione can also undergo transport across plasma membrane into the extracellular space such as blood plasma and bile where can be utilized by other cells and tissues (Ballatori *et al.*, 2009).

Glutathione plays essential role as the most efficient intracellular thiol substrate for GPX, and a protective molecule against oxidants and xenobiotics (Dickinson and Forman, 2002; Brigelius-Flohé, 1999; Meister and Anderson, 1983). Glutathione is maintained in its reduced form by NADPH dependent enzyme GR. Synthetized GSH can go across cell membrane into the extracellular compartment. Impaired response to severe cellular OS may lead to accumulation of GSSG within the cytosol followed by depletion of cellular GSH (Lu, 1999). To protect the cell from redox imbalance, GSSG can be exported out of the cell or react with a sulfhydryl group of proteins forming protein-glutathione mixed disulphides (Deneke and Fanburg, 1989).

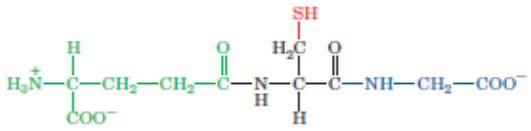

Figure 1-6. Structure of glutathione (Voet and Voet, 2011)

Glutathione is major thiol "redox buffer" that maintains thiol/disulphide redox potential (Sies, 1999). Homeostasis of glutathione is regulated by controlling the rates of its synthesis and export from cells. Cellular GSH levels are also affected by agents that modify the thiol-redox state which results in the formation of glutathione S-conjugates and /or disrupt the GSH distribution among intracellular organelles. In addition, it can be influenced by nutritional status and hormonal levels (Ballatori *et al.*, 2009).

1.3.1 Synthesis

Reduced glutathione is synthesized in the cytosol of all mammalian cells from L-glutamate, L- cysteine and glycine in the two steps sequentially catalysed by two ATP - requiring enzymes: (1) glutamate cysteine ligase (GCL) (formerly γ-glutamyl-cysteine synthetase), which is supposed to be the rate limiting enzyme, and (2) GSH synthase (formerly glutathione synthetase) (Figure 1-7) (Sies, 1999; Meister and Anderson, 1983). The liver is the main source of GSH synthesis (Sies and Akerboom, 1984). Erythrocytes also can synthesize GSH from cysteine, glutamate and glycine, because they contain all of the enzymes important for its biosynthesis, daily produce significant percentage of GSH by *de novo* synthesis (Giustarini *et al.*, 2008) that depends on two factors, cysteine availability as a substrate and GCL activity (Lu, 2000). Cysteine seems to be the limiting amino acid for GSH synthesis in humans (Wu *et al.*, 2004).

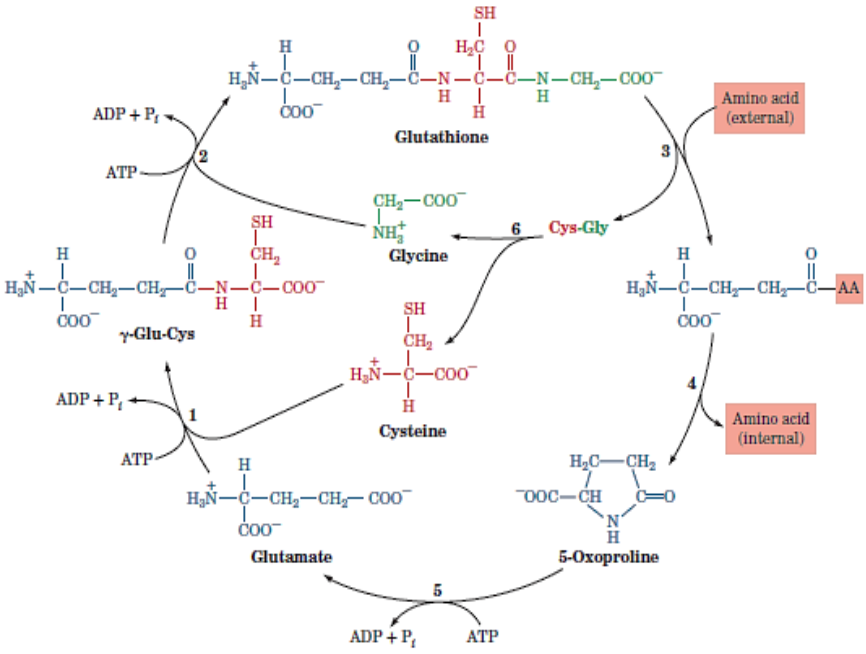

Figure 1-7. Glutathione synthesis as part of the γ-glutamyl cycle of glutathione metabolism. (1) γ-glutamylcysteine synthetase, (2) glutathione synthetase, (3) γ-glutamyl transpeptidase, (4) γ-glutamyl cyclotransferase, (5) 5-oxoprolinase, and (6) cysteinylglycine dipeptidase. (Voet and Voet, 2011).

The γ-glutamyl cycle, as a part of GSH synthesis provides mechanism for amino acids transport into cells through the synthesis and breakdown of GSH (Voet and Voet, 2011). Synthesis of GSH is two-step reaction; in the first reaction GCL catalyses the formation of γ-glutamyl cysteine from L-glutamate and L-cysteine followed by second reaction catalysed by glutathione synthase where γ-glutamylcysteine reacts with glycine to form GSH (Anderson, 1998). Steps 1 and 2 involved ATP hydrolysis, where the γ-carboxyl group of glutamate is activated for reaction with the amino group of cysteine and synthesis of peptide bond to form an acyl phosphate intermediate (Voet and Voet, 2011). The specific γ-amide bond protects GSH against hydrolysis by intracellular aminopeptidases (Wu *et al.*, 2004).

The degradation of GSH is catalysed by γ-glutamyl transpeptidase, γ-glutamyl cyclotransferase, 5-oxoprolinase, and cysteinylglycine dipeptidase. Glutathione is found and synthesized intracellularly, whereas degradation of GSH occurs only in the extracellular space and in the cells expressing γ-glutamyl transpeptidase (Hammond *et al.*, 2001). Additionally, GSH is transported to the external surface of the cell membrane where

is the γ-glutamyl group of GSH cleaved by γ-glutamyl transpeptidase to form γ-glutamyl enzyme. This enzyme intermediate then accepts an external amino acid, followed by transport of cysteine γ-glutamyl amino acid back into the cell by sodium-dependent aminoacid transporter system and where is immediately converted to glutamate by two steps (Singh *et al.*, 2012). Transported amino acid is released by reaction catalysed by γ-glutamyl cyclotransferase that forms 5-oxoproline (a cyclic form of glutamate) and the corresponding amino acid. Finally, the ring of 5-oxoproline is opened by 5-oxoprolinase using ATP to form glutamate (Voet and Voet, 2011). GSH synthase catalyses the ATP-dependent formation of GSH from γ-glutamylcysteine and glycine.

Synthesis of GSH can be regulated by GCL and amino acids. Nuclear factor κB (NFκB) cause the up-regulation of GCL expressions in response to OS and inflammation (Wu *et al.*, 2004). Formation of GSSG between cysteine of protein and glutathione prevents further oxidation of thiols in cell signalling, it is thus protective mechanism for thiols (Bindolli *et al.*, 2008). Reduced glutathione is regenerated from the reaction catalysed by GR (Michiels *et al.*, 1994). GSH deficiency can be caused by using a selective irreversible inhibitor of GCL L-buthionine sulphoximine (BSO) which inhibit GSH synthesis (Meister, 1988). In addition, it was shown the link between NO and GSH metabolism by S-nitrosation of GCL protein by S-nitrosocysteine or S-nitroso-L-cysteinylglycine (NO donors) followed by reduction of GCL activity and thus GSH depletion (Dalle-Donne *et al.*, 2008).

1.3.2 Biological function

Reduced glutathione is the main intracellular non-protein thiol and redox buffer that maintains reducing environment important for the activity of the most enzymes and macromolecules within the cell. Glutathione and enzymes related to its synthesis form a system that maintains reducing environment in the cells and acts as a primary defence against ROS overproduction (Morales *et al.*, 1998). This system is involved in the metabolism and maintenance of sulfhydryl groups of proteins and low molecular weight compounds such as cysteine and coenzyme A (Anderson, 1998; Lu, 1999). Cysteine can be autoxidized to cystine, forming toxic oxygen radicals, thus most of the non-protein cysteine is stored as GSH to avoid this toxic autoxidation reaction (Olney *et al.*, 1990; Wu *et al.*, 2004; Hammond *et al.*, 2001). Thus GSH serves as a main storage and transport form of cysteine in the body (Anderson, 1998; Hammond *et al.*, 2001). Factors e.g. insulin

and growth factors increase intracellular GSH levels by stimulating uptake of cysteine into cells (Wu *et al.*, 2004).

Reduced glutathione is involved in many cellular reactions, having number physiological roles because of its high reactivity (Balendiran *et al.*, 2004; Sies 1999; Townsend *et al.*, 2003). Glutathione is also important non-enzymatic antioxidant; it is able to scavange ROS and peroxides non-enzymatically via formation of mixed disulphide (Anderson, 1998; Singh *et al.*, 2012). Glutathione plays an essential role in antioxidant defence such as scavenger of free radicals and RONS (e.g. superoxide and hydroxyl radical, hydrogen peroxide, peroxynitrite, lipid peroxyl radical) through enzymatic reactions catalysed by e.g. GPX, glutathione S-transferase, formaldehyde dehydrogenase and glyoxalase I (Wu *et al.*, 2004; Estrela *et al.*, 2006); in detoxification of electrophiles (e.g. alkenes, alkyl halides, lactones, epoxides, and heavy metals); physiologic metabolites (e.g. estrogens, prostaglandins, leukotrines) as well as xenobiotics (e.g. bromobenzene, acetaminophen, etc.). Xenobiotics form conjugates in reactions catalysed by glutathione-S-transferase; GSH conjugation irreversibly consumes intracellular GSH (Lu and Holmgren, 2009). The sulfhydryl group of GSH cysteine has a high affinity for metals, forming *via* non-enzymatic reactions thermodynamically stable but kinetically labile mercaptides with a number of metals, such as copper, zinc, selenium, chromium, mercury, silver, cadmium, arsenic, lead, gold (Ballatori *et al.*, 2009; Hammond *et al.*, 2001).

Glutathione is involved in nitric oxide (NO) metabolism, where reacts with NO to form S-nitrosoglutathione (Balendiran *et al.*, 2004). It maintains reduced form of ascorbic acid (Anderson, 1998). Glutathione and ascorbate play a role in detoxification of ROS that may cause DNA modification. It was shown negative correlation between both GSH and ascorbate and oxidative DNA damage in human lymphocytes (Lenton *et al.*, 1999).

Furthermore, GSH regulates protein and DNA synthesis via thiol-disulphide exchange reactions and by maintaining reduced glutaredoxin and thioredoxin, which are essential for ribonucleotide reductase, the rate-limiting enzyme in DNA synthesis (Holmgren, 1981). It is involved in activation of transcription factors within the cells and also may influence caspase activity and S-glutathionylation of proteins (e.g. peroxiredoxin 1, thioredoxin, cytochrome c oxidase, and ubiquitin-conjugating enzyme) (Sies, 1999; Townsend *et al.*, 2003; Wu *et al.*, 2004; Ballatori *et al.*, 2009; Singh *et al.*, 2012).

Protein S-glutathionylation is post-translational modification of protein cysteine residues by reversible GSH conjugation to cysteine sulfhydryl groups of proteins. It yields mixed disulphides during oxidative and nitrosative stress (Dalle Donne *et al.*, 2008;

Townsend *et al.*, 2003). Protein cysteinyl residues are more susceptible to ROS attack; thus GSH is involved in the control of protein function by this modification, storage of glutathione and particularly in cell signalling processes associated with tumor necrosis factor alpha (TNF-α) induced apoptosis and viral infection (Pan and Berk, 2007; Prinarakis, 2008; Dalle-Donne *et al.*, 2009). This reversible oxidation may also protect protein thiols from irreversible oxidation (Dalle-Donne *et al.*, 2007). S glutathionylated protein may be formed from exchange between protein thiols and GSSG, direct interaction between partially oxidized protein thiols and GSH; reactions between protein thiols and S-nitrosothiols (Dale-Donne *et al.*, 2008). It is supposed that S-glutathionylation is the general mechanism of protein redox regulation that can lead to phenotypic changes such as increased cell proliferation and apoptosis (Townsend *et al.*, 2003; Singh *et al.*, 2012). Moreover, S-glutathionylation may serve as a storage mechanism for GSH in pro-oxidant conditions within the cell because GSH oxidized to GSSG could be rapidly excluded from the cell (Dalle-Donne *et al.*, 2007; Sies and Akerboom, 1984).

Reduced glutathione also plays an important role in modulation of cell proliferation and apoptosis, in regulation of the immune response and cytokine production, in the regulation of signal transduction and in gene expression. Decreased GSH is capable to stimulate activation of NF-κB by regulation the expression of genes containing the NF-κB binding sites (Balendiran *et al.*, 2004). An increase in GSH is associated with an early proliferative response in e.g. lymphocytes and fibroblasts and is important for the cell to enter the S phase, thus is important in the regulation of cellular proliferation (Shaw and Chou, 1986; Hamilos *et al.*, 1989).

It has been shown that GSH is one of the important factors in the apoptosis pathway and depletion of the intracellular GSH inducing cell death in various cell types (Cotgreave and Gerdes, 1998; Armstrong and Jones, 2002; Lu and Holmgren 2009). The role of GSH in the apoptosis pathway depends on the cell type and pro-apoptotic stimuli. Glutathione regulates redox state of specific thiol residues of proteins such as NFκB, caspases and kinases involved in the cell death. Decreased glutathione in the course of apoptosis is accompanying by increased extracellular GSH levels, indicating that GSH is exported in its reduced form into the extracellular space, but the precise mechanism is not well known (Hammond *et al.*, 2007). Glutathione is extruded by the cells undergoing apoptosis before the lost of plasma membrane integrity indicating that depletion in apoptosis is not a consequence of OS and ROS (Franco and Cidlowski, 2006). It is known that reduction of GSH might be the cause of OS by altering the redox status of cells (Ghibelli *et al.*, 1998,

Hammond *et al.*, 2001). Glutathione release during apoptosis is mediated by the transport proteins (multidrug resistance-associated protein family) and it is required for the activation of specific apoptotic signalling pathways (Franco and Cidlowski, 2006; Hammond *et al.*, 2007). Conversely, elevation of intracellular GSH levels caused by cysteine precursors, drugs, growth regulatory protein and protein synthesis inhibitors, leads to increased resistance of cells against apoptosis (Cotgreave and Gerdes 1998; Singh *et al.*, 2012; Chiba *et al.*, 1996).

Reduced glutathione is involved in apoptotic process by affecting activation of caspases and transcription factor, ceramide production and thiol-redox signalling (Hammond *et al.*, 2001). Caspases are aspartate specific cysteine proteases that contain cysteine residues in their active sites and require a presence of GSH. Reduced glutathione as reductant can provide activation of caspases to prevent oxidation of these residues and thus maintain their catalytic activity (Hentze *et al.*, 2002; Singh *et al.*, 2012). It has been shown that low levels of intracellular GSH can prevent apoptosis and lead to the cell survival and recovery by regulating the activity of the redox sensitive caspases. The depletion of mitochondrial as well as cellular GSH opens the permeability transition pore inducing apoptosis (Armstrong and Jones, 2002). The levels of GSH in apoptosis are regulated by the specific pro- and anti-apoptotic proteins such as Bcl-2, heat-shock proteins (HSP25 and HSP27) and protein p53. Protein Bcl-2 inhibits apoptosis and release of cytochrome c and also regulates the activation of the caspase cascade of apoptosis (Singh *et al.*, 2012). Increased expression of anti-apoptotic protein Bcl-2 may lead to the enhanced level of intracellular GSH, whereas experimentally induced GSH depletion causes degradation and loss of Bcl-2 protein (Celli *et al.*, 1998). Similarly heat-shock proteins prevent apoptosis, their overexpression increases levels of intracellular GSH that facilitates the inhibition of apoptosis (Baek *et al.*, 2000).

Oxidative stress has effect on the progression of apoptosis. It has also been shown that physiologic levels of GSH maintain cytochrome c in a reduced and thus in inactive form and thus block the association of cytochrome c with apoptosome. Oxidized form of cytochrome c is required for its apoptotic activation and thus development of apoptosis (Jefferies *et al.*, 2003, Singh *et al.*, 2012). During OS, cytochrome c is released from mitochondria because of the depletion of cytoplasmic GSH which leads to oxidation of cytochrome c. It was shown in *in vitro* studies that depletion in GSH is important for apoptosome formation (Singh *et al.*, 2012). Reduction of GSH efflux out of cells leads to inhibition of apoptosis (Ghibbelli *et al.*, 1998).

Finally, GSH is required for the synthesis of leukotrienes and prostaglandins being essential in conversion of prostaglandin H_2 (derived from arachidonic acid) into prostaglandin D_2 and E_2, in reactions catalysed by prostaglandin isomerase (Lu, 2000).

2. Aims and Scopes

1. The aim of the doctoral thesis study was to ascertain the importance of antioxidant enzymes - glutathione peroxidase, glutathione reductase and non-enzymatic antioxidant reduced glutathione in relation to oxidative stress markers in different pathophysiologic states - depressive disorder, metabolic syndrome, sepsis, chronic and acute pancreatitis and pancreatic cancer compared with healthy controls.
2. Antioxidant status together with oxidative stress markers have been followed up in acute phase of sepsis, septic shock and acute pancreatitis, in the course of these diseases and after clinical recovery phase.
3. This doctoral thesis is focused on the state of antioxidant defense system in various pathophysiologic states. We assume that oxidative stress plays a key role in pathophysiology of above mentioned diseases.

3. Materials and Methods

3.1 Subjects and Studies

Basic characteristics of enrolled patients and inclusion criteria of individual studies are presented in Table 3-1. All of these prospective studies were carried out at the 4th Department of Internal Medicine of General University Hospital in Prague except patients with depressive disorder (DD). Patients with DD were recruited from the consecutive outpatients of the Psychiatric Department of General University Hospital in Prague and 1st Faculty of Medicine of Charles University in Prague. All patients were compared to healthy controls (CON) matched for sex and age. Healthy controls were defined as individuals without known signs and symptoms of the disease and major disease. The protocols of all studies were approved by the institutional review board and the Ethics Committee of the First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague. Written informed consent was obtained from all participants.

Table 3-1. Basic characteristic of enrolled patients into the individual studies

Study	Number of subjects (Male/Female)	Inclusion criteria according to	References
Depressive disorder	35 (0/35)	Diagnostic and statistical Manual of Mental Disorders, 4th Edition, American Psychiatric Association, 1994 (American Psychiatric association,1994)	Kodydkova *et al.*, 2009 Supplement 1
Metabolic syndrome	40 (20/20)	International Diabetes Federation criteria. (Alberti *et al.*, 2005)	Vavrova *et al.*, 2013 Supplement 2
Sepsis **- course** **- sepsis (septic shock)** **- critically ill patients**	 19 (10/9) 15 (9/6) 15 (9/6)	The Society of Critical Care Medicine/American College of Chest Physicians definitions (Bone *et al.*, 1992)	Vavrova *et al.*, 2013 now under review Supplement 3

Acute pancreatitis	13 (9/4)	Classification of the severity of acute pancreatitis, 2010 (Petrov and Windsor, 2010)	Vavrova *et al*., 2012 Supplement 4
Pancreatic cancer **Chronic pancreatitis**	50 (40/10) 50 (40/10)	PC staging according to the TNM system (Clasification of Malignant Tumors) and Union Internationale Contre le Cancer with American Joint Committee on Cancer (Greene *et al.*, 2003) The grade of according to M-ANNHEIM pancreatic imaging criteria (Schneider *et al*, 2007)	Kodydkova *et al*, 2013 Supplement 5

The common exclusion criteria for all studied subjects were the following: current antioxidant therapy (e.g. vitamin C, vitamin E, allopurinol, N-acetylcysteine, supplementation with n-3 polyunsaturated fatty acids), chronic dialysis, kidney disease (creatinine > 150 μmol/l), clinically manifest proteinuria (urinary protein > 500 mg/l), and liver cirrhosis, decompensate DM, concomitant malignancies, chronic, immunosuppressive and anti-inflammatory therapy, as well as chemotherapy. Further exclusion criteria were history of cardiovascular and cerebrovascular disease, hepatic and/or renal diseases, hypothyroidism, macroalbuminuria, treatment with hypolipidemic medications, unstable angina pectoris, stage within 1 year after acute myocardial infarction, respectively coronaro-aorto bypass grafting, or percutaneous coronary intervention, and stroke.

For patients with pancreatic cancer (PC) were further exlusion criteria: acute pancreatitis, endocrine disease or acute relapse of chronic pancreatitis. Patients who were operated for gastrointestinal tract (in the previous 1 year) and subjects after systemic inflammation in the previous 6 months were also excluded. Patients with chronic pancreatitis enrolled into the study were re-examined after 2 years to exclude the

development of PC and thus to avoid enrolment of patients with initial stages of PC into the study.

3.2 Blood sample collection and preparation

Blood samples were obtained after overnight fasting. Samples were processed immediately after collection. For plasma samples we used K_2EDTA as anticoagulant and centrifuged the anticoagulated blood for at least 15 min at 2500 *g* at 4 °C to obtain cell-free plasma. After separation of plasma, fraction of red blood cells was further processed. The remaining erythrocytes were washed three times in proportion 1:10 with physiological saline (0.9% sodium chloride) solution and centrifuged at 2500 *g* at 4 °C for 5 min. For analysis of GPX1 and GR activities was used hemolysate of washed erythrocytes. Hemolysate was prepared by suspending washed RBC in cold redistilled water in a ratio 1:4. Prepared hemolysate was diluted before measurement 30 times with phosphate buffer. Serum samples were prepared after coagulation in vacutainer tubes by centrifugation at 2500 *g* at 4 °C for 10 min. The plasma, serum samples and suspension of washed erythrocytes were stored at -80 °C until assay.

3.3 Measurement of antioxidant enzyme activities

Glutathione peroxidase 1

The glutathione peroxidase activity was measured by the modified method of Paglia and Valentine using tert-butyl hydroperoxide as a substrate (Paglia and Valentine, 1867). Briefly, 580 μL of 172.4 mM tris–HCl buffer containing 0.86 mM EDTA, pH = 8.0; 100 μL of 20 mM GSH, 100 μL of 10 U/mL GR, 100 μL of 2 mM NADPH and 100 μL of diluted sample were pipetted into the cuvettes. The reaction was started after 10 min of incubation at 37 °C by the addition of 20 μL of 9.99 mM tert-butyl hydroperoxide. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm. Blank was run for each sample. Activity of GPX1 was calculated using the molar extinction coefficient of NADPH 6220 M^{-1} cm^{-1} and expressed as U/g haemoglobin. One unit of GPX1 (U) is defined as 1 μmol of NADPH oxidized to NADP per min.

Glutathione reductase

The activity of GR was measured according to the method of Goldberg (Goldberg, 1983). Briefly, 700 μL of 0.127 M potassium phosphate buffer containing 0.633 mM $Na_2EDTA{\cdot}2H_2O$, pH = 7.2 was added to cuvettes followed by 100 μL of 22 mM oxidized

glutathione and 100 μL of diluted hemolysate. Hemolysate was prepared by suspending washed RBC in cold redistilled water in a ratio 1:4. Prepared hemolysate was diluted before measurement 30 times with phosphate buffer. The reaction was started after 10 min of incubation at 37 °C by addition of 100 μL of 1.7 mM NADPH. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm. Blank was run for each sample. Activity of GR was calculated using the molar extinction coefficient of NADPH 6220 M^{-1} cm^{-1} and expressed as U/g haemoglobin. One unit of GR (U) is defined as the amount of enzyme catalysing the reduction of 1 μmol of GSSG per minute.

3.4 Measurement of markers of oxidative stress

Conjugated dienes in precipitated low density lipoproteins

Serum low density lipoproteins were isolated by precipitation method of Ahotupa *et al.* (Ahotupa *et al*, 1996). The concentration of CD in precipitated LDL (CD/LDL) was measured by the modified method of Wieland and Seidel (Wieland and Seidel, 1983). Serum samples were stabilized with EDTA in portion 10:1 (v/v) and analysed within 2 weeks. Briefly, 110 μL of sample was added to 1 mL of the heparin-citrate precipitation buffer consisting of 0.064M trisodium citrate adjusted to pH 5.05 by addition of 5 M HCl and contained 50000 IU/L heparin. Precipitated lipoproteins were separated by centrifugation at 2800 rpm for 10 min after 10 min incubation. The pellet was resuspended in 100 μL of NaCl solution (9 g/L). Lipids were extracted by chloroform–methanol mixture (2:1) and then were incubated for 10 min with intermittent mixing. For phase separation was used 250 μL of redistilled water. After 5 min centrifugation the 800 μL of lower layer was dried under nitrogen, redissolved in 300 μL of cyclohexane. Conjugated dienes were analysed spectrophotometrically at 234 nm. The concentration of CD was calculated using the molar extinction coefficient 2.95×10^4 M^{-1} cm^{-1} and expressed as mmol/L serum.

Oxidized low density lipoproteins

The measurement of oxidized LDL (ox-LDL) was performed by commercially available kits Oxidized LDL Competitive ELISA (Enzyme-Linked Immunosorbent Assay) (Mercodia, Sweden). Oxidized LDL in the sample competes with a fixed amount of ox-LDL bound to the microtiter wells for the binding of the biotin-labelled specific antibodies. Unreacted sample components were removed and bound biotin-labelled antibody was detected by Streptavidin HRP enzyme conjugate. After incubation and washings steps the

bound conjugate was detected by reaction with 3,3′,5,5′- tetramethylbenzidine. The reaction was stopped by addition of stop solution (0.5 M H_2SO_4) and measured spectrophotometrically at 450 nm. Oxidized-LDL reflects concentration of malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), the highly reactive end-products of lipid peroxidation that are bound as adducts to the ε-amino-group of lysine in molecule of apolipoprotein B.

3.5 Measurement of non-enzymatic antioxidants and selenium

Reduced glutathione

Reduced glutathione was measured by the modified spectrophotometric method described earlier (Griffith, 1985). Suspension of washed erythrocytes (500 μL) was mixed with 100 μL of diluted acetic acid in water (6%, v/v), haemolysate was vortexed and 400 μL of 5-sulphosalicylic acid 10% (w/v) was immediately added. After centrifugation at 10 000 g for 2 min, supernatant solution was collected for analysis. This method is based on the determination of 5,5′ - dithio-bis(2-nitrobenzoic acid) (DTNB), relatively stable yellow product of reduction by sulfhydryl compounds. Briefly, 50 μL of 0.125 M potassium phosphate buffer containing 6.3 mmol/L $Na_2EDTA{\cdot}2H_2O$, pH = 7.5 was added to micro-cuvettes followed by 37.5 μL of the sample and 12.5 μL of 6 mmol/L DTNB. The absorbance of the yellow product (reduced chromogen) was measured at 412 nm. Concentration was calculated by means of calibration curve and was expressed as μg/g haemoglobin.

Vitamins and selenium

Vitamins A and E were assayed by high performance liquid chromatography in laboratories of Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague. Selenium was analysed by atomic absorption spectrometry with thermal atomization in the same abovementioned laboratories.

The Total Peroxyl Radical Trapping (cTRAP) was calculated according to formula of Roth et al. (Roth et al. 2004) - cTRAP (μmol/l) = [(albumin) 0.63 + (uric acid) 1.02 + (bilirubin) 1.50)].

3.6 Statistical analysis

Data are expressed as mean ± SD (standard deviation) for parametric, and as median and interquartile range (IQR, 25^{th}-75^{th} percentiles) for non-parametric data. Normality of data distribution was tested with the Shapiro-Wilks W test. Difference between compared groups (PC, CP and CON; S, NC and CON) were tested with the one-way ANOVA with Scheffé and Newman-Keuls post-tests or Dunnett´s post-hoc test (in septic studies). For nonparametric analysis, the Kruskal-Wallis ANOVA was used. Comparison between two independent groups was carried out by the independent t-test for parametric variables or Mann-Whitney U test for non-parametric data e.g. patients with depression or with metabolic syndrome. Friedman ANOVA was used for dependent analysis e.g. patients with acute pancreatitis. For correlation analysis, the Spearmen coefficient was used.

All above described statistical analyses were performed using version 8.0, 9.0 and 10.0 of Statistica programme (StatSoft software CZ). $P < 0.05$ was considered to be statistically significant.

4. Results

4.1 Depressive disorder

Subject and biochemical characteristic as well as other biochemical data are shown in the publication of Kodydkova *et al.*, 2009; Supplement 1, Table 1 and Table 2.
Thirty five women with DD were enrolled in the study and compared with 35 sex- and age-matched CON. We have observed activities of GPX1 and GR as well as concentration of GSH and oxidative stress marker levels (CD/LDL) in women with DD. Increased erythrocyte GR activities were observed in patients with DD than in CON ($p < 0.05$), whereas GPX1 activities were lower in DD patients ($p < 0.05$). Similarly concentration of GSH was significantly decreased in depressive women compared to controls ($p < 0.05$). In the contrast CD/LDL levels were higher in patients with DD when they were compared to CON ($p < 0.05$). Furthermore, we found positive correlation of GPX1 with GSH ($r = 0.284$, $p < 0.05$) in patients with DD, whereas there was no significant correlation between GPX1 and GSH in healthy controls.

4.2 Metabolic syndrome

Clinical and biochemical characteristics and other results of subjects with MetS and that of healthy controls are shown in the publication of Vavrova *et al.*, 2013; Supplement 2, Table 1.

In 40 patients with MetS, activities of antioxidant enzymes and concentrations of GSH and CD/LDL together with levels of calculated cTRAP were observed and compared with 40 sex and age matched CON. All patients fulfilled the inclusion criteria for diagnosis of MetS abdominal obesity (waist circumference ≥ 94cm for men and ≥ 80cm for women) and fulfilled any two of the following four factors: a) raised TG level (≥ 1.7 mmol/l) b) reduced HDL-C (< 1.03 mmol/l in males and < 1.29 mmol/l in females), or specific treatment for these abnormalities, c) raised blood pressure (BP): systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg, or treatment of previously diagnosed hypertension, d) raised fasting plasma glucose (≥ 5.6 mmol/l), or previously diagnosed type 2 of diabetes mellitus. In patients with MetS, 21 patients had three, 13 patients four and 6 patients had all five components of metabolic syndrome.

Patients with MetS had significantly increased activities of GR than CON ($p < 0.001$), whereas no significant differences were observed in GPX1 activities in comparison

with CON. In the contrast, concentration of GSH was found to be decreased in MetS patients compared to CON ($p < 0.05$). Levels of CD/LDL and c TRAP were significantly higher in MetS than in CON ($p < 0.001$, $p < 0.01$, respectively). Furthermore, Spearmen correlations between selected variables were observed. Concentrations of CD/LDL significantly correlated with concentrations of TG and HDL-C.

Activities of GR and GPX1 correlated positively with those of CuZnSOD ($r = 0.341$, $p < 0.01$) and ($r = 0.260$, $p < 0.05$). Concentrations of CD/LDL correlated positively with total cholesterol ($r = 0.565$, $p < 0.001$), apo B ($r = 0.597$, $p < 0.001$) and LDL ($r = 0.384$, $p < 0.001$) and negatively with CAT ($r = -0.233$, $p < 0.05$).

4.3 Sepsis

Thirty septic patients (S) were enrolled into this study; 15 sex, age and APACHE II matched non-septic (NC) critically ill patients with systemic inflammatory response syndrome (SIRS) (non-septic control group) and 30 age and sex matched healthy controls without clinical and laboratory signs of inflammation, sepsis or known major disease. Samples from septic patients were collected three times: 24 – hours after intensive care unit admission (S1), 7 days after first sampling (S7) and finally after recovery (7 days after absence of clinical signs and normal values of CRP and temperature) (R7). These free samplings were available only from 19 septic patients, because 8 patients died and 3 patients never fully recovered from sepsis. Firstly, we compared these 19 septic patients with all three samplings with 19 sex and age matched CON. Furthermore, we compared 15 septic patients with 15 sex-, age- and APACHE II score matched NC with SIRS and 15 sex- and age- matched CON.

Course of sepsis

Demographic and clinical characteristics of 19 septic patients in all three samplings (S1, S7, R7) and 19 sex- and age- matched CON are shown in the publication of Vavrova *et al.* (now under review); Supplement 4 and Table 1.

Activities of antioxidant enzymes, levels of non-enzymatic antioxidants, vitamins and markers of oxidative stress were observed. Decrease in GPX1 activity persisted in all three samplings in comparison with controls. In the contrast, no significant difference in GR activity between individual S samplings and CON was found. The GPX1 activity and selenium (Se) level were substantially decreased in all three septic samplings and never reached CON values. In addition lower concentrations of Se were found in both S1 and S7

samplings compared to R7. There was also decrease in concentrations of vitamin E, vitamin A and bilirubin in S1 only and the decline of uric acid was found only in S7 compared to CON. The levels of ox-LDL and CD/LDL were increased in S1, culminated in S7 and returned to CON values in R7.

Calculated TRAP (cTRAP) closely followed the values of GPX1, the decrease was found in all three samplings in comparison with CON; S1 and S7 values were significahtly higher compared to R7.

Activity of GPX1 in S1 group positively correlated with CRP ($r = 0.467$; $p < 0.05$). Positive correlations between GPX1 activity and levels of CRP ($r = 0.584$; $p < 0.01$) and PCT ($r = 0.617$; $p < 0.01$) were found in S7 group, but GPX1 negatively correlated with vitamin A ($r = -0.457$; $p < 0.05$) and cTRAP ($r = -0.574$; $p < 0.05$). After recovery there was negative correlation between GPX1 activity and vitamin A ($r = -0.458$; $p < 0.05$), vitamin E ($r = -0.460$; $p < 0.05$), markers of oxidative stress CD/LDL ($r = -0.553$; $p < 0.05$) and ox-LDL/LDL ($r = -0.528$; $p < 0.05$), and IL-6 ($r = -0.654$; $p < 0.01$).

Septic patients versus non-septic critically ill patients

In this part of the study we compared erythrocyte activities of GPX1, GR and GSH as well as markers of oxidative stress CD/LDL and ox-LDL and vitamins A and E in 15 septic patients with 15 sex, age and APACHE II score matched NC and 15 sex- and age-matched CON. Basic clinical data and characteristic of patients are shown in Table 4-1. These results have not yet been published.

Table 4-1. Clinical and biochemical data of septic, on-septic critically ill patients and healthy controls

	S	NC	CON
N (M/F)	15 (9/6)	15 (9/6)	15 (9/6)
Age (years)	74 (61 - 79)	70 (57 - 79)	71 (58 - 79)
APACHE II score	16 (13 - 20)	17 (13 - 20)	-
CRP (mg/L)	96.0 (47.0 - 185.5)***	84.8 (4.8 - 130.6)**	2.1 (2.8 - 7.8)
PCT (mg/L)	2.39 (0.79 - 10.0)*$^{+++}$	0.28 (0.14 - 0.73)	0.585 (0.32 - 0.90)
IL-6 (µg/L)	114.0 (51.0 - 313.1)***	21.5 (10.9 - 48)**	1.15 (0.58 - 2.86)
IL-10 (µg/L)	8.58 (5.12 - 16.57)***	5.16 (1.76 - 6.98)***	0.79 (0.00 - 1.03)
TNF- α (µg/L)	21.8 (11.9 - 39.2)***	6.54 (4.16 - 9.50)	11.89 (6.82 - 14.47)
TC(mmol/L)	3.04 ± 0.71***	3.31 ± 1.14***	5.77 ± 1.05
HDL-C(mmol/L)	0.66 ± 0.29***$^{+}$	0.96 ± 0.45**	1.41 ± 0.42
LDL-C(mmol/L)	1.76 ± 0.55***	1.77 ± 0.93***	3.67 ± 0.75
TG (mmol/L)	1.30 ± 0.48	1.28 ± 0.51	1.41 ± 0.68
Ferritin (µg/L)	336.6 (196.9 - 1297.5)**	356.1 (222.2 - 1346.8)	278.4 (193.9 - 646.4)
Transferin (g/L)	1.58 (1.46 - 1.91)***	1.92 (1.40 - 2.47)***	2.50 (2.45 - 2.65)
Albumin (µmol /L)	416.3 ± 98.2***$^{++}$	513.9 ± 105.9***	685.8 ± 56.6
Bilirubin (µmol/L)	14.8 (9.6 - 45.1)	10.5 (8.1 - 13.5)	12.9 (7.3 - 15.8)
Uric acid (µmol/L)	261.9 ± 94.4^{+}	363.7 ± 117.3	324.3 ± 61.3

Comments and abbreviations used:
S: septic patients after onset of sepsis, NC: non-septic critically ill patients (non-septic control), CON: healthy controls; APACHE II score: Acute Physiology and Chronic Health Evaluation II score, CRP: C- reactive protein, PCT: procalcitonin, IL: interleukin TNF-α: tumor necrosis factor-α, LDL: low density lipoprotein, HDL-C: high density lipoprotein, TC: total cholesterol, TG: triglycerides. Data are expressed as mean ± standard deviation (SD) for parametric and median (IQR) for non-parametric variables. * S or NC vs. CON,

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; $^{+}$ S vs. NC: $^{+++}$ $p < 0.001$, $^{++}$ $p < 0.01$, $^{+}$ $p < 0.05$; (one-way ANOVA with Newman-Keuls post-test for parametric and Kruskal-Wallis ANOVA for non-parametric analysis).

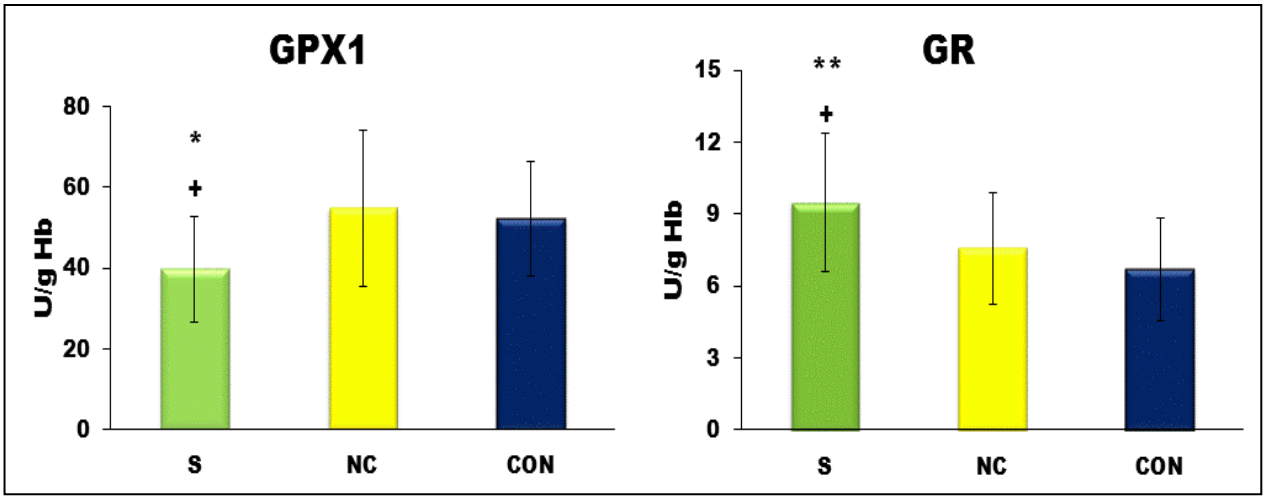

Figure 4-1. Erythrocyte GPX1 and GR activities.
Legend and abbreviations used S: septic patients after onset of sepsis, NC: non-septic critically ill patients (non-septic control group), CON: healthy controls; GPX1: glutathione peroxidase 1, GR: glutathione reductase. Data are expressed as mean ± S.D. * S or NC vs. CON, ** $p < 0.01$, * $p < 0.05$; $^{+}$ S vs. NC: $^{+}$ $p < 0.05$; (one-way ANOVA with Newman-Keuls post-test)

Figure 4-1 shows erythrocyte activities of GPX1 and GR in S, NC and CON. Activity of GPX1 in S patients was decreased to those found in NC and controls (both $p < 0.05$). In the contrast, GR activity was increased in S patients compared to NC patients and CON ($p < 0.05$, $p < 0.01$, respectively). No significant difference in activities of GPX1 and GR were found between NC patients and healthy controls.

The mean serum vitamin A, E and selenium concentrations as well as levels of c TRAP are presented in Figure 4-2. The serum concentration of Se was significantly lower in S patients compared with NC and CON ($p < 0.01$, $p < 0.001$, respectively). Critically ill patients also possesed the decrease in Se concentration compared to CON ($p < 0.001$). Similarly to these findings the value of c TRAP was the lowest in septic patients. Concentrations of vitamin A and E were decreased in patients with S as well as in NC group in comparison with CON ($p < 0.01$).

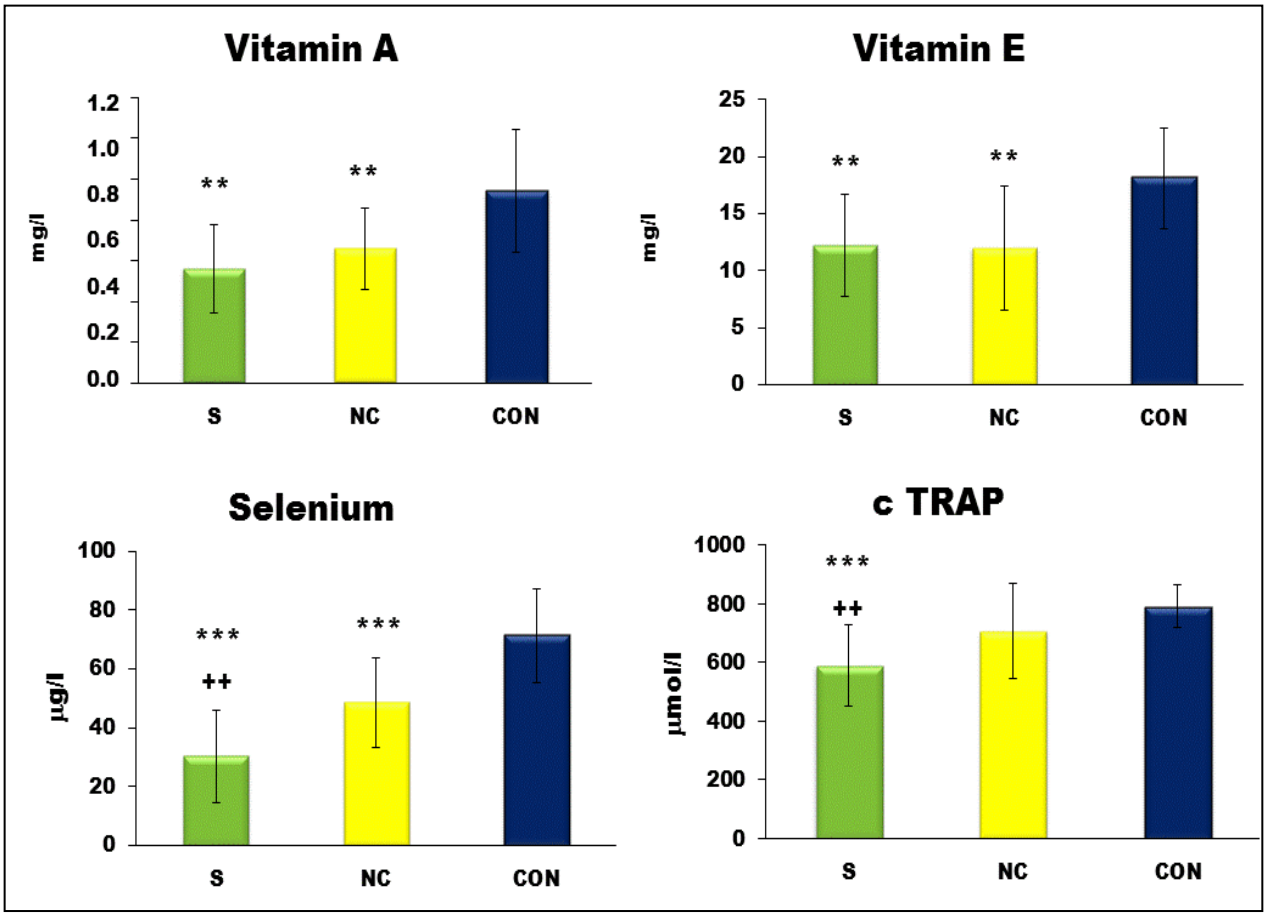

Figure 4-2. Concentration of vitamin A, E, selenium and calculated TRAP.
Legend and abbreviations used: S: septic patients after onset of sepsis, NC: non-septic critically ill patients (non-septic control group), CON: healthy controls; cTRAP: calculated total peroxyl radical trapping – calculation: [0.63 (albumin) + 1.02 (uric acid) + 1.50 (bilirubin)]. Data are expressed as mean ± S.D. * S or NC vs. CON, *** $p < 0.001$, ** $p < 0.01$, $^{+}$ S vs. NC: $^{++}p < 0.01$, $^{+}p < 0.05$; (one-way ANOVA with Newman-Keuls post-test)

Figure 4-3 shows levels of oxidative stress markers - ox-LDL/LDL and CD/LDL, respectively, observed for the S and NC patients in comparison with CON. For S patients, levels of ox-LDL/LDL and CD/LDL were similar to those found in NC patients, whereas concentration of ox-LDL in both S and NC group was higher than in CON (both $p < 0.001$). Furthermore, levels of CD/LDL were also increased in S and NC subjects in comparison with CON ($p < 0.01$).

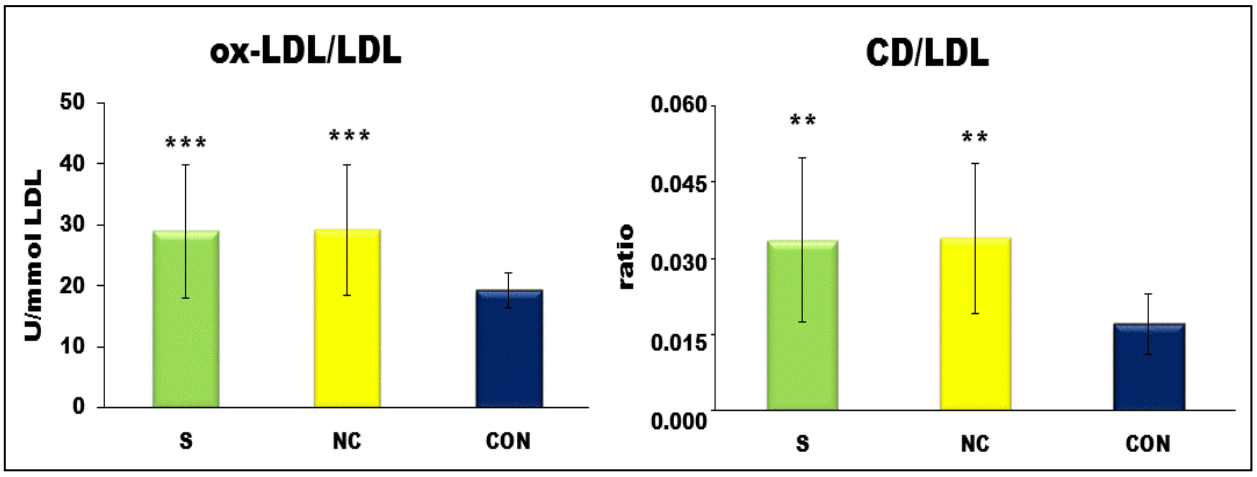

Figure 4-3. Levels of oxidative stress markers.

Legend and abbreviations used: S: septic patients after onset of sepsis, NC: non-septic critically ill patients (non-septic control group), CON: healthy controls; ox-LDL/LDL: oxidized low density lipoproteins, CD: conjugated dienes in precipitated LDL, LDL: low density lipoprotein cholesterol. Data are expressed as mean ± S.D. * S or NC vs. CON, *** $p < 0.001$, ** $p < 0.01$; (one-way ANOVA with Newman-Keuls post-test)

4.4 Acute pancreatitis

Into the study were enrolled 13 patients with acute pancreatitis (AP) diagnosis and two sex- and age- matched control groups: 13 healthy controls (CON) and group of 13 patients that within 2-3 years prior to sampling had an acute pancreatitis and during the study were without signs of acute pancreatitis (PAP). In patients with AP were taken 4 samplings: first sampling within 24 hours after onset of acute pancreatitis (AP1), AP after 72 hours (AP3), AP five days after onset of signs (AP5) and finally 10 days after admission (AP10). Main antioxidant enzymes, non-enzymatic antioxidants and markers of oxidative stress were followed up in the course of the acute pancreatitis and compared with controls.

Basic anthropometric and clinical data are shown in the publication of Vavrova *et al.*, 2012; Supplement 3 and Table 1. In the course of acute pancreatitis, activities of GPX1 and GR did not differ among individual samplings. However patients with AP had decrease in GPX1 activity in all individual samplings in comparison with CON. The decreased GPX1 activity was found in PAP patients compared to CON ($p < 0.05$). Furthermore, no differences in GR activities among individual AP samplings and in individual AP samplings in comparison with both control groups were found.

We have also observed levels of main non-enzymatic antioxidant GSH. Decreased levels of GSH were found in AP1 sampling in comparison with sampling the third day (AP3) ($p < 0.05$); in the contrast concentration of GSH in AP3 sampling was higher than in AP5 and AP10 samplings (both $p < 0.05$). Increased levels of GSH were also in AP3 sampling compared to CON ($p < 0.01$). No significantly different levels of GSH have been found between PAP patients and CON. There were no significant differences in the CD/LDL ratio among individual AP samplings; however increased levels of CD/LDL in all AP samplings in comparison with CON were observed (all $p < 0.05$). Concentration of ox-LDL/LDL was rising in course of acute pancreatitis; the highest level was found in AP5 (all $p < 0.05$).

Furthermore, we observed Se and vitamins A and E levels. Concentration of Se was lower in AP and PAP patients than those in CON (both $p < 0.01$). Patients with AP had also decreased levels of vitamin A and E in comparison with both PAP and CON groups. ($p < 0.001$, $p < 0.05$ respectively). Moreover, vitamin A was lower in AP patients than in PAP patients ($p < 0.01$). Trend to lowered concentration of vitamin E was found in PAP patients compared to CON ($p = 0.07$).

4.5 Chronic pancreatitis and pancreatic cancer

Clinical and biochemical characteristics of the 50 patients in studied groups - patients with chronic pancreatitis (CP), pancreatic cancer (PC) and CON are summarizes in the publication of Kodydkova et al., 2013; Supplement 5 and Table 1. In patients with CP, PC and CON were measured activities of antioxidant enzymes, concentration of GSH and markers of OS. The erythrocyte activities of GPX1 and GR in PC and CP patients were significantly different from those in CON. Both groups of patients had lower GPX1 activity than did CON (both $p < 0.001$). Activities of GR were decreased in PC subjects in comparison with CON ($p < 0.05$). Similarly to these findings, patients with CP had lower activity of GR compared to CON ($p < 0.01$).

When we compared patients with pancreatic cancer and those with chronic pancreatitis, patients with PC had significantly lower levels of GSH than those with CP ($p < 0.05$). Moreover, patients with PC had decreased GSH concentrations in comparison with CON ($p < 0.001$). No significant differences of GSH were found between patients with chronic pancreatitis and healthy controls.

Mean serum vitamin A concentrations were lower in PC patients than in the CP and CON ($p < 0.05$, $p < 0.05$, respectively), whereas no significant differences in vitamin E levels was found between PC and CP patients and compared to CON. The lower Se levels were found in both PC and CP groups as compared to CON ($p < 0.001$, $p < 0.05$, respectively). No significant difference was found in c TRAP among PC, CP groups and CON. Furthermore, when we compared difference in CD/LDL ratio between both groups of patients and CON, an increase was observed in PC and CP groups compared to CON ($p < 0.05$, $p < 0.01$, respectively), though CD/LDL levels did not significantly differ between groups of patients. In addition, significantly increased levels of ox-LDL/LDL were found in patients with PC in comparison with CP and CON (both $p < 0.001$). Patients with chronic pancreatitis also had higher levels of ox-LDL/LDL than CON group ($p < 0.05$).

There was a significant correlation between Se and GPX1 in the whole group ($r = 0.319$; $p < 0.01$) and also in CP patients ($r = 0.470$; $p < 0.01$). Positive correlation was found between vitamin A and E in the whole group ($r = 0.580$; $p < 0.01$) as well as in PC and CP groups ($r = 0.790$; $p < 0.001$; $r = 0.638$; $p < 0.001$, respectively). Serum levels of CD/LDL positively correlated with ox-LDL/LDL levels in PC and CP group ($r = 0.438$; $p < 0.01$; $r = 0.445$; $p < 0.01$, respectively), but not in controls.

5. Discussion

We have studied activities of main antioxidant enzymes such as GPX1 and GR as well as non-enzymatic, low molecular-weight antioxidant GSH in six different pathophysiologic states - depressive disorder, metabolic syndrome, sepsis, acute and chronic pancreatitis and pancreatic cancer. In all aforementioned pathophysiologic states, levels of CD/LDL were also measured. In patients with S, CP, PC and AP we also analysed levels of ox-LDL. Furthermore, serum concentrations of Se and serum levels of vitamin A and E were determined in some studies. Our results demonstrated increased levels of OS markers in all aforementioned studies.

In the pathogenesis of abovementioned states we have assumed the role of OS, because it has been implicated in variety of human diseases such as neurodegenerative, cardiovascular diseases and cancer (Sies, 1991). Critical illnesses, such as sepsis or acute respiratory distress syndrome are characterized by ROS overproduction and increased production of other radical species with subsequent OS (Gutteridge and Mitchel *et al*., 1999). The prolonged OS may lead to reduction of antioxidant enzymes activities and excessive peroxidation of lipids. Sepsis and acute pancreatitis have similar pathogenetic mechanisms that have been implicated in the progression of multiple organ failure.

5.1 Glutathione peroxidase

Glutathione peroxidase is important ubiquitous selenoenzyme, which catalyses degradation of not only H_2O_2, but also a wide range of hydroperoxides to water and corresponding alcohol, respectively. Therefore this enzyme plays a role in the protection of cells against RONS induced OS. We have studied activities of erythrocyte GPX1 in patients with depressive disorder in comparison with healthy controls. Decrease in GPX1 activities has been found in patients with major depression than those in healthy controls (Kodydkova *et al.*, 2009). Major depression is characterized by decreased levels of a number of important antioxidants and by lowered antioxidant status. Moreover lowered GPX1 activity is supposed to be one of the characteristic features for depression and play a role in pathogenesis of depression (Maes *et al.*, 2011a). Similarly to our findings Maes *et al.* observed that whole blood GPX1 was significantly decreased in patients with major depressive disorder (MDD) compared to controls (Maes *et al.*, 2011b). In the contrast according to study of Bilici *et al.* (Bilici *et al.*, 2001), patients with major depression had

increased erythrocyte GPX1 activity, especially patients with melancholia. They suggested that depressive disorder is associated with overproduction of RONS. Another studies on patients with MDD reported that activity of GPX1 did not differ between patients with MDD and healthy subjects (Sarandol *et al.,* 2007; Galecki *et al.,* 2009). Srivastava *et al.* observed no significant changes in activities of GPX1 in polymorphonuclear leukocytes from the patients with depression (Srivastava *et al.,* 2002). Decreased GPX1 activity was also found in animal models with induced depression in cortex of the brain (Eren *et al.*, 2007a; Eren *et al.,* 2007b). It has been shown that GPX1 exhibit neuroprotective effects against cell death, DNA and neuronal damage (Leonard and Maes, 2012).

Furthermore, there are several studies focused on activities of antioxidant enzymes in different neuropsychiatric disorders (e.g. schizophrenia, affective disorder and bipolar disorder). In schizophrenic patients have been shown decreased erythrocyte GPX1 activities compared to control groups (Li *et al.*, 2006; Ben Othmen *et al.*, 2008; Yapişlar, 2012). On the other hand, there are also some contradictory results. Some studies showed increased GPX1 activities in schizophrenic patients than in controls (Herken *et al.*, 2001; Kuloglu *et al.*, 2002). Ozcan *et al.* reported significantly lower GPX1 activity in patients with affective disorder than had healthy controls (Ozcan *et al.*, 2004). No changes in GPX1 activities have been shown in bipolar disorder (Kuloglu *et al.*, 2002; Andrezza *et al.*, 2009).

Metabolic syndrome is associated with a number of pathophysiologic processes such as increased OS, activation of inflammatory cytokines and prothrombic mediators. The erythrocyte GPX1 activity in our study was not altered in MetS patients in comparison with healthy controls (Vávrová *et al.*, 2013). In accordance with our findings, in other studies also observed no significant changes in GPX1 in erythrocytes of patients with MetS compared to healthy controls (Dimitrijevic-Sreckovic *et al*., 2007; Broncel *et al*., 2010; Sánchez-Rodríguez *et al.*, 2010; Mansego *et al*., 2011; Kowalczyk *et al.*, 2012). On the contrary, Cardona *et al.* found lower activities of GPX1 in a group of subjects with hypertriglyceridemia, a component of MetS, that is associated with increased OS, and the decrease of its activity was almost to 75 % of the control group (Cardona *et al.*, 2008a, b). Bougoulia *et al.* showed decreased activity of GPX1 in obese subjects with its increase after weight reduction (Bougoulia *et al.*, 2006). Similarly in the study of Koziróg *et al.* and Chen *et al.* found that patients with MetS had significantly lower GPX1 activities compared to healthy controls (Koziróg *et al*., 2011, Chen *et al*., 2012). In the contrast Ferro

et al. investigated activities of erythrocyte GPX1 in obese women with metabolic syndrome and compared to healthy controls. They found higher GPX1 activity in obese women with MetS than in controls (Ferro *et al.*, 2011). Similarly, higher activities of erythrocyte GPX1 have been observed in obese children and in elderly subjects with MetS in comparison with elderly controls without MetS (Erdeve *et al.*, 2004; Pizent *et al.*, 2010).

The key role of OS has been shown in pathogenesis of sepsis (Crimi *et al.*, 2006b). In the part of study dealing with sepsis, we compared patients in the course of sepsis with healthy controls. We have shown decreased GPX1 activities in erythrocytes at the onset of sepsis in the comparison with healthy controls. This decrease in activity of GPX1 persisted still after recovery. Decreased GPX1 activity was accompanied by depletion in selenium levels at the onset of sepsis as well as after recovery in comparison with healthy controls. When we compared septic patients with critically ill patients (non-septic patients) and healthy controls, septic patients exhibited decreased GPX1 activity in comparison with critically ill and control subjects, whereas there was no difference in erythrocyte GPX1 activity between critically ill patients and controls. Decrease in Se levels has been observed in both septic and critically ill patients compared to healthy controls. Moreover, septic patients had lower selenium levels than did critically ill. It is known that GPX1 requires GSH as a substrate in the milimolar range, which is at intracellular space. The decrease in GPX1 activity can be possibly explained by two main reasons: low level of GSH observed in erythrocytes of septic patients by others (Mühl *et al.*, 2011) and/or decreased concentration of selenium that is bound at the active site of the enzyme in the form of SeCys residue and is essential for its activity (Brigelius-Flohé, 1999; Burk and Hill, 2010).

By contrast to our findings, Mishra *et al.* compared patients with systemic inflammatory response syndrome (SIRS) with those of severe sepsis. Erythrocyte GPX1 activities did not differ between these groups (Mishra *et al.*, 2005). Similarly, Leff *et al.* found no significant difference in serum GPX activity between patients with sepsis with or without adult respiratory distress syndrome (Leff *et al.*, 1993). No significant difference has been observed in erythrocyte GPX1 activity between severe septic patients with high dose and normal selenium supplementation (Mishra *et al.*, 2007), whereas in the study of Valenta *et al.* septic patients with high dose selenium supplementation had increased whole blood GPX1 activity than did septic patients with normal selenium dose, except baseline levels (Valenta *et al.*, 2011). Further, Forceville *et al.* studied patients with septic shock or severe systemic SIRS with organ failure and patients without sepsis or SIRS, there were

found no significant differences in plasma GPX activity between septic patients, non-SIRS or healthy controls (Forceville *et al.*, 2009), whereas Manzanares *et al.* observed decreased plasma GPX activity in patients with SIRS compared to patients without SIRS and controls (Manzanares *et al.*, 2009). Critically ill patients without SIRS had similar plasma GPX activity compared to healthy controls (Manzanares *et al.*, 2009). Higher serum GPX activities were found in premature neonates with septicaemia, where increased activities might be a protective mechanism against higher free radicals production (Bartra *et al*., 2000; Kapoor *et al.*, 2006).

In accordance with our findings, there are some studies where decreased levels of selenium in septic patients have been also found (Weber *et al.*, 2008; Sakr *et al.*, 2007; Forceville *et al.*, 1998, Forceville *et al.*, 2009), while in patients with SIRS, Sakr *et al.* found higher selenium levels at admission compared with patients without SIRS and those with severe sepsis (Sakr *et al.*, 2007). Furthermore, in patients with SIRS, severe sepsis and septic shock has been shown an early significant decrease in plasma selenium levels (Hawker *et al.*, 1990; Forceville *et al.*, 1998), which correlates with the severity of the disease and mortality (Manzanares *et al.*, 2009; Sakr *et al.*, 2007). Significant decrease of selenium levels has been shown during the stay at intensive care unit in patients with SIRS and severe SIRS (Sakr *et al*., 2007). Supplementation with selenium improved antioxidant capacity, as demonstrated by increased GPX activity (Mishra *et al.*, 2007). Levels of vitamin A and E were declined in septic patients in the course of sepsis only at the onset of sepsis, in septic patients and critically ill patients compared to healthy controls. In accordance with these findings, Weber *et al*. observed lower vitamin E levels in severe septic patients than did critically ill without sepsis and healthy controls (Weber *et al.*, 2008).

The aim of our study focused on acute pancreatitis was to observe changes in the antioxidant system during the course of the acute pancreatitis, which is rapidly developing inflammatory process associated with significant metabolic changes and clinical response. Oxidative stress plays an important role in progression of AP and its intensity correlates with the severity of disease. Our study showed that the activity of GPX1 was lower at the onset of acute pancreatitis and persisted lowered in all AP samplings (after 72 hours, five days after onset of signs and finally 10 days after admission) in comparison with healthy controls (Vávrová *et al.*, 2012). Decrease in GPX1 activity has also been observed in post acute pancreatitis patients. Decreased GPX1 activity was accompanied by lower plasma

levels of selenium in patients with AP and PAP in comparison with healthy controls (Vávrová *et al.*, 2012). In accordance with our results, Musil *et al.* measured antioxidants such as GPX1 activity and selenium in the course of severe and mild acute pancreatitis compared to healthy controls. They found a significant decrease in erythrocyte GPX 1 in patients who had severe AP from admission to day 8 of the study, same findings has been obtained for selenium concentrations (Musil *et al.,* 2005). Another study focused on patients with AP determined the concentration of GPX activity and selenium levels in serum with respect to AP severity (Wereszczynska-Siemiatkowska *et al.,* 2004). They observed significantly lower serum GPX in patients with severe form of AP during the 10 days observation period. The decrease was most pronounced during the first 2 days of hospitalization compared to the serum concentrations in patients with the mild type of AP and healthy controls. Furthermore, decreased serum GPX concentration in acute pancreatitis was found in other studies (Czeczot *et al.,* 2009; Modzelewski, 2005), whereas in the study of Szuster- Ciesielska *et al.,* the serum level of GPX was comparable to that of controls (Szuster-Ciesielska *et al.,* 2001a).

Our findings are in accordance with other studies in patients with AP where has been shown the decrease in selenium levels (Musil *et al.,* 2005; Wereszcynska-Siemiatkowska *et al.,* 2004), whereas in the study of Morris *et al.* there were no differences in plasma selenium levels in AP patients in comparison with patients with chronic pancreatitis and healthy controls (Morris-Stiff *et al.*, 1999). Furthermore, there are several studies showing that chronic pancreatitis is associated with a decrease in plasma selenium concentration (Mathew, 1996; Quillot, 2000; Quillot, 2005). Other antioxidants that have been measured were vitamins A and E. Vitamin E plays an essential role in the protection of cell membranes against free radical damage and affects the response of cells to OS. Vitamin A is also known to have antioxidant capacity (Niki, 1989). We have found decreased vitamin A and E levels in patients with AP compared to controls. The decrease in levels of vitamin A and E during acute pancreatitis has been demonstrated in study of Curran *et al.* (Curran *et al.*, 2002). Decreased levels of vitamin A have also been observed in the study of AP patients by Musil *et al.,* while the concentration of vitamin E did not differ (Musil *et al.*, 2005; Morris-Stiff *et al.,* 1999). Plasma levels of vitamins A and E also significantly differed among patients with chronic pancreatitis, lower levels of vitamin A and E has been found in CP patients compared to controls (Van Gossum *et al.*1996; Quillot *et al.,* 2000; Quillot *et al.,* 2005).

Decrease in erythrocyte GPX1 activity we have been also observed in pancreatic cancer and in patients with chronic pancreatitis compared to healthy controls (Kodydkova *et al.*, 2013). Similarly to our findings, Girish *et al*. found lower erythrocyte GPX1 activity in patients with tropical and alcoholic chronic pancreatitis as compared with healthy controls (Girish *et al.*, 2011). In the contrast to our study, Quillot *et al.* showed that erythrocyte GPX1 activity did not differ significantly between patients with CP and healthy controls (Quillot *et al.*, 2000; Ouillot *et al.*, 2005). Published results in serum and plasma GPX activities are inconsistent. Szuster-Ciesielska *et al.* and Van Gossum *et al.* found significantly decreased GPX concentration in patients with chronic pancreatitis in serum and plasma (Van Gossum et al., 1996; Szuster-Ciesielska *et al.*, 2001 a, b), while in other studies observed no significant difference (Mathew *et al.*, 1996; Quillot *et al.*, 2000; Ouillot *et al.*, 2005). Lowered GPX1 activity may be explained by the depletion in selenium levels in both groups and/or decreased concentrations of GSH found in PC patients.

Depression, sepsis, acute and chronic pancreatitis are inflammatory disorders. It is known that inflammatory response leads to the increased production of RONS and induction of OS. Insufficient protection against RONS due to lowered GPX1 activity may lead to oxidative damage of membrane lipids (lipid peroxidation) and DNA. Depletion in GPX1 activity in aforementioned diseases may be explained by decreased GSH levels in these pathophysiologic states.

5.2 Glutathione reductase

Glutathione reductase is the enzyme responsible for reduction of GSSG to the GSH. It has been shown that GR is up-regulated in response to OS (Schuliga *et al.*, 2002; Gawryluk *et al.*, 2011). We have found increased GR erythrocyte activities in depressive women than in healthy controls (Kodydkova *et al.*, 2009*)*. Similarly to our findings, Bilici *et al.* also found increased plasma GR activities in MDD with melancholia compared to controls (Bilici *et al.*, 2001). They suggested that major depression is associated with elevated antioxidant enzymes activities (Bilici *et al*., 2001). Andrezza *et al*. observed significant increase in the late stage of bipolar disorder in comparison with controls (Andrezza *et al.*, 2009). Gibson *et al.* assayed GR protein expression in cultured fibroblasts under glucose conditions in patients with MDD and showed increased GR protein expression in MDD patients group than in controls (Gibson *et al.*, 2012).

In patients with metabolic syndrome we have found elevated GR activities in comparison with healthy controls (Vávrová *et al.*, 2013). On the other hand, Cardona *et al.* observed a significant depletion in GR activity in patients with hypertriglyceridemia with and without MetS compared to control subjects and also in other study in patients with MetS compared to controls (Cardona *et al.*, 2008 a, b). Increased activity of GR could be attributed to a compensatory protective mechanism of the cells against RONS.

We have measured GR activities in septic patients, critically ill patients and healthy controls. In the course of sepsis there were no significant difference in all samplings of septic patients in comparison with healthy controls, but at the onset there is a trend to increased GR activities in septic patients in comparison with healthy controls. On the other hand, there was an increase in GR activity in comparison with critically ill and healthy controls. GR activities did not differ between critically ill and controls. There are no studies on GR activity in septic patients, whereas it has been shown a higher activity of GR in liver of septic rats compared to pair-fed rats (Malmezat *et al.*, 2000). This is in accordance with the results of Hunter and Grimble who also observed that rats treated with tumor necrosis factor alpha had increased GR activity (Hunter and Grimble, 1997). This response of liver to an inflammatory challenge may lead to the maintenance of a high GSH/GSSG ratio.

In patients with acute pancreatitis we have not found significantly different activities of GR in the course of AP compared with healthy controls (Vávrová *et al.*, 2012). Our findings are in accordance with only once study of Czeczot *et al.* where activity of GR did not differ in patients with acute pancreatitis and controls (Czeczot, 2009).

We have shown decreased erythrocyte GR activity in patients with pancreatic cancer and chronic pancreatitis in comparison with healthy controls (Kodydkova *et al.*, 2013). To our knowledge there are no studies focused on analysis of GR activity in patients with pancreatic cancer. Decrease in GR activity may lead to a reduction in a GSH content, which we have found in patients with pancreatic cancer.

Increase in GR activities could be a compensatory mechanism to reduce further oxidative damage and progression of illness during oxidative stress.

5.3 Reduced glutathione

Reduced glutathione represents a major intracellular defence system against oxidative stress. It is one of the most important intracellular redox regulators in the body protecting brain against oxidative damage by free radicals (Samuelsson *et al.*, 2012). It recycles inactive vitamin C and E to their active form and may act as antioxidants. Reduced glutathione is the major scavenger of ROS in the brain. We have described disturbed GSH metabolism in depressive patients (Kodydkova *et al.*, 2009). We have shown decreased levels of reduced glutathione in patients with depressive disorder compared to controls. In the study of Samuelsson *et al.*, they did not found differences in total GSH in plasma or blood GSH levels of depressive patients before and after electronvulsive therapy (Samuelsson *et al.*, 2012). Gawryluk *et al.* examined post-mortem brain tissues of patients with bipolar disorder, MDD, schizophrenia and compared with non-psychiatric, non-neurological control group. Supporting our findings GSH levels were also reduced in psychiatric illness in this study. Decreased levels of GSH have been measured in animal models with stress induced depression (Pal *et al.*, 1994; Eren *et al.*, 2007b).

We have found significant depletion of erythrocyte GSH levels in patients with MetS (Vávrová *et al.*, 2013). Decreased concentrations of GSH with opposite changes in GSSG levels were also found in MetS subjects in the study of Cardona *et al.*, where patients with hypertriglyceridemia with or without MetS had lower GSH levels than control group (Cardona *et al.*, 2008 a, b). Furthermore, in other study also observed decreased levels of GSH in subjects with different cardiovascular risk factors such as hypertension (HT) with and without MetS, familial hypercholesterolemia (FH) and familial combined hyperlipidemia (FCH), where patients with HT had the lowest GSH concentration among FH, FCH and control groups (Mansego *et al.*, 2011). Furthermore, our expected increase in the GSSG/GSH ratio due to lower levels of GSH may stimulate compensatory increase in GR activity to reduce increased levels of GSSG in GSH.

The erythrocyte GSH concentration of patients with AP was increased during the first 3 days of hospitalization compared to other days and controls and the most pronounced was 3rd day. Other samplings of AP patients did not differ significantly from the values found in erythrocytes of control subjects (Vávrová *et al.*, 2012). In the study of Bansal *et al.* measured levels of antioxidants in patients with severe acute pancreatitis that were randomly assigned to antioxidant treatment group (received vitamin C, E and A) or to

a control group. Levels of reduced glutathione did not significantly differ at baseline in both the groups, increase in GSH levels were observed after 7 days in both the groups, but these changes from baseline were not stastically significant (Bansal *et al.*, 2011). In the contrast to our study, significantly reduced GSH levels have been found in patients with mild and severe form of acute pancreatitis compared with healthy controls (Rahman *et al*, 2004; Rahman *et al.*, 2009). Also in serum has been observed decreased GSH levels in AP patients in comparison with control subjects (Czeczot *et al*, 2009).

It is known that deficiency of glutathione may lead to progression of many pathologic states (Balendiran *et al.*, 2004). We have observed in patients with pancreatic cancer decreased levels of GSH compared to chronic pancreatitis and healthy controls, whereas we have found no significant difference in patients with CP compared to controls (Kodydkova *et al.*, 2013). In the contrast to our findings, Girish *et al.* found decreased concentration of GSH in patients with tropical and alcoholic chronic pancreatitis in comparison with controls; moreover patients with alcoholic form of CP had lower GSH levels than patients with tropical form (Girish *et al.*, 2010, 2011). Similarly, Czeczot *et al.* observed lower GSH concentration in CP patients than in control group (Van Gossum *et al*,. 1996; Czeczot *et al.*, 2009). To our knowledge, there are no studies on concentration of reduced glutathione and pancreatic cancer.

Increased oxidation could be explained by the impaired GSH function and weakened GSH redox efficiency. Increase in GSH levels could be a part of an adaptive response to elevated oxidative stress.

6. Conclusion

This doctoral thesis is dealing with activities of antioxidant enzymes - glutathione peroxidase, glutathione reductase and non-enzymatic antioxidant reduced glutathione in various pathophysiologic states. These parameters were measured in patients with depressive disorder, metabolic syndrome, sepsis, acute and chronic pancreatitis and in pancreatic cancer.

- In women with depressive disorder were observed decreased erythrocyte activities of GPX1 and levels of GSH, while activities of GR were elevated in comparison with healthy controls.
- Patients with metabolic syndrome had increased activities of GR, but decreased concentrations of GSH in erythrocytes compared to healthy controls. We have found no significant difference in erythrocyte activities of GPX1 between patients with metabolic syndrome and controls.
- The decrease in GPX1 activity has been found in septic patients in the course of sepsis (persisted in all three samplings) in comparison with controls. In the contrast, no significant difference in GR activity in the course of sepsis has been observed between individual S samplings and controls. The decrease in activities of GPX1 has been also found among septic patients, critically ill non-septic patients and control subjects. In the contrast, GR activity was increased in sepsis compared to critically ill patients and CON. No significant changes in activities of GPX1 and GR were found between critically ill patients and healthy controls.
- Patients with chronic pancreatitis and pancreatic cancer had lower GPX1 activities than did controls. Similarly, activities of GR were decreased in pancreatic cancer and chronic pancreatitis in comparison with controls. No significant differences have been found between pancreatic cancer and chronic pancreatitis. Moreover, patients with pancreatic cancer had lower GSH concentration than those with chronic pancreatitis and controls. No significant differences were found in GSH concentration between patients with chronic pancreatitis and healthy controls.
- In the course of acute pancreatitis activities of GPX1 and GR did not differ among individual samplings. However, patients with acute pancreatitis had decrease in GPX1 activity in all individual samplings in comparison with healthy controls. Furthermore, no difference in GR activities among individual AP samplings and

controls. Decreased levels of GSH were found in patient with acute pancreatits at baseline in comparison with AP3 sampling where the concentration of GSH was the highest

In conclusion, our findings indicate that the cumulative effect of continuous oxidative stress results in the imbalance of oxidant/antioxidant system. Increased oxidative stress leads to decrease and exhaustion of antioxidant defence system. These findings show that oxidative stress may have pathophysiologic role in aforementioned diseases.

7. References

Ahotupa M, Ruutu M, Mantyla E. Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. Clin Biochem.1996; 29: 139-44.

Alberti KG, Zimmet P, Shaw J. The metabolic syndrome-a new worldwide definition. Lancet . 2005;366:1059-1062.

American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (4th edition)Washington, D.C (1994)

Andreazza AC, Kapczinski F, Kauer-Sant'Anna M, Walz JC, Bond DJ, Gonçalves CA, Young LT, Yatham LN. 3-Nitrotyrosine and glutathione antioxidant system in patients in the early and late stages of bipolar disorder. J Psychiatry Neurosci. 2009;34(4):263-71.

Anderson ME. Glutathione: an overview of biosynthesis and modulation. Chem Biol Interact. 1998;111-112:1-14.

Armstrong JS, Jones DP. Glutathione depletion enforces the mitochondrial permeability transition and causes cell death in Bcl-2 overexpressing HL60 cells. FASEB J. 2002;16(10):1263-5.

Arthur JR. The glutathione peroxidases. Cell Mol Life Sci. 2000;57(13-14):1825-35.

Awasthi YC, Beutler E, Srivastava SK. Purification and properties of human erythrocyte glutathione peroxidase. J Biol Chem. 1975;250(13):5144-9.

Baek SH, Min JN, Park EM, Han MY, Lee YS, Lee YJ, Park YM. Role of small heat shock protein HSP25 in radioresistance and glutathione-redox cycle. J Cell Physiol. 2000;183(1):100-7.

Ballatori N, Krance SM, Marchan R, Hammond CL. Plasma membrane glutathione transporters and their roles in cell physiology and pathophysiology. Mol Aspects Med. 2009;30(1-2):13-28.

Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem. 2009 Mar;390(3):191-214.

Balendiran GK, Dabur R, Fraser D. The role of glutathione in cancer. Cell Biochem Funct. 2004;22(6):343-52.

Bansal D, Bhalla A, Bhasin DK, Pandhi P, Sharma N, Rana S, Malhotra S. Safety and efficacy of vitamin-based antioxidant therapy in patients with severe acute pancreatitis: a randomized controlled trial. Saudi J Gastroenterol. 2011;17(3):174-9.

Bashir A, Perham RN, Scrutton NS, Berry A. Altering kinetic mechanism and enzyme stability by mutagenesis of the dimer interface of glutathione reductase. Biochem J. 1995;312 (Pt 2):527-33.

Batra S, Kumar R, Kapoor AK, Ray G. Alterations in antioxidant status during neonatal sepsis. Ann Trop Paediatr. 2000;20(1):27-33.

Ben Othmen L, Mechri A, Fendri C, Bost M, Chazot G, et al.. Altered antioxidant defense system in clinically stable patients with schizophrenia and their unaffected siblings. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32(1):155-9.

Bergendi L, Benes L, Duracková Z, Ferencik M. Chemistry, physiology and pathology of free radicals. Life Sci. 1999;65(18-19):1865-74.

Berger SJ, Gosky D, Zborowska E, Willson JK, et al. Sensitive enzymatic cycling assay for glutathione: measurements of glutathione content and its modulation by buthionine sulfoximine in vivo and in vitro in human colon cancer. Cancer Res. 1994;54(15):4077-83.

Berkholz DS, Faber HR, Savvides SN, Karplus PA. Catalytic cycle of human glutathione reductase near 1 A resolution. J Mol Biol. 2008;382(2):371-84.

Bermano G, Arthur JR, Hesketh JE. Selective control of cytosolic glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase mRNA stability by selenium supply. FEBS Lett. 1996;387(2-3):157-60.

Beutler E. Effect of flavin compounds on glutathione reductase activity: In vivo and in vitro studies. J Clin Invest. 1969;48:1957-1966.

Bilici M, Efe H, Köroğlu MA, Uydu HA, Bekaroğlu M, Değer O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. J Affect Disord. 2001;64(1):43-51.

Bindoli A, Fukuto JM, Forman HJ. Thiol chemistry in peroxidase catalysis and redox signaling. Antioxid Redox Signal. 2008;10(9):1549-64.

Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The

ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992; 101:1644-1655.

Bonizzi G, Piette J, Schoonbroodt S, Greimers R, Havard L, et al. Reactive oxygen intermediate-dependent NF-kappaB activation by interleukin-1beta requires 5-lipoxygenase or NADPH oxidase activity. Mol Cell Biol. 1999;19(3):1950-60.

Borrás C, Gambini J, Gómez-Cabrera MC, et al. 17beta-oestradiol up-regulates longevity-related, antioxidant enzyme expression via the ERK1 and ERK2 [MAPK]/NFkappaB cascade. Aging Cell. 2005; 4(3):113-8.

Bougoulia M, Triantos A, Kolioakos G. Plasma Interleukin-6 levels, glutathione peroxidase and isoprostane in obese women before and after weight loss. Association with cardiovascular risk factors. Hormones. 2006;5:192-199.

Brigelius-Flohé R, Aumann KD, Blöcker H, Gross G, Kiess M, Klöppel KD, Maiorino M, Roveri A, Schuckelt R, Usani F, et al. Phospholipid-hydroperoxide glutathione peroxidase. Genomic DNA, cDNA, and deduced amino acid sequence. J Biol Chem. 1994;269(10):7342-8.

Brigelius-Flohé R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med. 1999; 27(9-10):951-65.

Brigelius-Flohé R, Flohé L. Is there a role of glutathione peroxidases in signaling and differentiation? Biofactors. 2003; 17(1-4):93-102.

Brigelius-Flohé R, Kipp A. Glutathione peroxidases in different stages of carcinogenesis. Biochim Biophys Acta. 2009;1790(11):1555-68.

Brenneisen P, Steinbrenner H, Sies H. Selenium, oxidative stress, and health aspects. Mol Aspects Med. 2005;26(4-5):256-67.

Broncel M, Kozirog M, Duchnowicz P, Koter-Michalak M, Sikora J, Chojnowska-Jezierska J. Aronia melanocarpa extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome. Med Sci Monit. 2010;16(1):CR28-34.

Burk RF, Hill KE. Comprehensive Toxicology. 2nd edition. Oxford University Press, Elsevier. Uuburn, USA. 2010. ISBN: 978-0-08-046884-6.

Cardona F, Túnez I, Tasset I, Montilla P, Collantes E, et al. Fat overload aggravates oxidative stress in patients with the metabolic syndrome. Eur J Clin Invest. 2008 a;38:510-515.

Cardona F, Tunez I, Tasset I, Murri M, Tinahones FJ. Similar increase in oxidative stress after fat overload in persons with baseline hypertriglyceridaemiawith or without the metabolic syndrome. Clin Biochem. 2008 b;41:701–705.

Celli A, Que FG, Gores GJ, LaRusso NF. Glutathione depletion is associated with decreased Bcl-2 expression and increased apoptosis in cholangiocytes. Am J Physiol. 1998;275(4 Pt 1):G749-57.

Cohen HJ, Chovaniec ME, Mistretta D, Baker SS. Selenium repletion and glutathione peroxidase--differential effects on plasma and red blood cell enzyme activity. Am J Clin Nutr. 1985;41(4):735-47.

Cotgreave IA, Gerdes RG. Recent trends in glutathione biochemistry--glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? Biochem Biophys Res Commun. 1998;242(1):1-9.

Crimi E, Sica V, Williams-Ignarro S, Zhang H, Slutsky AS, et al. The role of oxidative stress in adult critical care. Free Radic Biol Med. 2006a;40(3):398-406.

Crimi E, Sica V, Slutsky AS, Zhang H, Williams-Ignarro S, et al. Role of oxidative stress in experimental sepsis and multisystem organ dysfunction. Free Radic Res. 2006b;40(7):665-72.

Cullen JJ, Mitros FA, Oberley LW. Expression of antioxidant enzymes in diseases of the human pancreas: another link between chronic pancreatitis and pancreatic cancer. Pancreas. 2003;26(1):23-7.

Curran FJ, Sattar N, Talwar D, Baxter JN, Imrie CW. Relationship of carotenoid and vitamins A and E with the acute inflammatory response in acute pancreatitis. Br J Surg. 2000;87(3):301-5.

Czeczot H, Majewska M, Skrzycki M, Podsiad M. Activity of GSH-dependent enzymes in blood serum of patients with acute and chronic pancreatitis. Wiad Lek. 2009;62(2):87-92.

Dalle-Donne I, Rossi R, Giustarini D, Colombo R, Milzani A. S-glutathionylation in protein redox regulation. Free Radic Biol Med. 2007;43(6):883-98.

Dalle-Donne I, Milzani A, Gagliano N, Colombo R, et al. Molecular mechanisms and potential clinical significance of S-glutathionylation. Antioxid Redox Signal. 2008;10(3):445-73.

Dalle-Donne I, Rossi R, Colombo G, Giustarini D, et al. Protein S-glutathionylation: a regulatory device from bacteria to humans. Trends Biochem Sci. 2009;34(2):85-96.

Dear TN, Campbell K, Rabbitts TH. Molecular cloning of putative odorant-binding and odorant-metabolizing proteins. Biochemistry. 1991; 30(43):10376-82.

Deneke SM, Fanburg BL. Regulation of cellular glutathione. Am J Physiol. 1989;257(4 Pt 1):L163-73.

Dickinson DA, Forman HJ. Cellular glutathione and thiols metabolism. Biochem Pharmacol. 2002;64(5-6):1019-26.

Dimitrijevic-Sreckovic V, Colak E, Djordjevic P, Gostiljac D, Sreckovic B, et al. Prothrombogenic factors and reduced antioxidative defense in children and adolescents with pre-metabolic and metabolic syndrome. Clin Chem Lab Med. 2007;45:1140-1144.

Drevet JR. The antioxidant glutathione peroxidase family and spermatozoa: a complex story. Mol Cell Endocrinol. 2006;250(1-2):70-9.

Driscoll DM, Copeland PR. Mechanism and regulation of selenoprotein synthesis. Annu Rev Nutr. 2003;23: 17-40.

Dym O, Eisenberg D. Sequence-structure analysis of FAD-containing proteins. Protein Sci. 2001;10(9):1712-28.

Epp O, Ladenstein R, Wendel A. The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution. Eur J Biochem. 1983;133(1):51-69.

Erdeve O, Siklar Z, Kocaturk PA, Dallar Y, Kavas GO. Antioxidant superoxide dismutase activity in obese children. Biol Trace Elem Res. 2004;98(3):219-28.

Eren I, Naziroğlu M, Demirdaş A, Celik O, Uğuz AC, et al. Venlafaxine modulates depression-induced oxidative stress in brain and medulla of rat. Neurochem Res. 2007a;32(3):497-505.

Eren I, Naziroğlu M, Demirdaş A. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. Neurochem Res. 2007 b;32(7):1188-95.

Estrela JM, Ortega A, Obrador E. Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci. 2006;43(2):143-81.

Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872-9.

Ferro FE, de Sousa Lima VB, Soares NR, de Sousa Almondes KG, Pires LV, et al. Parameters of metabolic syndrome and its relationship with zincemia and activities of superoxide dismutase and glutathione peroxidase in obese women. Biol Trace Elem Res. 2011;143(2):787-93.

Filomeni G, Rotilio G, Ciriolo MR. Cell signalling and the glutathione redox system. Biochem Pharmacol. 2002;64(5-6):1057-64.

Fisher AB, Dodia C, Manevich Y, Chen JW, Feinstein SI. Phospholipid hydroperoxides are substrates for non-selenium glutathione peroxidase. J Biol Chem. 1999 ;274(30):21326-34.

Flohé L. Glutathione peroxidase: enzymology and biological aspects. Klin Wochenschr. 1971;49(12):669-83.

Flohe L, Günzler WA, Schock HH. Glutathione peroxidase: a selenoenzyme. FEBS Lett. 1973; 32(1):132-4.

Flohé L. Glutathione peroxidase. Basic Life Sci. 1988;49:663-8.

Flohé L, Budde H, Hofmann B. Peroxiredoxins in antioxidant defense and redox regulation. Biofactors. 2003;19(1-2):3-10.

Forceville X, Vitoux D, Gauzit R, Combes A, Lahilaire P, et al. Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients. Crit Care Med. 1998; 26(9):1536-44.

Forceville X, Mostert V, Pierantoni A, Vitoux D, Le Toumelin P, et al. Selenoprotein P, rather than glutathione peroxidase, as a potential marker of septic shock and related syndromes. Eur Surg Res. 2009;43(4):338-47.

Forstrom JW, Zakowski JJ, Tappel AL. Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry. 1978;17(13):2639-44.

Franco R, Cidlowski JA. SLCO/OATP-like transport of glutathione in FasL-induced apoptosis: glutathione efflux is coupled to an organic anion exchange and is necessary for the progression of the execution phase of apoptosis. J Biol Chem. 2006;281(40):29542-57.

Gaetani GF, Galiano S, Canepa L, Ferraris AM, Kirkman HN. Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood. 1989;73(1):334-9.

Gałecki P, Szemraj J, Bieńkiewicz M, Florkowski A, Gałecka E. Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. Pharmacol Rep. 2009;61(3):436-47.

Ganther HE, Hafeman DG, Lawrence RA, Serfass RE, Hoekstra WG. In Trace Elements in Human Health and Disease. Prasad AS, Ed. Academic Press, Inc. New York, 1976; Vol.II, pp.165-218.

Garcia-Ruiz C, Fernández-Checa JC. Redox regulation of hepatocyte apoptosis. J Gastroenterol Hepatol. 2007;Suppl 1:S38-42.

Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. Int J Neuropsychopharmacol. 2011;14(1):123-30.

Georgiou G, Masip L. Biochemistry. An overoxidation journey with a return ticket. Science. 2003;300(5619):592-4.

Ghibelli L, Fanelli C, Rotilio G, Lafavia E, Coppola S, et al. Rescue of cells from apoptosis by inhibition of active GSH extrusion. FASEB J. 1998;12(6):479-86.

Ghyselinck NB, Jimenez C, Dufaure JP. Sequence homology of androgen-regulated epididymal proteins with glutathione peroxidase in mice. J Reprod Fertil. 1991; 93(2):461-6.

Ghyselinck NB, Dufaure I, Lareyre JJ, Rigaudière N, Mattéi MG, Dufaure JP. Structural organization and regulation of the gene for the androgen-dependent glutathione peroxidase-like protein specific to the mouse epididymis. Mol Endocrinol. 1993;7(2):258-72.

Gibson SA, Korade Ž, Shelton RC. Oxidative stress and glutathione response in tissue cultures from persons with major depression. J Psychiatr Res. 2012;46(10):1326-32.

Girish BN, Vaidyanathan K, Rao NA, Rajesh G, Reshmi S, et al. Chronic pancreatitis is associated with hyperhomocysteinemia and derangements in transsulfuration and transmethylation pathways. Pancreas. 2010;39(1):e11-6.

Girish BN, Rajesh G, Vaidyanathan K, Balakrishnan V. Assessment of oxidative status in chronic pancreatitis and its relation with zinc status. Indian J Gastroenterol. 2011;30(2):84-8.

Giustarini D, Milzani A, Dalle-Donne I, Rossi R. Red blood cells as a physiological source of glutathione for extracellular fluids. Blood Cells Mol Dis. 2008;40(2):174-9.

Goetz ME, Luch A. Reactive species: a cell damaging rout assisting to chemical carcinogens. Cancer Lett. 2008;266(1):73-83.

Goldberg DM, SRJ. Assay of glutathione reductase. In Bergameyer Hu, Bergmeyer J, Grassi M, editors. In Methods of Enzymatic Analysis. Verlag Chemie: Weimheim Chemie: Wenheim; 1983: 258-64.

Greene FL, Page DL, Fleming ID, Fritz CM, Balch DG, et al (eds). AJCC Cancer Staging Manual 6 th edition. Ann Oncol 2003; 14 (2): 345-346.

Griffith OW. Glutathione and glutathione disulphide. In Bergameyer HU, editor. Methods of Enzymatic analysis. Verlag Chemie: Weinheim; 1985: 521-9.

Grossmann A, Wendel A. Non-reactivity of the selenoenzyme glutathione peroxidase with enzymatically hydroperoxidized phospholipids. Eur J Biochem. 1983; 135(3):549-52.

Gutteridge JM, Mitchell J. Redox imbalance in the critically ill. Br Med Bull. 1999;55(1):49-75.

Halliwell B. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. Br J Exp Pathol. 1989;70(6):737-57.

Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 4th edition. Oxford University Press. Oxford.2007. ISBN13: 978-0-19-856869-8, ISBN10: 0-19-856869-X.

Hamilos DL, Zelarney P, Mascali JJ. Lymphocyte proliferation in glutathione-depleted lymphocytes: direct relationship between glutathione availability and the proliferative response. Immunopharmacology. 1989;18(3):223-35.

Hammond CL, Marchan R, Krance SM, Ballatori N. Glutathione export during apoptosis requires functional multidrug resistance-associated proteins. J Biol Chem. 2007;282(19):14337-47.

Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. J Hepatol. 2001;34(6):946-54.

Harington CR, Mead TH. Synthesis of glutathione. Biochem.J. 1935;29:1602–1611.

Hawker FH, Stewart PM, Snitch PJ. Effects of acute illness on selenium homeostasis. Crit Care Med. 1990;18:442–446.

Hawkes WC, Alkan Z. Regulation of redox signaling by selenoproteins. Biol Trace Elem Res. 2010;134(3):235-51.

Hentze H, Schmitz I, Latta M, Krueger A, Krammer PH,et al. Glutathione dependence of caspase-8 activation at the death-inducing signaling complex. J Biol Chem. 2002;277(7):5588-95.

Herken H, Uz E, Ozyurt H, Söğüt S, Virit O, et al. Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. Mol Psychiatry. 2001;6(1):66-73.

Holmgren A. Regulation of ribonucleotide reductase. Curr Top Cell Regul. 1981;19:47-76.

Hunter EA, Grimble RF. Dietary sulphur amino acid adequacy influences glutathione synthesis and glutathione-dependent enzymes during the inflammatory response to endotoxin and tumor necrosis factor-alpha in rats. Clin Sci (Lond). 1997;92(3):297-305.

Chen SJ, Yen CH, Huang YC, Lee BJ, Hsia S, Lin PT. Relationships between Inflammation, Adiponectin, and Oxidative Stress in Metabolic Syndrome. PLoS One. 2012;7(9):e45693.

Cheng WH, Ho YS, Valentine BA, et al. Cellular glutathione peroxidase is the mediator of body selenium to protect against paraquat lethality in transgenic mice. J Nutr. 1998;128(7):1070-6.

Chiba T, Takahashi S, Sato N, Ishii S, Kikuchi K. Fas-mediated apoptosis is modulated by intracellular glutathione in human T cells. Eur J Immunol. 1996;26(5):1164-9.

Chu FF, Doroshow JH, Esworthy RS. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. J Biol Chem. 1993;268(4):2571-6.

Chung SW, Toriba A, Chung HY, Yu BP, Kameda T, et al. Activation of 5-lipoxygenase and NF-kappa B in the action of acenaphthenequinone by modulation of oxidative stress. Toxicol Sci. 2008;101(1):152-8.

Janes W, Schulz GE. Role of the charged groups of glutathione disulfide in the catalysis of glutathione reductase: crystallographic and kinetic studies with synthetic analogues. Biochemistry. 1990;29(16):4022-30.

Jatana M, Giri S, Ansari MA, Elango C, Singh AK, Singh I, Khan M. Inhibition of NF-kappaB activation by 5-lipoxygenase inhibitors protects brain against injury in a rat model of focal cerebral ischemia. J Neuroinflammation. 2006;3:1-13.

Jefferies H, Bot J, Coster J, Khalil A, Hall JC, McCauley RD. The role of glutathione in intestinal dysfunction. J Invest Surg. 2003;16(6):315-23.

Jones DP, Eklöw L, Thor H, et al. Metabolism of hydrogen peroxide in isolated hepatocytes: relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H2O2. Arch Biochem Biophys. 1981;210(2):505-16.

Kamerbeek NM, van Zwieten R, de Boer M, Morren G, Vuil H, Bannink N, Lincke C, Dolman KM, Becker K, Schirmer RH, Gromer S, Roos D. Molecular basis of glutathione reductase deficiency in human blood cells. Blood. 2007;109(8):3560-6.

Kapoor K, Basu S, Das BK, Bhatia BD. Lipid peroxidation and antioxidants in neonatal septicemia. J Trop Pediatr. 2006;52(5):372-5.

Karplus PA, Schulz GE. Refined structure of glutathione reductase at 1.54 A resolution. J Mol Biol. 1987;195(3):701-29.

Klotz LO, Sies H. Defenses against peroxynitrite: selenocompounds and flavonoids. Toxicol Lett. 2003;140-141:125-32.

Kodydková J, Vávrová L, Staňková B, Macášek J, Krechler T, Žák A. Changes in antioxidants and oxidative stress markers in pancreatic diseases. Pancreas. 2013;42(4):614-621.

Kodydková J, Vávrová L, Zeman M, Jirák R, Macásek J, et al. Antioxidative enzymes and increased oxidative stress in depressive women. Clin Biochem. 2009;42(13-14):1368-74.

Kowalczyk E, Kowalski J, Błaszczyk J, Gwoździński L, Ciećwierz J, et al. Estimation of cell membrane properties and erythrocyte red-ox balance in patients with metabolic syndrome. Mol Biol Rep. 2012;39(12):11113-8.

Koziróg M, Poliwczak AR, Duchnowicz P, Koter-Michalak M, Sikora J, et al. Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. J Pineal Res. 2011;50(3):261-6.

Krauth-Siegel RL, Blatterspiel R, Saleh M, Schiltz E,et al. Glutathione reductase from human erythrocytes. The sequences of the NADPH domain and of the interface domain. Eur J Biochem. 1982;121(2):259-67.

Krauth-Siegel RL, Arscott LD, Schönleben-Janas A, Schirmer RH, Role of active site tyrosine residues in catalysis by human glutathione reductase. Biochemistry. 1998;37(40):13968-77.

Kretz-Remy C, Mehlen P, Mirault ME, Arrigo AP. Inhibition of I kappa B-alpha phosphorylation and degradation and subsequent NF-kappa B activation by glutathione peroxidase overexpression. J Cell Biol. 1996;133(5):1083-93.

Kretz-Remy C, Arrigo AP. Selenium: a key element that controls NF-kappa B activation and I kappa B alpha half-life. Biofactors. 2001;14(1-4):117-25.

Kulmacz RJ. Regulation of cyclooxygenase catalysis by hydroperoxides. Biochem Biophys Res Commun. 2005;338(1):25-33.

Kuloglu M, Ustundag B, Atmaca M, Canatan H, Tezcan AE, Cinkilinc N. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. Cell Biochem Funct. 2002;20(2):171-5.

Leff JA, Parsons PE, Day CE, Taniguchi N, Jochum M, et al. Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis. Lancet. 1993;341(8848):777-80.

Lenton KJ, Therriault H, Fülöp T, Payette H, et al. Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. Carcinogenesis. 1999;20(4):607-13.

Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neurosci Biobehav Rev. 2012 ;36(2):764-85.

Li S, Yan T, Yang JQ, Oberley TD, Oberley LW. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. Cancer Res. 2000;60(14):3927-39.

Li HC, Chen QZ, Ma Y, Zhou JF. Imbalanced free radicals and antioxidant defense systems in schizophrenia: a comparative study. J Zhejiang Univ Sci B. 2006;7(12):981-6.

Little C, O'Brien PJ. An intracellular GSH-peroxidase with a lipid peroxide substrate. Biochem Biophys Res Commun. 1968;31(2):145-50.

Lu SC. Regulation of glutathione synthesis. Curr Top Cell Regul. 2000;36:95-116.

Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J. 1999;13(10):1169-83.

Lu SC. Regulation of glutathione synthesis. Curr Top Cell Regul. 2000;36:95-116.

Lu J, Holmgren A. Selenoproteins. J Biol Chem. 2009; 284(2):723-7.

Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2011; 15(7):1957-97.

Macásek J, Zeman M, Vecka M, Vávrová L, Kodydková J, Tvrzická E, Zák A. Reactive oxygen and nitrogen species in the clinical medicine. Cas Lek Cesk 2011; 150(8): 423-32.

Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog Neuropsychopharmacol Biol Psychiatry. 2011a;35(3):676-92.

Maes M, Mihaylova I, Kubera M, Uytterhoeven M, Vrydags N, Bosmans E. Lower whole blood glutathione peroxidase (GPX) activity in depression, but not in myalgic encephalomyelitis / chronic fatigue syndrome: another pathway that may be associated with coronary artery disease and neuroprogression in depression. Neuro Endocrinol Lett. 2011b;32(2):133-40.

Malmezat T, Breuillé D, Capitan P, Mirand PP, Obled C. Glutathione turnover is increased during the acute phase of sepsis in rats. J Nutr. 2000;130(5):1239-46.

Mansego ML, Redon J, Martinez-Hervas S, Real JT, Martinez F, Blesa S, Gonzalez-Albert V, Saez GT, Carmena R, Chaves FJ. Different impacts of cardiovascular risk factors on oxidative stress. Int J Mol Sci. 2011;12(9):6146-63.

Manzanares W, Biestro A, Galusso F,Torre MH, Manay N, Pittini G, FacchinG, Hardy G Serum selenium and glutathione peroxidase-3 activity: biomarkers of systemic inflammation in the critically ill? Intensive Care Med. 2009;35:882–889

Margis R, Dunand C, Teixeira FK et al. Glutathione peroxidase family - an evolutionary overview. FEBS J. 2008; 275(15):3959-70.

Marinho HS, Antunes F, Pinto RE. Role of glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase in the reduction of lysophospholipid hydroperoxides. Free Radic Biol Med. 1997;22(5):871-83.

Matés JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000;153(1-3):83-104.

Mathew P, Wyllie R, Van Lente F, et al. Antioxidants in hereditary pancreatitis. Am J Gastroenterol. 1996; 91: 1558-62.

Meister A, Anderson ME. Glutathione. Annu Rev Biochem.1983;52:711-760.

Meister A. Glutathione metabolism and its selective modification. J Biol Chem. 1988; 263(33):17205-8.

Mills GC. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. Biol Chem. 1957; 229(1):189-97.

Michiels C, Raes M, Toussaint O, et al. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic Biol Med. 1994;17(3):235-48.

Mishra V, Baines M, Wenstone R, Shenkin A. Markers of oxidative damage, antioxidant status and clinical outcome in critically ill patients. Ann Clin Biochem. 2005;42(Pt 4):269-76.

Mishra V, Baines M, Perry SE, McLaughlin PJ, Carson J. Effect of selenium supplementation on biochemical markers and outcome in critically ill patients. Clin Nutr. 2007;26(1):41-50.

Mittl PR, Schulz GE Structure of glutathione reductase from Escherichia coli at 1.86 A resolution: comparison with the enzyme from human erythrocytes. Protein Sci. 1994;3(5):799-809.

Miwa T, Adachi T, Ito Y, Hirano K, Sugiura M. Purification and properties of glutathione peroxidase from human liver. Chem Pharm Bull (Tokyo). 1983; 31(1):179-85.

Modzelewski B. Serum anti-oxidative barrier in acute pancreatitis. Pol Merkur Lekarski. 2005;18(106):418-20.

Morales A, Miranda M, Sanchez-Reyes A, Colell A, Biete A, et al. Transcriptional regulation of the heavy subunit chain of gamma-glutamylcysteine synthetase by ionizing radiation. FEBS Lett. 1998;427(1):15-20.

Morris-Stiff GJ, Bowrey DJ, Oleesky D, Davies M, Clark GW, Puntis MC. The antioxidant profiles of patients with recurrent acute and chronic pancreatitis. Am J Gastroenterol. 1999 Aug;94(8):2135-40.

Musil F, Zadák Z, Solichová D, Hyspler R, Kaska M, Sobotka L, Manák J. Dynamics of antioxidants in patients with acute pancreatitis and in patients operated for colorectal cancer: a clinical study. Nutrition. 2005;21(2):118-24.

Mühl D, Woth G, Drenkovics L, Varga A, Ghosh S, Csontos C, Bogár L, Wéber G, Lantos J. Comparison of oxidative stress & leukocyte activation in patients with severe sepsis & burn injury. Indian J Med Res. 2011;134:69-78.

Niki E, Yamamoto Y, Takahashi M, Komuro E, Miyama Y. Inhibition of oxidation of biomembranes by tocopherol. Ann N Y Acad Sci. 1989;570:23-31.

Nordhoff A, Tziatzios C, van den Broek JA, Schott MK. Denaturation and reactivation of dimeric human glutathione reductase--an assay for folding inhibitors. Eur J Biochem. 1997;245(2):273-82.

Olney JW, Zorumski C, Price MT, Labruyere J. L-cysteine, a bicarbonate-sensitive endogenous excitotoxin. Science. 1990;248(4955):596-9.

Ozcan ME, Gulec M, Ozerol E, Polat R, Akyol O. Antioxidant enzyme activities and oxidative stress in affective disorders. Int Clin Psychopharmacol. 2004;19(2):89-95.

Padmaja S, Squadrito GL, Pryor WA. Inactivation of glutathione peroxidase by peroxynitrite. Arch Biochem Biophys. 1998;349(1):1-6.

Pai EF, Schulz GE. The catalytic mechanism of glutathione reductase as derived from x-ray diffraction analyses of reaction intermediates. J Biol Chem. 1983; 258(3):1752-7.

Pai EF, Karplus PA, Schulz GE. Crystallographic analysis of the binding of NADPH, NADPH fragments, and NADPH analogues to glutathione reductase. Biochemistry. 1988;27(12):4465-74.

Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70: 158–69.

Pal SN, Dandiya PC. Glutathione as a cerebral substrate in depressive behavior. Pharmacol Biochem Behav. 1994;48(4):845-51.

Pan S, Berk BC. Glutathiolation regulates tumor necrosis factor-alpha-induced caspase-3 cleavage and apoptosis: key role for glutaredoxin in the death pathway. Circ Res. 2007;100(2):213-9.

Peng D, Belkhiri A, Hu T, Chaturvedi R, Asim M, et al. Glutathione peroxidase 7 protects against oxidative DNA damage in oesophageal cells. Gut. 2012;61(9):1250-60.

Petrov MS, Windsor JA. Classification of the severity of acute pancreatitis: how many categories make sense? Am J Gastroenterol, 2010;105:74–76.

Prabhakar R, Morokuma K, Musaev DG. Computational modelling for homogeneous and enzymatic catalysis. A knowledge base for designing efficient catalysis. Chapter. Computational insights into the structural properties and catalytic functions of selenoprotein glutathione peroxidase. 2008. 1-25. WYLEI-VCH Verlag GmBH&Co. KGaA, Weinheim. ISBN: 978-3-527-31843-8.

Pizent A, Pavlovic M, Jurasovic J, Dodig S, Pasalic D, Mujagic R. Antioxidants, trace elements and metabolic syndrome in elderly subjects. J Nutr Health Aging. 2010;14(10):866-71.

Poli G, Leonarduzzi G, Biasi F, Chiarpotto E. Oxidative stressand cell signalling, Curr. Med. Chem. 2004;11: 1163–1182.

Prinarakis E, Chantzoura E, Thanos D, Spyrou G. S-glutathionylation of IRF3 regulates IRF3-CBP interaction and activation of the IFN beta pathway. EMBO J. 2008;27(6):865-75.

Quilliot D, Dousset B, Guerci B, et al. Evidence that diabetes mellitus favors impaired metabolism of zinc, copper, and selenium in chronic pancreatitis. Pancreas. 2001; 22: 299-306.

Quillot D, Walters E, Bontze JP, et al. Diabetes mellitus worsens antioxidant status in pateints with chronic pancreatitis. Am J Clin Nutr. 2005; 81: 1117-25.

Rahman SH, Ibrahim K, Larvin M, Kingsnorth A, McMahon MJ. Association of antioxidant enzyme gene polymorphisms and glutathione status with severe acute pancreatitis. Gastroenterology. 2004;126(5):1312-22.

Rahman SH, Srinivasan AR, Nicolaou A. Transsulfuration pathway defects and increased glutathione degradation in severe acute pancreatitis. Dig Dis Sci. 2009;54(3):675-82.

RCSB Protein Data Bank. http://www.rcsb.org. Accessed December 12 2012.

Ren B, Huang W, Akesson B, et al. The crystal structure of seleno-glutathione peroxidase from human plasma at 2.9 A resolution. J Mol Biol. 1997; 268(5):869-85.

Rescigno M, Perham RN. Structure of the NADPH-binding motif of glutathione reductase: efficiency determined by evolution. Biochemistry. 1994;33(19):5721-7.

Rhee SG, Chae HZ, Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic Biol Med. 2005;38(12):1543-52.

Robertson RP, Harmon JS. Pancreatic islet beta-cell and oxidative stress: the importance of glutathione peroxidase. FEBS Lett. 2007; 581(19):3743-8.

Roth E, Manhart N, Wessner B. Assessing the antioxidative status in critically ill patients. Curr Opin Clin Nutr Metab Care. 2004 Mar;7(2):161-8.

Rotruck JT, Pope AL, Ganther HE,et al. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179(4073):588-90.

Roveri A, Maiorino M, Ursini F. Enzymatic and immunological measurements of soluble and membrane-bound phospholipid-hydroperoxide glutathione peroxidase. Methods Enzymol. 1994;233:202-12.

Sakr Y, Reinhart K, Bloos F, Marx G, Russwurm S, et al. Time course and relationship between plasma selenium concentrations, systemic inflammatory response, sepsis, and multiorgan failure. Br J Anaesth. 2007;98(6):775-84.

Samuelsson M, Gerdin G, Ollinger K, Vrethem M. Taurine and glutathione levels in plasma before and after ECT treatment. Psychiatry Res. 2012;198(1):53-7.

Sánchez-Rodríguez MA, Martínez-Cruz M, Correa-Muñoz E, Mendoza-Núñez VM. Relationship between metabolic syndrome components and oxidative stress in elderly community-dwelling Mexicans. Ann Nutr Metab. 2010;56(4):302-7.

Sappey C, Legrand-Poels S, Best-Belpomme M,et al.. Stimulation of glutathione peroxidase activity decreases HIV type 1 activation after oxidative stress. AIDS Res Hum Retroviruses. 1994;10(11):1451-61.

Sarandol A, Sarandol E, Eker SS, Erdinc S, Vatansever E, Kirli S. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. Hum Psychopharmacol. 2007;22(2):67-73.

Sevanian A, Muakkassah-Kelly SF, Montestruque S. The influence of phospholipase A2 and glutathione peroxidase on the elimination of membrane lipid peroxides. Arch Biochem Biophys. 1983;223(2):441-52.

Seo MS, Kang SW, Kim K, Baines IC, Lee TH, Rhee SG. Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate. J Biol Chem. 2000; 275(27):20346-54.

Shaw JP, Chou IN. Elevation of intracellular glutathione content associated with mitogenic stimulation of quiescent fibroblasts. J Cell Physiol. 1986;129(2):193-8.

Schneider A., Löhr JM, Winter MV. The M-ANNHEIM classification of chronic pancreatitis: introduction of a unifying classification system based on a review of previous classification of the disease. J Gastroenterol. 2007; 42: 101-119.

Schönleben-Janas A, Kirsch P, Mittl PR, Schirmer RH. Inhibition of human glutathione reductase by 10-arylisoalloxazines: crystalline, kinetic, and electrochemical studies. J Med Chem. 1996 Mar 29;39(7):1549-54.

Schuliga M, Chouchane S, Snow ET. Upregulation of glutathione-related genes and enzyme activities in cultured human cells by sublethal concentrations of inorganic arsenic. Toxicol Sci. 2002;70(2):183-92.

Sies H, Akerboom TP. Glutathione disulfide (GSSG) efflux from cells and tissues. Methods Enzymol. 1984;105:445-51.

Sies H. Oxidative stress: from basic research to clinical application. Am J Med. 1991;91(3C):31S-38S.

Sies H. Strategies of antioxidant defense. Eur J Biochem. 1993;215(2):213-9.

Sies H, Sharov VS, Klotz LO, Briviba K. Glutathione peroxidase protects against peroxynitrite-mediated oxidations. A new function for selenoproteins as peroxynitrite reductase. J Biol Chem. 1997;272(44):27812-7.

Sies H. Glutathione and its role in cellular functions. Free Rad Biol Med.1999;27:916-921.

Singh S, Khan AR, Gupta AK. Role of glutathione in cancer pathophysiology and therapeutic interventions. J Exp Ther Oncol. 2012;9(4):303-16.

Spolarics Z, Wu JX. Role of glutathione and catalase in H2O2 detoxification in LPS-activated hepatic endothelial and Kupffer cells. Am J Physiol. 1997;273(6 Pt 1):G1304-11.

Srivastava N, Barthwal MK, Dalal PK, Agarwal AK, Nag D, Seth PK, Srimal RC, Dikshit M. A study on nitric oxide, beta-adrenergic receptors and antioxidant status in the polymorphonuclear leukocytes from the patients of depression. J Affect Disord. 2002;72(1):45-52.

Straif D, Werz O, Kellner R, Bahr U, Steinhilber D. Glutathione peroxidase-1 but not -4 is involved in the regulation of cellular 5-lipoxygenase activity in monocytic cells. Biochem J. 2000;349(Pt 2):455-61.

Sunde, RA. Intracellular glutathione peroxidases - structure, regulation and function. In Selenium in Biology and Human Health (Burk, R. F., ed.), 1994: 45-77, Springer-Verlag, New York.

Szuster-Ciesielska A, Daniluk J, Kandefer-Szerszeń M. Oxidative stress in blood of patients with alcohol-related pancreatitis. Pancreas. 2001a;22(3):261-6.

Szuster-Ciesielska A, Daniluk J, Kandefer-Szerszeń M. Alcohol-related cirrhosis with pancreatitis. The role of oxidative stress in the progression of the disease. Arch Immunol Ther Exp (Warsz). 2001b;49(2):139-46.

Sweet WL, Blanchard JS. Human erythrocyte glutathione reductase: chemical mechanism and structure of the transition state for hydride transfer. Biochemistry. 1991;30(35):8702-9.

Takahashi K, Cohen HJ. Selenium-dependent glutathione peroxidase protein and activity: immunological investigations on cellular and plasma enzymes. Blood. 1986; 68(3):640-5.

Takahashi K, Avissar N, Whitin J, Cohen H. Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch Biochem Biophys. 1987 Aug 1;256(2):677-86.

Tandoğan B, Ulusu NN. Kinetic mechanism and molecular properties of glutathione reductase. Fabad J Pharm Sci.2006; 31:230-237.

Toppo S, Flohé L, Ursini F, et al. Catalytic mechanism and specifities of glutathione peroxidase. Variation of basic scheme. Biochim Biophys Acta.2009; 1790:1486-1500.

Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003;57(3-4):145-55.

Untucht-Grau R, Schirmer RH, Schirmer I, Krauth-Siegel RL. Glutathione reductase from human erythrocytes: amino-acid sequence of the structurally known FAD-binding domain. Eur J Biochem. 1981;120(2):407-19.

Utomo A, Jiang X, Furuta S, Yun J, Levin DS, Wang YC, Desai KV, Green JE, Chen PL, Lee WH. Identification of a novel putative non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase (NPGPx) essential for alleviating oxidative stress generated from polyunsaturated fatty acids in breast cancer cells. J Biol Chem. 2004;279(42):43522-9.

Ursini F, Maiorino M, Gregolin C. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim Biophys Acta. 1985;839(1):62-70.

Valenta J, Brodska H, Drabek T, Hendl J, Kazda A. High-dose selenium substitution in sepsis: a prospective randomized clinical trial. Intensive Care Med. 2011;37(5):808-15.

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell. Biochem.2004; 266: 37–56.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160(1):1-40.

Van Gossum A, Closset P, Noel E, Cremer M, Neve J. Deficiency in antioxidant factors in patients with alcohol-related chronic pancreatitis. Dig Dis Sci. 1996;41(6):1225-31.

Vávrová L, Kodydková J, Zeman M, Dušejovská M, Macášek J, Staňková B, Tvrzická E, Žák A. Altered Activities of Antioxidant Enzymes in Patients with Metabolic Syndrome. Obesity Facts. 2013;6(1):39-47.

Vávrová L, Kodydková J, Macášek J, Ulrych J, Žák A. Oxidační stres v průběhu akutní pankreatitidy. Klin Biochem Metab. 2012; 20(41): 188-193.

Voet D, Voetová JG. Biochemie. Victoria Publishing a.s. Praha 1995. ISBN 80-85605-44-9.

Voet D, Voet JG. Biochemistry 4th Eddition. John Wiley & Sons, New Jersey, United States of America. 2011. ISBN 13 978-0470-57095-1 ISBN 13 978-0470-91745-9

Weber SU, Lehmann LE, Schewe JC, Thiele JT, Schröder S. Low serum alpha-tocopherol and selenium are associated with accelerated apoptosis in severe sepsis. Biofactors. 2008;33(2):107-19.

Werz O, Steinhilber D. Development of 5-lipoxygenase inhibitors--lessons from cellular enzyme regulation. Biochem Pharmacol. 2005;70(3):327-33.

Wereszczynska-Siemiatkowska U, Mroczko B, Siemiatkowski A, Szmitkowski M, Borawska M, et.al. The importance of interleukin 18, glutathione peroxidase, and selenium concentration changes in acute pancreatitis. Dig Dis Sci. 2004;49(4):642-50.

Wieland H, Seidel D. A simple specific method for precipitation of low density lipoproteins. J Lipid Res.1983; 24:904-9.

Wingler K, Böcher M, Flohé L, Kollmus H, Brigelius-Flohé R. mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. Eur J Biochem. 1999;259(1-2):149-57.

Wong KK, Vanoni MA, Blanchard JS. Glutathione reductase: solvent equilibrium and kinetic isotope effects. Biochemistry. 1988 Sep 6;27(18):7091-6.

Wood ZA, Schröder E, Robin Harris J, Poole LB. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci. 2003;28(1):32-40.

Wouters MA, Fan SW, Hawort NL. Disulfides as redox switches : from molecular mechanisms to functional significance. Antiox Redox Signal. 2010; 12:53-91.

Worthington DJ, Rosemeyer MA. Glutathione reductase from human erythrocytes. Molecular weight, subunit composition and aggregation properties. Eur J Biochem. 1975; 60(2):459-66.

Worthington DJ, Rosemeyer MA. Glutathione reductase from human erythrocytes. Catalytic properties and aggregation. Eur J Biochem. 1976; 67(1):231-8.

Wu G, Fang YZ, Yang S, Lupton JR. Glutathione metabolism and its implications for health. J Nutr. 2004;134:489-492.

Yan J, Meng X, Wancket LM, Lintner K, et al. Glutathione reductase facilitates host defense by sustaining phagocytic oxidative burst and promoting the development of neutrophil extracellular traps. J Immunol. 2012;188(5):2316-27.

Yapişlar H. Altered erythrocyte deformability and antioxidant status in patients with schizophrenia. Marmara Medical Journal. 2012;25:133-137.

Zachara BA, Gromadzińska J, Wasowicz W, et al. Red blood cell and plasma glutathione peroxidase activities and selenium concentration in patients with chronic kidney disease: a review. Acta Biochim Pol. 2006; 53(4):663-77.

Zhao R, Holmgren A. A novel antioxidant mechanism of ebselen involving ebselen diselenide, a substrate of mammalian thioredoxin and thioredoxin reductase. J Biol Chem. 2002;277(42):39456-62.

List of publications

Publications with impact factor

1. **Kodydková J**, Vávrová L, Staňková B, Macášek J, Krechler T, Žák A. Changes in antioxidants and oxidative stress markers in pancreatic diseases. Pancreas. 2013;42(4):614-621. **IF = 2.386**

2. Vávrová L, **Kodydková J**, Zeman M, Dušejovská M, Macášek J, Staňková B, Tvrzická E, Žák A. Altered Activities of Antioxidant Enzymes in Patients with Metabolic Syndrome. Obesity Facts. 2013;6(1):39-47. **IF = 1.856**

3. Vecka M, Dušejovská M, Staňková B, Zeman M, Vávrová L, **Kodydková J**, Slabý A, Žák A. N-3 polyunsaturated fatty acids in the treatment of atherogenic dyslipidemia. Neuro Endocrinol Lett. 2012;33 (Suppl 2):87-92. **IF=1.296**

4. Vecka M, Jáchymová M, Vávrová L, **Kodydková J**, Macášek J, Urbánek M, Krechler T, Slabý A, Dušková J, Muravská A, Žák A. Paraoxonase-1 (PON1) status in pancreatic cancer: relation to clinical parameters. Folia Biologica. 2012; 58(6):231-7. **IF = 1.151**

5. Novák F, Vávrová L, **Kodydková J**, Novák F Sr, Hynková M, Žák A, Nováková O.: Decreased paraoxonase activity in critically ill patients with sepsis. Clin Exp Med. 2010; 10(1):21-5. **IF = 1.581**

6. **Kodydková J**, Vávrová L, Zeman M, Jirák R, Macášek J, Staňková B, Tvrzická E, Žák A.: Antioxidative enzymes and increased oxidative stress in depressive women. Clin Biochem. 2009; 42(13-14):1368-74. **IF = 2.019**

7. Zeman M, Stopka P, Vecka M, Žák A, Písaříková A, Jirák R, Staňková B, Vávrová L, **Kodydková J**, Krizová J, Macášek J. Electron Spin Resonance Determination of Hydroxyl and Nitroxide Radicals in Depressions and Hyperlipidemia. Chemické listy, 2009; 103(8):667-671. **IF = 0.717**

8. Žák A, Tvrzická E, Vecka M, Jáchymová M, Duffková L, Staňková B, Vávrová L, **Kodydková J**, Zeman M. Severity of metabolic syndrome unfavorably influences oxidative stress and fatty acid metabolism in men. Tohoku J Exp Med. 2007; 212(4):359-71. **IF = 1.133**

Publications without impact factor

1. Vávrová L, **Kodydková J**, Macášek J, Ulrych J, Žák A. Oxidační stres v průběhu akutní pankreatitidy. Klin Biochem Metab. 2012; 20(41): 188-193.
2. Kocík M, Zimovjanová M, Petruželka L, **Kodydková J**, Vávrová L, Žák A. Oxidative stress after anthracycline therapy in patients with solid tumors. Cas Lek Cesk. 2012;151(10):463-7.
3. Macášek J, Zeman M, Vecka M, Vávrová L, **Kodydková J**, Tvrzická E, Žák A. Reaktivní kyslíkové a dusíkové sloučeniny v klinické medicíně. Cas Lek Cesk. 2011; 150:423-432.
4. Novák F, Borovská J, Vecka M, Vávrová L, **Kodydková J**, Mráčková M, Novák F, sr., Nováková O, Žák A. Alterations in fatty acid composition of plasma and erythrocyte lipids in critically ill patients during sepsis. Cas Lek Cesk. 2010; 149(7):324-31. Czech.
5. Zeman M, Jirák R, Žák A, Jáchymová M, Vecka M, Tvrzická E, Vávrová L, **Kodydková J**, Staňková B. Features of metabolic syndrome in patients with depressive disorder. Cas Lek Cesk. 2009; 148(7):309-14. Czech.
6. Žák A, Vecka M, Tvrzická E, Jáchymová M, Dušejovská M, Janíková L, Staňková B, Vávrová L, **Kodydková J**, Zeman M. Composition of the nonesterified fatty acids and lipid peroxidation in metabolic syndrome. Cas Lek Cesk. 2007; 146(5):484-91. Czech.

Publication under review

Vávrová L, **Kodydková J**, Mráčková M, Novák F, sr., Nováková O, Žák A, Novák F. Changes in antioxidant status and inflammatory response markers persist long after clinical recovery from severe sepsis. 2012

Abstracts on the topics of the doctoral thesis

1. **Kodydková J**, Vávrová L, Staňková B, Macášek J, Krechler T, Žák A. Změny aktivit antioxidačních enzymů a markerů oxidačního stresu u pacientů s karcinomem pankreatu a chronickou pankreatitidou. Šobrův den. 6.6. 2012.
2. **Kodydková J**, Vávrová L, Macášek J, Krechler T, Žák A. Antioxidační enzymy a karcinom pankreatu. Cas Lek Cesk. 2012; 151(1):30-38.
3. **Kodydková J**, Vávrová L, Macášek J, Krechler T, Žák A. Antioxidační enzymy a karcinom pankreatu. Sborník Atherosklerosa 2011; 58-61. ISBN: 978-80-254-8809-6.

4. **Kodydková J**, Vávrová L, Tvrzická E, Novák F, Krechler T, Žák A. Změny antioxidačních enzymů za různých patofyziologických stavů. Sborník abstrakt. Cor Vasa 2011; 53 (3): 202.
5. **Kodydková J**, Vávrová L, Krechler T, Žák A. Changes in antioxidant enzymes activities in patients with carcinoma of pancreas. European Journal of Internal Medicine. 2011; 22 (S1): S48-S49. (IF = 1.657)
6. **Kodydková J**, Vávrová L, Kocík M, Žák A. Vliv chemoterapie na aktivity antioxidačních enzymů u žen s karcinomem prsu. Sborník abstrakt XV. Kongres o Ateroskleróze. 2011; 22.
7. Vávrová L, **Kodydková J**, Macášek J, Krechler T, Žák A. Antioxidant enzymes activities in pancretic carcinoma and the influence of diabetes mellitus. European Journal of Internal Medicine, 2011, 22(S1): S98. (IF = 1.657)
8. Vávrová L, **Kodydková J**, Kocík M, Žák A. Změny aktivit antioxidačních enzymů u žen s karcinomem prsu. Sborník abstrakt XV. Kongres o Ateroskleróze. 2011; 34-35.
9. Staňková B, **Kodydková J**, Vávrová L, Macášek J, Krechler T, Žák A. Aktivita antioxidačních enzymů u pacientů s karcinomem pankreatu. Sborník abstrakt XV. Kongres o Ateroskleróze. 2011; 34-35.
10. Vávrová L, **Kodydková J**, Zeman M, Tvrzická E, Žák A. Aktivity antioxidačních enzymů u pacientů s metabolickým syndromem. Sborník abstraktů přednášek – Diagnostika, léčba a prevence závažných civilizačních onemocnění. 2010, ISBN: 978-80-7177-034-3, 58.
11. Novák F, Vávrová L, **Kodydková J**, Nováková O, Novák F, sr., Žák A. Decreased paraoxonase activity and increased lipid peroxidation in critically ill patients with sepsis and systemic inflammation. Intensive Care Medicine. 2010; 36 (Suppl. 2):430.
12. **Kodydková J**, Vávrová L, Krechler T, Tvrzická E, Žák A. Onemocnění pankreatu a změny aktivit antioxidačních enzymů. Sborník abstraktů přednášek – Diagnostika, léčba a prevence závažných civilizačních onemocnění. 2010; 56. ISBN: 978-80-7177-034-3.
13. Trávníčková J, **Kodydková J**, Vávrová L, Zeman M, Tvrzická E, Žák A. Aktivita antioxidačních enzymů u pacientů s metabolickým syndromem**.** Klin Biochem Metab 2010;18(2):119.
14. **Kodydková J**, Vávrová L, Tvrzická E, Zeman M, Krechler T, Žák A. Aktivita antioxidačních enzymů u pacientů s diabetes mellitus a metabolickým syndromem. Vnitř Lék. 2009; 55(12):5-6.

15. Vávrová L, **Kodydková J**, Krechler T., Tvrzická E, Žák A. Aktivita antioxidačních enzymů u pacientů s pankreatopatií. Cas Lek Cesk. 2009; 148(11):569.
16. Vávrová L, **Kodydková J**, Krechler T, Tvrzická E, Žák A. Aktivita antioxidačních enzymů u pacientů s pankreatopatií. Sborník Atherosklerosa 2009; ISBN: 978-80-254-5389-6,86-88.
17. **Kodydková J**, Vávrová L, Zeman M, Tvrzická E, Žák A. Aktivity antioxidačních enzymů u metabolického syndromu. Vnitř Lék. 2008; 54(7&8):791. Šobrův den 19.6.
18. **Kodydková J**, Vávrová L, Zeman M, Tvrzická E, Žák A. Aktivita antioxidačních enzymů u pacientů s metabolickým syndromem. Sborník Atherosklerosa. 2008; 30-33. ISBN: 978-80-254-2834-4.
19. Vávrová L, **Kodydková J**, Zeman M, Tvrzická E, Žák A. Aktivita antioxidačních enzymů u pacientů s depresí. Sborník Atherosklerosa. 2008;78-80. ISBN: 978-80-254-2834-4.
20. Vávrová L, **Kodydková J**, Tvrzická E, Zeman M, Žák A. Metabolický syndrom a některé parametry oxidačního stresu. Vnitř Lék. 2008; 54(12): 1236-1237.
21. Novák F, Kodydková J, Vávrová L, Novák F, sr. Aktivita antioxidačních enzymů u kriticky nemocných pacientů v sepsi. Sborník 9. studentské vědecké konference 21. 5. 2008; 40-41.
22. **Kodydková J**, Vávrová L, Novák F, Novák F, jr., Žák A. Vliv sepse na aktivity glutathionperoxidasy a glutathionreduktasy. Sborník 8. studentská konference 1. LF UK, 2007;43.
23. **Kodydková J**, Vávrová L, Nováková O, Novák F, Novák F, jr. Effect of Severe Sepsis on Erythrocyte Glutathione Peroxidase and Glutathione Reductase Activities. Sborník abstrakt, Second Central & Eastern Europe Conference on Health and the Environment, SR, Bratislava, Oct. 22-25, 2006, 36.

Supplements

Supplement 1

Kodydková J, Vávrová L, Zeman M, Jirák R, Macášek J, Staňková B, Tvrzická E, Žák A. Antioxidative enzymes and increased oxidative stress in depressive women. Clin Biochem. 2009; 42: 1368-74.

Supplement 2

Vávrová L, **Kodydková J**, Zeman M, Dušejovska M, Macášek J, Staňková B, Tvrzická E, Žák A. Altered Activities of Antioxidant Enzymes in Patients with Metabolic Syndrome. Obesity Facts. 2013; 6(1):39-47.

Supplement 3

Vávrová L, **Kodydková J**, Mráčková M, Novák F, sr., Nováková O, Žák A, Novák F. Changes in antioxidant status and inflammatory response markers persist long after clinical recovery from severe sepsis. 2012. (Under review).

Supplement 4

Vávrová L, **Kodydková J**, Macášek J, Ulrych J, Žák A. Oxidační stres v průběhu akutní pankreatitidy. Klin Biochem Metab. 2012; 20(41): 188-193.

Supplement 5

Kodydková J, Vávrová L, Staňková B, Macášek J, Krechler T, Žák A. Changes in antioxidants and oxidative stress markers in pancreatic diseases. Pancreas. 2013; 42(4):614-621.

Supplement 6

Macášek J, Zeman M, Vecka M, Vávrová L, **Kodydková J**, Tvrzická E, Žák A. Reactive oxygen and nitrogen species in the clinical medicine. Cas Lek Cesk 2011; 150(8): 423-32.

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CLINICAL BIOCHEMISTRY

Antioxidative enzymes and increased oxidative stress in depressive women

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Abstract

Objectives: To investigate the activities of the main antioxidative enzymes and oxidative stress in women with depressive disorder (DD).

Methods: In 35 drug-naive women with DD and 35 age matched healthy women enzymes superoxide dismutase (CuZnSOD), catalase (CAT), glutathione peroxidase (GPX1), glutathione reductase (GR) and paraoxonase (PON1), concentrations of conjugated dienes (CD), reduced glutathione (GSH) and anthropometric and clinical data were investigated.

Results: Women with DD were found to have decreased activities of GPX1 ($p<0.05$), decreased concentrations of GSH ($p<0.05$), and increased activities of GR ($p<0.05$), CuZnSOD ($p<0.001$), and concentrations of CD ($p<0.05$). Activity of GPX1 was positively correlated with concentration of GSH ($p<0.05$). Concentrations of CD were positively correlated with TG ($p<0.01$).

Conclusion: Our set of depressive women was characterized by changes indicating an increased oxidative stress, as well as by certain features of metabolic syndrome.

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Keywords: Depressive disorder; Oxidative stress; Antioxidative enzymes; Conjugated dienes

Introduction

Depressive disorder (DD) belongs to diseases, incidence of which is now increasing all around the world. In the USA, it was established, that about 16% of the population fall ill with major depressive disorder during the lifetime [1]. In Finland, 5% prevalence of the depression was described [2]. In 2006, 168 new cases of affective disorders per 100,000 inhabitants were noticed in the Czech Republic, the incidence being 2 times higher in women than in men [3]. The dysfunction of serotoninergic, noradrenergic and dopaminergic neurotransmission [4,5], abnormal regulation in the hypothalamic–pituitary–adrenal axis (HPA) [6], disturbance of cellular plasticity including reduced neurogenesis [7], or chronic inflammation, connected with higher oxidative stress [8] could play a role in the pathogenesis of DD.

Large consumption of oxygen (up to 20% of the total requirement of organism), high amount of polyunsaturated fatty acids, which are prone to oxidation, high amount of iron and low activities of antioxidant enzymes contribute to higher sensitivity of brain to oxidative stress [9]. Oxidative stress is defined as the imbalance between production of reactive oxygen and nitrogen species (RONS) and their insufficient decomposition by the antioxidative system [10]. This defence system involves enzymatic antioxidants — superoxide dismutase (EC 1.15.1.1.; SOD), glutathione peroxidase (EC 1.11.1.9; GPX), glutathione reductase (EC 1.6.4.2; GR), catalase (EC 1.11.1.6; CAT) and paraoxonase (EC 3.1.8.1; PON) as well as nonenzymatic antioxidants — reduced glutathione (GSH), provitamin A, vitamin C and E, coenzyme Q10, carotenoids and trace elements like copper, zinc or selenium. Increased production of RONS has been observed in patients with neurodegenerative and psychiatric diseases such as Alzheimer's and Parkinson's

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disease or schizophrenia [11–13]. Neurodegenerative changes, which are augmented by inflammation and oxidative stress, play an important role also in the pathogenesis of the DD [14,15]. The raised level of oxidative stress is supposed to be one of the factors, standing behind higher incidence of type 2 diabetes mellitus (DM2) and cardiovascular diseases (CVD), which were observed in patients with depression [16,17]. However, only few studies have studied an oxidative stress in DD and the results have been inconsistent. The aim of this study was to determine the activities of main antioxidative enzymes, concentrations of reduced glutathione and conjugated dienes (CD) as marker of lipoperoxidation, and their relations to anthropometric and selected metabolic parameters in women with DD in comparison with healthy controls.

Methods

Subjects

Thirty five women with DD, recruited from the consecutive outpatients of the Psychiatric Department of 1st Faculty of Medicine of Charles University in Prague from May 2006 to May 2008, and 35 age-matched healthy controls were included in the study. Depressive disorder was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, DSM-IV (American Psychiatric Association, 1994) [18]. All patients were evaluated using Hamilton Depression Rating Scale (HAM-D).

Exclusion criteria of the study were: history of cardiovascular and cerebrovascular disease, DM, hepatic and/or renal diseases, hypothyroidism, malignancies, macroalbuminuria (proteinuria higher than 300 mg/day), excessive alcohol consumption (>30 g/day), treatment with hypolipidemic medications, supplementation by vitamins, polyunsaturated fatty acids and/or antioxidants. Patients have completed the 7 days dietary questionnaire. Food intake was processed by the software NutriMaster. We have evaluated intake of total energy, protein, fat, carbohydrate, vitamins and minerals. The study protocol was approved by the Joint Ethical Committee of the General Teaching Hospital and the 1st Faculty of Medicine of Charles University in Prague. Written informed consent was obtained from all participants.

Blood samples

Blood samples were obtained after overnight fasting. Activities of CAT, GR, GPX1 and CuZnSOD were measured in haemolysed erythrocytes. The blood samples were collected into the tubes with K_2 EDTA, erythrocytes were washed three times with a NaCl isotonic solution (9 g/L). Serum was used for the determination of all other parameters. The samples were stored at −80 °C until assay. The haemotological parameters were measured by routine laboratory techniques using an autoanalyzer (Coulter LH750 — haematological analyzer, Beckman Coulter).

Measurement of enzyme activities

Glutathione peroxidase 1

The activity was measured by the modified method of Paglia and Valentine using tert-butyl hydroperoxide as a substrate [19]. Briefly, 580 μL of 172.4 mM tris–HCl buffer containing 0.86 mM EDTA, pH=8.0; 100 μL of 20 mM GSH, 100 μL of 10 U/mL GR, 100 μL of 2 mM NADPH and 100 μL of diluted sample were pipetted into the cuvettes. The reaction was started after 10 min of incubation at 37 °C by the addition of 20 μL of 9.99 mM tert-butyl hydroperoxide. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm. Blank was run for each sample. Activity of GPX1 was calculated using the molar extinction coefficient of NADPH 6220 M^{-1} cm^{-1} and expressed as U/g haemoglobin. One unit of GPX1 (U) is defined as 1 μmol of NADPH oxidized to NADP per minute.

Glutathione reductase

The activity was measured according to the method of Goldberg et al. [20]. Briefly, 700 μL of 0.127 M potassium phosphate buffer containing 0.633 mM $Na_2EDTA{\cdot}2H_2O$, pH=7.2 was added to cuvettes followed by 100 μL of 22 mM oxidized glutathione (GSSG) and 100 μL of diluted sample. The reaction was started after 10 min of incubation at 37 °C by addition of 100 μL of 1.7 mM NADPH. The rate of NADPH degradation was monitored spectrophotometrically at 340 nm. Blank was run for each sample. Activity of GR was calculated using the molar extinction coefficient of NADPH 6220 M^{-1} cm^{-1} and expressed as U/g haemoglobin. One unit of GR (U) is defined as the amount of enzyme catalyzing the reduction of 1 μmol of GSSG per minute.

Catalase

The activity was determined by the modified method of Aebi [21]. The reaction mixture in cuvettes contained 876 μL of 50 mM potassium phosphate buffer, pH=7.2 and 25 μL of diluted sample. The reaction was started after 10 min of incubation at 30 °C by addition of 99 μL of 10 mM H_2O_2. The rate of H_2O_2 degradation was monitored spectrophotometrically at 240 nm. Blank was run for each sample. Catalase activity was calculated using the molar extinction coefficient of H_2O_2 43.6 M^{-1} cm^{-1} and expressed as kU/g haemoglobin. One unit of CAT (U) is defined as 1 μmol of H_2O_2 decomposition per minute.

CuZn-Superoxide dismutase

The activity was determined according to the modified method of Štípek et al. [22]. The reaction mixture in cuvettes contained 700 μL of 50 mM potassium phosphate buffer, pH=7.2; 50 μL of xanthine oxidase; 100μL of NBT and 50 μL of diluted sample. The reaction was started after 10 min of incubation at 25 °C by addition of 100 μL of 1 mM xanthine. The rate of NBT-formazan generation was monitored spectrophotometrically at 540 nm. Blank was run for each sample. Superoxide dismutase activity was calculated by means of calibration curve and expressed as U/g haemoglobin. One unit

of SOD (U) is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Superoxide dismutase standard (Cat. No. S9636-1kU) was purchased from Sigma Aldrich (St. Louis, MO USA).

Paraoxonase 1

The arylesterase activity of PON1 was measured according to the method of Eckerson et al. using phenylacetate as a substrate [23]. Briefly, 900 μL of 20 mM Tris–HCl buffer containing 1 mM $CaCl_2$, pH=8.0 was added to cuvettes followed by 50 μL of diluted serum sample. The reaction was started by addition of 50μL of 100 mM phenylacetate. The rate of phenol generation was monitored spectrophotometrically at 270 nm. Blank was run for each sample. Arylesterase activity of PON1 was calculated using the molar extinction coefficient of the produced phenol, 1310 M^{-1} cm^{-1} and expressed as U/mL serum. One unit of PON1 (U) is defined as 1 μmol of phenylacetate degradation to phenol per minute.

Measurement of concentration of reduced glutathione

Reduced glutathione was measured by the modified spectrophotometric method according to Griffith [24]. Suspension of washed erythrocytes (500 μL) was mixed with 100 μL of diluted acetic acid in water (6%, v/v), haemolysate was vortexed and 400 μL of 5-sulphosalicylic acid 10% (w/v) was immediately added. After centrifugation at 10 000 g for 2 min, supernatant solution was collected for analysis. This method is based on the determination of relatively stable product of reduction of 5.5′ dithiobis-2-nitrobenzoic acid (DTNB) reduction by sulfhydryl compounds to yellow product. Briefly, 50 μL of 0.125 M potassium phosphate buffer containing 6.3 mmol/L $Na_2EDTA{\cdot}2H_2O$, pH=7.5 was added to micro-cuvettes followed by 37.5 μL of the sample and 12.5 μL of 6 mmol/L DTNB. The absorbance of the yellow product (reduced chromogen) was measured at 412 nm. Concentration was calculated by means of calibration curve and was expressed as μg/g haemoglobin.

Measurement of concentration of conjugated dienes

Serum low density lipoproteins were isolated by precipitation method of Ahotupa et al. [25]. Concentrations of CD in precipitated LDL were measured by the modified method of Wieland et al. [26]. Serum samples were stabilized with EDTA (10:1 v/v) and analyzed within 2 weeks. The precipitation buffer consisted of 0.064 M trisodium citrate adjusted to pH 5.05 with 5 M HCl, and contained 50,000 IU/L heparin. Sample (110 μL) of serum with EDTA (10:1 v/v) was added to 1 mL of the heparin-citrate buffer. After mixing, the suspension was incubated for 10 min at room temperature. The precipitated lipoproteins were then separated by centrifugation at 2800 rpm for 10 min. Supernatant was removed and the pellet was resuspended in 100 μL of NaCl isotonic solution (9 g/L); this process, individual for each sample, did not exceed 3 s to prevent LDL oxidation. Lipids were extracted by chloroform–methanol (2:1), the mixture was incubated for 10 min with intermittent mixing, 250 μL redistilled water was used for phase separation. The mixture was centrifuged at 3000 rpm for 5 min. The 800 μL of lower layer (infranatant) was dried under nitrogen, redissolved in 300 μL of cyclohexane, and analyzed spectrophotometrically at 234 nm. The concentration of CD was calculated using the molar extinction coefficient 2.95×10^4 M^{-1} cm^{-1} and expressed as mmol/L serum.

Statistical analysis

All data were expressed as median (25th–75th percentiles). Normality of distribution of data was tested with Shapiro–Wilks *W* test. Differences between compared groups were tested with one-way ANOVA. Mann–Whitney *U* test was used for non-parametric comparison of groups. The Spearman correlation coefficients were used for correlation analysis. All statistical analyses were performed using version 8.0 of StatSoft software Statistica (2007, CZ).

Results

The basic characteristics and essential biochemical parameters observed in the studied groups are shown in Table 1, parameters of oxidative stress are presented in Table 2. Patients with DD had significantly raised values of waist circumference, TG, glucose and index of insulin resistance (HOMA-IR) in comparison with control group. The mean systolic and diastolic blood pressure (SBP and DBP) did not differ significantly. There were also no significant differences in concentrations of HDL-C, LDL-C, CRP, apo A-I and apo B, as well as those of calcium, zinc and copper. We have found no statistical

Table 1
Subject characteristics.

	Depression	Controls
N (female)	35	35
Age (years)	64.5 (50.0–75.1)	65.0 (53.2–77.0)
BMI (kg/m^2)	26.1 (24.1–29.4)$^{+}$	24.7 (22.7–25.9)
Waist (cm)	87.0 (77.0–96.0)$^{+}$	80.5 (77.0–85.5)
Systolic BP (mm Hg)	120.0 (120.0–135.0)	127.5 (120.0–130.0)
Diastolic BP (mm Hg)	80.0 (70.0–80.0)	80.0 (75.0–80.0)
TC (mmol/L)	5.42 (4.55–6.57)	5.92 (4.99–6.48)
TG (mmol/L)	1.32 (0.95–1.8)$^{+}$	1.06 (0.87–1.46)
HDL-C (mmol/L)	1.42 (1.24–1.71)	1.68 (1.49–1.94)
LDL-C (mmol/L)	3.14 (2.54–4.05)	3.56 (2.73–4.27)
Apo A-I (g/L)	1.41 (1.26–1.56)	1.45 (1.33–1.61)
Apo B (g/L)	1.02 (0.86–1.34)	1.04 (0.90–1.25)
Glucose (mmol/L)	5.0 (4.6–5.9)$^{++}$	4.70 (4.6–4.9)
HOMA-IR	2.32 (1.19–4.35)$^{++}$	1.65 (1.19–1.95)
CRP (mmol/L)	3.3 (2.0–7.9)	2.2 (2.0–5.5)
Ca (mmol/L)	2.35 (2.29–2.47)	2.35 (2.28–2.42)
Cu (mmol/L)	21.3 (17.8–23.5)	19.5 (18.3–21.8)
Zn (mmol/L)	15.2 (13.8–16.7)	14.8 (13.7–16.8)
Cu/Zn	1.33 (1.15–1.64)	1.26 (1.11–1.53)

Abbreviations used: BMI: body mass index, BP: blood pressure, TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, CRP: C-reactive protein; Data were expressed as median (25th–75th percentiles). Statistical analysis: $^{+}p<0.05$; $^{++}p<0.01$.

Table 2
Parameters of oxidative stress.

	Depression	Controls
GPX1 (U/g Hb)	53.7 (42.7–65.7)$^{+}$	64.0 (52.9–70.7)
GR (U/g Hb)	7.95 (6.84–8.62)$^{+}$	7.00 (6.19–8.30)
GSH (μg/g Hb)	568.75 (387.93–3484.01)$^{+}$	2374.93 (515.16–5668.35)
CuZnSOD (U/g Hb)	2356.2 (2080.75–2586.5)$^{+++}$	1930.5 (1309.2–2249.7)
CAT (kU/g Hb)	174.0 (155.2–217.9)	189.0 (166.6–215.4)
PON1 (kU/L)	161.3 (140.8–196.2)	175.9 (146.2–207.3)
CD (mmol/L)	55.7 (47.7–80.8)$^{+}$	53.3 (43.8–62.1)

Abbreviations used: GPX1: glutathione peroxidase1, GR: glutathione reductase, GSH: reduced glutathione, CuZnSOD: CuZn-superoxide dismutase, CAT: catalase, PON1: paraoxonase1, CD: conjugated dienes, Hb: haemoglobin; Data were expressed as median (25th–75th percentiles). Statistical analysis: $^{+}$ $p<0.05$; $^{++}$ $p<0.01$; $^{+++}$ $p<0.001$.

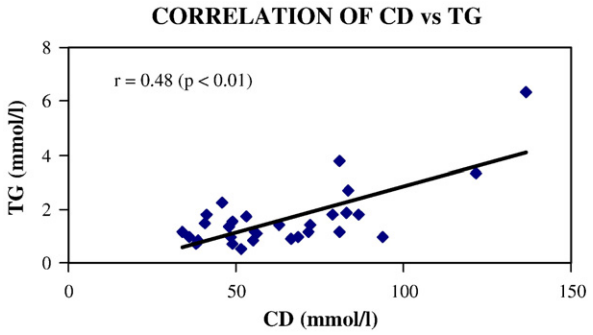

Fig. 2. Correlation of concentrations of conjugated dienes and concentration s of triglycerides. Abbreviation used: CD: conjugated dienes, TG: triglycerides; Statistical analysis: Spearman's rank correlation coefficient.

significant differences in nutritional habits between women with DD and control group (data not shown).

Erythrocyte activities of GR and CuZnSOD and concentrations of CD in precipitated LDL were increased in depressive women; however, activities of GPX1 were decreased. Reduced glutathione was significantly lower in depressive women than in the control group. Activities of CAT and PON1 were not altered in patients with DD.

In women with DD, activities of PON1 were positively correlated with concentrations of HDL-C ($r=0.457$, $p<0.01$), apo A-I ($r=0.379$, $p<0.05$) and calcium ($r=0.371$, $p<0.05$), but in control group we have found only positive correlation with apoA-I ($r=0{,}492$; $p<0.05$). Furthermore, activities of CuZnSOD were positively correlated with concentrations of zinc in DD (Fig. 1) and also in control group ($r=0.393$, $p<0.05$; $r=0.477$, $p<0.05$, respectively). There was no significant correlation of CuZnSOD with copper in both groups.

Activities of GPX1 were positively correlated with concentrations of GSH ($r=0.284$, $p<0.05$) in DD, but not in control group. There were no correlations observed between activities of individual antioxidant enzymes.

Concentrations of serum TG were positively correlated with concentrations of CD in precipitated LDL in the DD group (Fig. 2) and in the control one ($r=0.480$, $p<0.01$; $r=0.391$; $p<0.05$, respectively). We did not find any correlation between HAM-D score and any of observed parameters.

Discussion

The most important findings of this study were significantly increased concentrations of CD in precipitated LDL, indicating increased lipid peroxidation, accompanied by the decrease in activity of GPX1 and increase in activities of both CuZnSOD and GR in women with DD. The presence of IR and certain features of metabolic syndrome (MetS) in our set of women with DD were further important findings.

Oxidative stress was accepted to participate in the pathophysiology of neurodegenerative conditions such as Alzheimer's disease [27,28], HIV-associated dementia [29], Parkinson's disease [30]. Neurodegenerative changes of brain have been demonstrated in patients with DD, in which also markers of oxidative stress were previously described, such as altered activities of antioxidative enzymes and increased lipid peroxidation products [31–34].

Glutathione peroxidase is ubiquitous enzyme responsible for the degradation of lipid hydroperoxides and of H_2O_2 to hydroxyderivates and water. Decreased activities of GPX1 in erythrocytes were found in our depressive patients, similarly as in the study of Ozcan et al. [35], who described lower activities of GPX1 in patients with affective disorders in comparison with healthy controls. However, Bilici et al. [33] found increased

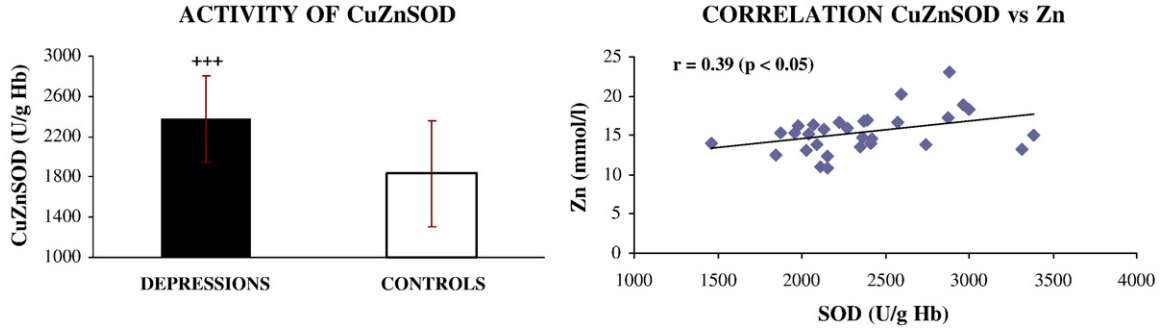

Fig. 1. Activity of CuZnSOD and its correlation with zinc in patients with depression. Abbreviation used: SOD: superoxide dismutase, Hb: haemoglobin; Statistical analysis: Spearman's rank correlation coefficient; $^{+++}p<0.001$ (Mann–Whitney U test).

activities of GPX1 in erythrocytes of patients with major depression, whereas Andrezza et al. [36] did not find any significant changes in patients with bipolar disorder. Activity of GPX1 could be decreased due to lower concentration of its substrate — GSH that we have found in women with DD. Reduced glutathione is one of the most important intracellular antioxidants in the cell and is enzymatically oxidized to GSSG in a number of biochemical pathways. In the present study we have observed significantly decreased concentrations of GSH in depressive women compared to control. To our knowledge, there has been no clinical study regarding data on GSH concentrations in patients with depressive disorders. The observed decrease of GSH were also described in patients with autism [37,38], schizophrenia [39] and Down syndrome [40] have reduced levels of total GSH. Reduced glutathione reacts also nonenzymatically with RONS leading to the glutathiol radical that reacts with further GSH to GSSG radical anion formation. Oxidized glutathione radical anion is involved in the conversion of oxygen to superoxide. The conversion of GSSG back to GSH is catalyzed by GR. In our study, we have found increased activities of GR in erythrocytes. Bilici et al. [33] described raised activities of GR in plasma, but no significant differences in erythrocytes in patients with major depression.

Studies have described a variety of intracellular sources of superoxide that include nitric oxide synthase, xanthine oxidase, cyclooxygenase and NADPH oxidase [41–44]. The most important source of superoxide in vascular cells is NADPH oxidase [45]. Decomposition of superoxide into H_2O_2 is catalyzed by SOD. We have found increased CuZnSOD activities in erythrocytes of depressive patients compared with healthy persons, similarly to Sarandol et al. [32]. They suggested that CuZnSOD activity is increased in response to increased ROS production. Bilici et al. [33] have also observed increased CuZnSOD activity in erythrocytes of depressive patients. Inconsistent results were published for serum CuZnSOD activities. Herken et al. [34] have found decreased, whereas Khanzode et al. [46] elevated CuZnSOD activities in patients with major depression. We have found positive correlation between CuZnSOD activity and concentration of zinc, which is responsible for the stability of CuZnSOD structure as its cofactor [47].

Activities of CAT in erythrocytes were not altered in our set of women with DD, in accordance with Bilici et al. [33]. However, Szuster-Ciesielska et al. [48] found raised activities of CAT in serum of patients with major depression and Ozcan et al. [35] described decreased CAT activities in erythrocytes of patients with affective disorders. Induction of CAT or SOD does not necessarily lead to the induction of the other one [49]. The increased activity of SOD leads to increased amounts of hydrogen peroxide that is then degraded by GPX in its low concentrations and by CAT in its high concentrations [50]. It could be supposed that the concentration of hydrogen peroxide wasn't enough high to increase activity of CAT, and that the task of H_2O_2 degradation remains on GPX. But GPX activity is dependent on GSH, as its substrate. This antioxidant is rapidly consumed in oxidative stress. It is problematic whether GPX could function appropriately in low GSH concentrations.

The activities of PON1 were not altered in women with DD, as well as levels of apo A-I, HDL-C and calcium. Apolipoprotein A-I plays a key role for PON1 because of the connection of PON1 to HDL is through apo A-I. We have found positive correlation between PON1 activity and both apo A-I and HDL-C concentrations. Paraoxonase is calcium dependent enzyme; calcium is located in the active site of enzyme. It is consistent with our finding of a positive correlation between the PON1 activity and calcium concentrations in patients with DD.

Increased concentrations of CD in LDL indicate an elevation of minimally modified (oxidized) LDL in vivo. Raised concentrations of CD in LDL were found in insulin-resistant states such as MetS and DM2 [51–53], however, different results were published by Gavella et al. [54].

Observed hypertriglyceridemia (HTG) and higher glycaemia, the accumulation of visceral fat and IR could play a role in changes of oxidant/antioxidant balance in our set of depressive women. In nondiabetic human subjects, both BMI and waist circumference were closely correlated with the markers of systemic oxidative stress (plasma TBARS, urinary 8-epi-PGF2α) [55]. Hypertriglyceridemia was associated with an increased oxidative stress in experimental rats [56] and also in humans [57]. Inconsistent results were obtained with regard to the activities of antioxidant enzymes in insulin-resistant states. In one study, increased activity of CAT, decreased of GPX and non-changed of SOD was found in type 2 diabetic patients [58] while in another study [59] the activities of GPX, SOD and CAT in red blood cells were significantly decreased in diabetic subjects when compared with healthy controls. Some authors suggest decreased GPX1 activity as cardiovascular risk factor that was in the prospective study associated with increased extent of atherosclerotic lesions [60].

In summary, we have found significant increase in CuZnSOD and GR activity and simultaneous decrease of GPX1 activity as well as elevated concentrations of CD in precipitated LDL, which positively correlated with TG in our set of depressive women. These findings are in accordance with hypothesis that oxidative stress may play an important role in the pathogenesis of depression. Metabolic changes and markers of IR in women with DD suggest the relationships between MetS and DD. Increased oxidative stress could be a possible connection between depression, IR and increased incidence of both DM2 and CVD.

Acknowledgments

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References

[1] Kessler RC, Berglund P, Demler O, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 2003;289:3095–105.

[2] Pirkola SP, Isometsa E, Suvisaari J, et al. DSM-IV mood-, anxiety- and alcohol use disorders and their comorbidity in the Finnish general population—results from the Health 2000 Study. Soc Psychiatry Psychiatr Epidemiol 2005;40:1–10.
[3] Czech Health Statistics Yearbook 2006 (2007), ÚZIS, Praha.
[4] Hindmarch I. Beyond the monoamine hypothesis: mechanisms, molecules and methods. Eur Psychiatry 2002;17(Suppl 3):294–9.
[5] Malhi GS, Parker GB, Greenwood J. Structural and functional models of depression: from sub-types to substrates. Acta Psychiatr Scand 2005;111: 94–105.
[6] Brown ES, Varghese FP, McEwen BS. Association of depression with medical illness: does cortisol play a role? Biol Psychiatry 2004;55:1–9.
[7] Kempermann G, Kronenberg G. Depressed new neurons—adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol Psychiatry 2003;54:499–503.
[8] Smith RS. The macrophage theory of depression. Med Hypotheses 1991;35:298–306.
[9] Evans PH. Free radicals in brain metabolism and pathology. Br Med Bull 1993;49:577–87.
[10] Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997;82: 291–5.
[11] Aslan M, Ozben T. Reactive oxygen and nitrogen species in Alzheimer's disease. Curr Alzheimer Res 2004;1:111–9.
[12] Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 1996;47:161–70.
[13] Mahadik SP, Mukherjee S. Free radical pathology and antioxidant defense in schizophrenia: a review. Schizophr Res 1996;19:1–17.
[14] Licinio J, Wong ML. The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate the biological substrate of depressive symptoms, regulate stress-responsive systems, and contribute to neurotoxicity and neuroprotection. Mol Psychiatr 1999;4:317–27.
[15] Moretti A, Gorini A, Villa RF. Affective disorders, antidepressant drugs and brain metabolism. Mol Psychiatr 2003;8:773–85.
[16] Mezuk B, Eaton WW, Albrecht S, Golden SH. Depression and type 2 diabetes over the lifespan: a meta-analysis. Diabetes Care 2008;31: 2383–90.
[17] Rugulies R. Depression as a predictor for coronary heart disease. A review and meta-analysis. Am J Prev Med 2002;23:51–61.
[18] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th edition. Washington, D.C; 1994.
[19] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–69.
[20] Goldberg DM, SRJ. Assay of glutathione reductase. In: Bergmeyer HU, Bergmeyer J, Grassl M, editors. In Methods of Enzymatic Analysis. Verlag Chemie: Weinheim; 1983. p. 258–64.
[21] Aebi H. Catalase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. Verlag Chemie: Weinheim; 1974. p. 673–84.
[22] Štípek S, Crkovská J, Dvořák V. Spectrophotometric assay for superoxide dismutase controlled by PC programme developed in LabWindows system. Klin Biochem Metab 1995;3:93–7.
[23] Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet 1983;35:1126–38.
[24] Griffith OW. Glutathione and glutathione disulphide. In: Bergemeyer HU, editor. Methods of Enzymatic Analysis. Verlag Chemie: Weinheim; 1985. p. 521–9.
[25] Ahotupa M, Ruutu M, Mantyla E. Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. Clin Biochem 1996;29:139–44.
[26] Wieland H, Seidel D. A simple specific method for precipitation of low density lipoproteins. J Lipid Res 1983;24:904–9.
[27] Mattson MP. Pathways towards and away from Alzheimer's disease. Nature 2004;430:631–9.
[28] Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta 2000;1502:139–44.
[29] Koutsilieri E, ter MV, Riederer P. Neurotransmission in HIV associated dementia: a short review. J Neural Transm 2001;108:767–75.
[30] Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 1996;47:161–70.
[31] Maes M, De VN, Pioli R, et al. Lower serum vitamin E concentrations in major depression. Another marker of lowered antioxidant defenses in that illness. J Affect Disord 2000;58:241–6.
[32] Sarandol A, Sarandol E, Eker SS, Erdinc S, Vatansever E, Kirli S. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. Hum Psychopharmacol 2007;22:67–73.
[33] Bilici M, Efe H, Koroglu MA, Uydu HA, Bekaroglu M, Deger O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. J Affect Disord 2001;64: 43–51.
[34] Herken H, Gurel A, Selek S, et al. Adenosine deaminase, nitric oxide, superoxide dismutase, and xanthine oxidase in patients with major depression: impact of antidepressant treatment. Arch Med Res 2007;38: 247–52.
[35] Ozcan ME, Gulec M, Ozerol E, Polat R, Akyol O. Antioxidant enzyme activities and oxidative stress in affective disorders. Int Clin Psychopharmacol 2004;19:89–95.
[36] Andreazza AC, Cassini C, Rosa AR, et al. Serum S100B and antioxidant enzymes in bipolar patients. J Psychiatr Res 2007;41:523–9.
[37] Geier DA, Kern JK, Garver CR, et al. Biomarkers of environmental toxicity and susceptibility in autism. J Neurol Sci 2009;280:101–8.
[38] Vojdani A, Mumper E, Granpeesheh D, et al. Low natural killer cell cytotoxic activity in autism: the role of glutathione, IL-2 and IL-15. J Neuroimmunol 2008;205:148–54.
[39] Altuntas I, Aksoy H, Coskun I, Cayköylü A, Akçay F. Erythrocyte superoxide dismutase and glutathione peroxidase activities, and malondialdehyde and reduced glutathione levels in schizophrenic patients. Clin Chem Lab Med 2000;38:1277–81.
[40] Muchova J, Garaiova I, Sustrova M. The redox state of glutathione in erythrocytes of individuals with Down syndrome. Bratisl Lek Listy 2007;108:70–4.
[41] Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Cirk Res 2000;86:494–501.
[42] Kojda G, Harrison D. Interactions between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. Cardiovasc Res 1999;43:562–71.
[43] Harrison R. Physiological roles of xanthine oxidoreductase. Drug Metab Rev 2004;36:363–75.
[44] Armstead WM. Vasopressin induced cyclooxygenase dependent superoxide generation contributes to K^+ channel function impairment after brain injury. Brain Res 2001;910:19–28.
[45] Babior BM. NADPH oxidase: an update. Blood 1999;93:1464–76.
[46] Khanzode SD, Dakhale GN, Khanzode SS, Saoji A, Palasodkar R. Oxidative damage and major depression: the potential antioxidant action of selective serotonin re-uptake inhibitors. Redox Rep 2003;8: 365–70.
[47] Forman HJ, Fridovich I. On the stability of bovine superoxide dismutase. The effects of metals. J Biol Chem 1973;248:2645–9.
[48] Szuster-Ciesielska A, Slotwinska M, Stachura A, et al. Accelerated apoptosis of blood leukocytes and oxidative stress in blood of patients with major depression. Prog.Neuropsychopharmacol. Biol Psychiatry 2008;32: 686–94.
[49] Amstad P, Peskin A, Shah G, et al. The balance between Cu,Zn-superoxide dismutase and catalase affects the sensitivity of mouse epidermal cells to oxidative stress. Biochemistry 1991;30:9305–13.
[50] Halliwell B, Gutteridge JMC. Free Radical in Biology and Medicine. 4th ed. New York: Oxford University Press; 2007. p. 123.
[51] Rabini RA, Tesei M, Galeazzi T, Dousset N, Ferretti G, Mazzanti L. Increased susceptibility to peroxidation of VLDL from non-insulin-dependent diabetic patients: a possible correlation with fatty acid composition. Mol Cell Biochem 1999;199:63–7.
[52] Zak A, Tvrzicka E, Vecka M, et al. Severity of metabolic syndrome unfavorably influences oxidative stress and fatty acid metabolism in men. Tohoku J Exp Med 2007;212:359–71.
[53] Zeman M, Zak A, Vecka M, Tvrzicka E, Pisarikova A, Stankova B. N-3

fatty acid supplementation decreases plasma homocysteine in diabetic dyslipidemia treated with statin-fibrate combination. J Nutr Biochem 2006;17:379–84.
[54] Gavella M, Lipovac V, Car A, Vucic M. Baseline diene conjugation in LDL lipids from newly diagnosed type 2 diabetic patients. Diabetes Metab 2002;28:391–6.
[55] Furukawa S, Fujita T, Shimabukuro M, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114: 1752–61.
[56] Kazdová L, Zák A, Vrána A. Increased lipoprotein oxidability and aortic lipid peroxidation in an experimental model of insulin resistance syndrome. Ann NY Acad Sci 1997;827:521–5.
[57] Zeman M, Zak A, Vecka M, Tvrzicka E, Romaniv S, Konarkova M. Treatment of hypertriglyceridemia with fenofibrate, fatty acid composition of plasma and LDL, and their relations to parameters of lipoperoxidation of LDL. Ann NY Acad Sci 2002;967:336–41.
[58] Kesavulu MM, Giri R, Kameswara Rao B, Apparao C. Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. Diabetes Metab 2000;26:387–92.
[59] Ramakrishna V, Jailkhani R. Oxidative stress in non-insulin-dependent diabetes mellitus (NIDDM) patients. Acta Diabetol 2008;45:41–6.
[60] Espinola-Klein C, Rupprecht HJ, Bickel C, et al. Glutathione peroxidase-1 activity, atherosclerotic burden, and cardiovascular prognosis. Am J Cardiol 2007;99:808–12.

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Original Article

Altered Activities of Antioxidant Enzymes in Patients with Metabolic Syndrome

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Key Words
Metabolic syndrome · Antioxidant enzymes · Reduced glutathione · Conjugated dienes

Abstract
Objective: In the pathogenesis of the metabolic syndrome (MetS), an increase of oxidative stress could play an important role which is closely linked with insulin resistance, endothelial dysfunction, and chronic inflammation. The aim of our study was to assess several parameters of the antioxidant status in MetS. ***Methods:*** 40 subjects with MetS and 40 age- and sex-matched volunteers without MetS were examined for activities of superoxide dismutase (CuZnSOD), catalase (CAT), glutathione peroxidase 1 (GPX1), glutathione reductase (GR), paraoxonase1 (PON1), concentrations of reduced glutathione (GSH), and conjugated dienes in low-density lipoprotein (CD-LDL). ***Results:*** Subjects with MetS had higher activities of CuZnSOD ($p < 0.05$) and GR ($p < 0.001$), higher concentrations of CD-LDL ($p < 0.001$), lower activities of CAT ($p < 0.05$) and PON1 ($p < 0.05$), and lower concentrations of GSH ($p < 0.05$), as compared with controls. Activity of GPX1 was not significantly changed. ***Conclusions:*** Our results implicated an increased oxidative stress in MetS and a decreased antioxidative defense that correlated with some laboratory (triglycerides, high-density lipoprotein cholesterol (HDL-C)) and clinical (waist circumference, blood pressure) components of MetS.

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Introduction

Currently, the prevailing notion of the metabolic syndrome (MetS) is that it is characterized by a cluster of risk factors for atherosclerosis and type 2 diabetes mellitus and can be regarded as a physiological and clinical entity [1]. The main components of MetS are accumulation of intra-abdominal fat, impaired metabolism of glucose, atherogenic dyslipidemia (low high-density lipoprotein cholesterol (HDL-C), hypertriglyceridemia), and arterial hypertension. In pathogenesis, several mechanisms were shown to take part, namely insulin resistance, chronic low-grade inflammation, endothelial dysfunction, and oxidative stress; their interactions have not been fully elucidated at present. Elevated levels of oxidative stress in subjects with MetS were demonstrated in many experimental and clinical studies [2].

Oxidative stress is defined as an imbalance between the production of reactive oxygen and nitrogen species (RONS) and their insufficient decomposition by the antioxidant system which results in macromolecular damage and disruption of redox signaling and control [3]. Free radicals and non-radical oxidants belong to RONS. Free radicals could induce DNA mutations, structural disorders in proteins, and peroxidative damage of cell membrane and plasma lipids [4]. RONS play an important role in the pathogenesis of many cardiovascular and neurodegenerative diseases as well as in type 2 diabetes mellitus and its complications [5].

The defense mechanisms of the human body against oxidative stress are complex and involve cellular and extracellular antioxidant systems which are regulated at multiple levels [6]. Various enzymes, e.g. superoxide dismutase (CuZnSOD), glutathione peroxidase 1 (GPX1), catalase (CAT), paraoxonase 1 (PON1), glutathione reductase (GR), as well as nonenzymatic antioxidant compounds (e.g. metal chelators, low-molecular-weight antioxidants) take part in the antioxidant defense.

In the first step of the defense mechanism against superoxide anions (O_2^-), the enzyme CuZnSOD catalyzes their dismutation into oxygen and H_2O_2. In the second step, CAT and GPX1 independently convert H_2O_2 to water. Any increase in the CuZnSOD catalytic activity produces an excess of H_2O_2 that must be efficiently neutralized by either CAT or GPX1; otherwise, H_2O_2 reacts with O_2^- producing in a two-step reaction (the Haber-Weiss reaction) hydroxyl radicals OH which are even more dangerous [5]. Cytosolic GPX1 detoxifies H_2O_2 in the presence of reduced glutathione (GSH), which is oxidized to oxidized glutathione (GSSG) and subsequently recycled by GR. GPX1 with the aid of GSH protects lipids against peroxidation. The pool of GSH has to be replenished by de novo synthesis that is catalyzed by the enzyme glutamate-cystein ligase. The PON1 enzyme as HDL-associated enzyme is implicated in the anti-inflammatory and antioxidant activities of HDL and impedes oxidative modification of low-density lipoprotein (LDL) thus protecting cell membranes from the damage caused by products of lipoperoxidation [7].

This study is focused on the state of the antioxidant defense system in patients with MetS. We intend to investigate the wide variety of known antioxidants in association with MetS. The activities of several antioxidant enzymes as well as the concentration of GSH were determined in the erythrocytes. It has been noted that these cells maintain fairly constant concentrations of enzymes throughout the life span which had been synthesized during the maturation of erythroid precursors [8]. Furthermore, levels of albumin, bilirubin, and calculated total peroxyl radical trapping (cTRAP) were assessed in serum. As a global marker of systemic oxidative stress, conjugated dienes in precipitated low-density lipoproteins (CD-LDL) were determined.

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Participants and Methods

Participants

40 Caucasian subjects with MetS (20 male / 20 female) were recruited from outpatients who had been subsequently examined (from January 2008 until August 2010) at the Lipid Clinic of the 4th Department of Medicine, First Faculty of Medicine, Charles University in Prague. This study group was compared with a control group constituted from 40 volunteers without MetS matched for sex and age (20 male / 20 female), all Caucasian.

MetS was diagnosed according to the International Diabetes Federation criteria [9]. To be included, patients had to have central obesity (waist circumference ≥ 94 cm for men and ≥ 80 cm for women) and fulfill any two of the following four criteria: i) raised TG level (≥1.7 mmol/l), ii) reduced HDL-C (<1.03 mmol/l in males and <1.29 mmol/l in females) or specific treatment for these abnormalities, iii) raised blood pressure (BP) with systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension, and iv) raised fasting plasma glucose (≥5.6 mmol/l) or previously diagnosed type 2 diabetes mellitus. All samples were marked with unique anonymized identification numbers, and the data was merged only after the assays had been completed.

In the MetS group, 21 patients (52.5%) had three, 13 patients (32.5%) four, and 6 patients (15.0%) had all five of the above mentioned components of MetS. In the control group, only three subjects (7.5%) met two components of MetS, 15 (37.5%) controls met one, and the 22 (55.0%) volunteers showed no components of MetS. In the MetS group, 35 patients suffered from hypertension, and of these patients, 21 were under antihypertensive treatment. Among them, 12 were treated with an angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor type 1 blockers, and the 9 remaining subjects were on a combination of ACE inhibitor with calcium channel blockers.

Exclusion criteria for both groups were the following: current antioxidant therapy, excessive alcohol consumption (>30 g/day), hormonal replacement therapy, supplementation with polyunsaturated fatty acids, manifestation of cardiovascular and/or cerebrovascular diseases, type 1 diabetes mellitus, liver (with exception of nonalcoholic fatty liver disease) and kidney diseases (creatinine >130 μmol/l), microalbuminuria (urinary albumine 30–300 mg/day), hypothyroidism as well as recent infections and malignancies.

Informed consent was obtained from all participants. The study protocol was approved by the Ethical Committee of the First Faculty of Medicine, Charles University in Prague.

Blood Samples

Blood samples were collected after a 12-hour overnight fast. Activities of antioxidant enzymes (with exception of PON1) and concentrations of GSH were measured in hemolysed erythrocytes which had been separated from the EDTA plasma and washed three times with saline. Serum was used for all other parameters. Samples were stored at –80 °C until the assay.

Methods

Activities of antioxidant enzymes were measured spectrophotometrically using kinetic methods previously described [10]. Briefly, the activity of GPX1 was measured using tert-butyl hydroperoxide as a substrate, and the rate of NADPH degradation was monitored. The molar extinction coefficient of NADPH (6,220 mol/l/cm) was used for calculation of activity which was then expressed as U/g hemoglobin. The activity of GR was measured by monitoring the rate of NADPH degradation. Activity was calculated using the molar extinction coefficient of NADPH and expressed as U/g hemoglobin. The CAT activity was calculated using the molar extinction coefficient of H_2O_2 (43.6 mol/l/cm), whose degradation rate was monitored at 240 nm. Activity is expressed as kU/g hemoglobin. The method of CuZnSOD activity assessment is based on the monitoring of the rate of NBT-formazan generation. Superoxide dismutase activity was calculated by means of a calibrating curve; superoxide dismutase standard (Cat. No. S9636-1kU) was purchased from Sigma Aldrich (St. Louis, MO, USA). Activity was expressed as U/g hemoglobin. The arylesterase activity of PON1 was measured using phenylacetate as a substrate. Arylesterase activity of PON1 was calculated using the molar extinction coefficient of the produced phenol (1,310 mol/l/cm) and expressed as U/ml serum.

GSH was assessed by the modified spectrophotometric method according to Griffith [11]; this method is based on the determination of the relatively stable product of the reduction of 5,5′ dithiobis-2-nitrobenzoic acid (DTNB). The concentration of CD-LDL was assessed by the modified method of Wieland and Seidel at 234 nm [12]; both methods have been fully described in the previously mentioned paper [10].

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All routine clinical tests were performed at the Institute for Clinical Biochemistry and Laboratory Diagnostics of General University Hospital in Prague: C-reactive protein (CRP) was determined by an immunoturbidimetric method using a K-ASSAY CRP kit (Kamiya Biomedical Company, Seattle, WA, USA; cv = max. 7.6%) on a Hitachi Modular analyzer (Tokyo, Japan). Copper and zinc were measured using atomic absorption spectrometry, uric acid by an enzymatic colorimetric method with the uricase-peroxidase system, and bilirubin by the 2,5-dichlorophenyldiazonium method with a Hitachi Modular analyzer. Plasma albumin was assessed by a colorimetric method using bromocresol green. Plasma concentrations of total cholesterol (TC) and triglycerides (TG) were measured by enzymatic-colorimetric methods (Boehringer, Mannheim, Germany). HDL-C was determined in the supernatant after precipitation of lipoproteins B by PTA/Mg^{2+}, using the kit from the same manufacturer; LDL-C was calculated according to Friedewald's formula. Concentrations of apolipoproteins apo B and apo A1 were measured by the Laurell rocket electroimmunoassay using standard and specific antibodies (Behringwerke, Marburg, Germany). The concentrations of insulin and C-peptide were determined with an electrochemiluminescence immunoassay (Roche, Basel, Switzerland). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as HOMA-IR = (fasting serum glucose (mmol/l) × fasting serum insulin (μU/ml)) / 22.5 [13]. The TRAP was calculated according to the formula: (0.63 (albumin) + 1.02 (uric acid) + 1.50 (bilirubin)) [14].

Statistical Analysis

Data was expressed as mean and standard deviation or median (25th–75th percentile) for data different from normal distribution. Normality of the distribution was tested by the Shapiro-Wilks W test. Comparisons between the groups were carried out by the independent t-test. Mann-Whitney U test was used for nonparametric comparisons and Spearman correlation coefficients for correlation analyses. All analyses were performed using version 8.0 of StatSoft Statistica software (2007, Czech version). The p value < 0.05 was considered statistically significant.

Results

Clinical and biochemical characteristics of the group of subjects with MetS and that of healthy controls are shown in table 1. The groups did not differ in age. In both groups there were no subjects with either type 1 or type 2 diabetes mellitus . The subjects included in the MetS group suffer from insulin resistance when the metabolism of glucose was impaired.

As expected, subjects with MetS had significantly higher values of body mass index and waist circumference. They also had higher values of systolic BP and diastolic BP, glucose, TC, TG, apolipoprotein B (apo B), and uric acid as well as a higher level of insulin and insulin resistance, as assessed by the homeostatic model HOMA-IR. Decreased values were observed for plasma concentrations of HDL-C and apo A1. The difference in CRP did not reach statistical significance. As expected, men have decreased levels of HDL-C and Cu and increased values of waist circumference compared to women.

Activities of antioxidant enzymes and concentrations of GSH and CD-LDL together with levels of cTRAP are presented in table 2. In the group of subjects with MetS, activities of CuZnSOD and GR as well as concentrations of CD-LDL and levels of cTRAP were significantly elevated. On the other hand, activities of CAT and PON1 as well as concentrations of GSH were found to be decreased.

Spearmen correlations (after Bonferroni adjustment) between selected variables are shown in table 3. All risk factors of MetS correlated significantly with the number of components of MetS, namely abnormal levels of glucose, waist circumference, TG, HDL-C, and SBP. Concentrations of CD-LDL significantly correlated with concentrations of TG and HDL-C.

Activities of CuZnSOD correlated positively with those of GR ($r = 0.341$, $p < 0.01$) and GPX1 ($r = 0.260$, $p < 0.05$), and with concentrations of Zn ($r = 0.363$, $p < 0.01$) as well as negatively with the ratio Cu/Zn ($r = -0.278$, $p < 0.05$). Activities of PON1 correlated positively with

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Table 1. Clinical and biochemical characteristics of subjects with the metabolic syndrome and of healthy controls[a]

	Metabolic syndrome			Controls		
	all	M	F	all	M	F
N	40	20	20	40	20	20
Age, years	58.4 (53.4–62.2)	57.0 (50.1–63.1)	58.7 (55.9–61.6)	58.5 (52.6–64.5)	57.8 (50.1–64.0)	59.5 (54.0–65.0)
Waist, cm	101.4 ± 9.1***	104.4 ± 6.4***+	98.3 ± 10.5***	82.5 ± 11.0	86.9 ± 13.2+	78.3 ± 6.6
BMI, kg/m^2	29.4 (27.4–31.7)***	29.0 (27.7–30.3)**	30.6 (25.9–32.4)***	23.9 (21.9–25.5)	25.0 (21.8–26.3)	23.8 (21.9–25.4)
Smoking, N (%)	10 (25.0)	6 (30.0)	4 (20.0)	4 (10.0)	0 (0.0)	4 (20.0)
Hypertension, N (%)	35 (87.5)	18 (90.0)	17 (85.0)	8 (20.0)	5 (25.0)	3 (15.0)
Systolic BP, mm Hg	140 (130–143)**	140 (130–140)***	140 (130–145)	130 (120–130)	128 (120–130)	130 (120–140)
Diastolic BP, mm Hg	90 (88–95)***	90 (90–95)***	90 (83–95)***	80 (80–80)	80 (80–85)	80 (80–85)
Glucose, mmol/l	5.4 (4.8–6.1)***	5.0 (4.7–5.8)	5.6 (5.1–6.3)**	4.7 (4.5–5.1)	4.9 (4.5–5.4)	4.7 (4.3–5.0)
Insulin, mU/l	11.4 (8.7–14.8)***	11.4 (8.6–15.1)*	11.3 (9.4–14.3)*	7.8 (4.6–9.5)	8.5 (4.8–10.1)	7.6 (4.6–9.1)
C-peptid, nmol/l	0.97 (0.84–1.19)***	0.99 (0.86–1.14)***	0.93 (0.81–1.28)***	0.64 (0.51–0.75)	0.59 (0.46–0.75)	0.68 (0.55–0.73)
HOMA-IR	3.0 (1.9–3.8)***	3.0 (1.8–3.7)*	3.0 (2.1–4.5)**	1.6 (1.0–2.1)	1.8 (1.0–2.2)	1.6 (1.0–1.9)
TC, mmol/l	6.3 (5.2–7.3)*	6.2 (5.2–7.1)*	6.5 (5.2–7.4)	5.7 (5.0–6.2)	5.7 (4.8–6.1)	5.8 (5.0–6.6)
TG, mmol/l	2.6 (1.9–3.7)***	2.3 (1.9–3.6)**	2.7 (1.7–3.9)***	1.1 (0.9–1.4)	1.1 (0.8–1.4)	1.0 (0.9–1.3)
HDL-C, mmol/l	1.2 (1.1–1.3)***	1.1 (1.0–1.2)***+	1.2 (1.1–1.3)***	1.6 (1.3–1.8)	1.5 (1.3–1.8)	1.6 (1.5–1.9)
LDL-C, mmol/l	3.6 (3.1–4.3)	3.5 (3.2–4.2)	3.6 (3.0–4.3)	3.5 (2.8–4.3)	3.4 (2.9–3.8)	3.6 (2.8–4.3)
Apo A1, g/l	1.26 ± 0.25**	1.24 ± 0.25	1.28 ± 0.26**	1.43 ± 0.21	1.36 ± 0.20+	1.50 ± 0.20
Apo B, g/l	1.34 ± 0.32***	1.39 ± 0.26***	1.28 ± 0.37	1.09 ± 0.25	1.06 ± 0.22	1.13 ± 0.28
NEFA, mmol/l	0.50 (0.39–0.72)	0.43 (0.35–0.68)	0.51 (0.43–0.75)	0.55 (0.43–0.71)	0.59 (0.435–0.83)	0.55 (0.40–0.61)
CRP, mg/l	2.7 (2.0–6.3)	2.8 (2.0–4.3)	2.7 (2.0–7.4)	2.3 (2.0–6.5)	2.0 (2.0–4.3)	4.6 (2.1–7.3)
Cu, µmol/l	17.7 (16.0–20.5)	17.0 (15.5–18.4)	19.6 (16.3–21.7)	18.5 (16.3–21.5)	16.3 (14.3–18.6)++	19.9 (18.5–23.4)
Zn, µmol/l	16.0 (13.4–17.7)	15.8 (13.4–17.8)	16.3 (13.8–17.1)	15.4 (14.6–19.9)	16.0 (14.5–18.3)	15.2 (14.7–20.8)
Bilirubin, µmol/l	9.1 (6.8–12.9)	10.9 (7.9–13.8)+	7.2 (6.1–9.6)	10.6 (8.0–15.2)	13.9 (9.8–18.2)++	9.0 (7.5–12.3)
Uric acid, µmol/l	346 (290–390)**	355 (312–420)*	329 (275–352)**	293 (236–346)	320 (291–370)+++	251 (195–293)

BP = Blood pressure; TC = total cholesterol; TG = triglycerides; HDL-C = high density lipoprotein; LDL-C = low density lipoprotein; Apo = apolipoprotein; HOMA-IR = homeostasis model assessment of insulin resistance; QUICKI = quantitative insulin sensitivity check index; NEFA = non-esterified fatty acids; CRP = C-reactive protein; Met = metabolic syndrome.

[a]Data presented as mean ± standard deviation (SD) for parametric and median (IQR) for non-parametric variables; MetS versus controls: *p < 0.05, ** p < 0.01, ***p < 0.001. Female versus male: +p < 0.05, ++p < 0.01, +++p < 0.001.

apo A1 (r = 0.479, p < 0.001). Concentrations of CD-LDL correlated positively with TC (r = 0.565, p < 0.001), apo B (r = 0.597, p < 0.001), and LDL-C (r = 0.384, p < 0.001), and negatively with CAT (r = –0.233, p < 0.05).

Discussion

In this study, comparing MetS patients with an age- and sex-matched control group, increased activities of CuZnSOD (+15%, p < 0.05) and GR (+19%; p < 0.001) and increased levels of CD-LDL (+14.4%; p < 0.001) and cTRAP (+6.5%; p < 0.01) were found in MetS

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Table 2. Parameters of oxidative stress of studied groups

	Metabolic syndrome			Controls		
	all	M	F	all	M	F
GPX1, U/g Hb	59.4 ± 15.8	57.6 ± 18.1	61.1 ± 13.4	59.1 ± 17.7	55.4 ± 19.2	62.8 ± 15.7
GR, U/g Hb	8.19 ± 1.54***	8.74 ± 1.21***$^{+}$	7.63 ± 1.67	6.88 ± 1.66	6.92 ± 1.76	6.83 ± 1.60
GSH, mg/g Hb	0.57 (0.38–2.73)*	0.56 (0.40–0.70)	1.51 (0.38–5.01)	1.46 (0.41–5.22)	1.22 (0.43–5.40)	1.70 (0.40–5.05)
CAT, kU/g Hb	189.6 ± 31.8*	192.5 ± 27.8	186.7 ± 35.8	204.6 ± 33.0	206.1 ± 32.5	203.1 ± 34.4
CuZnSOD, kU/g Hb	2.3 (1.9–2.5)**	2.3 (2.2–2.5)*	2.0 (1.7–2.5)$^{+}$	2.0 (1.2–2.5)	2.2 (1.1–2.6)	2.0 (1.3–2.4)
PON1, kU/l	158.9 ± 41.9*	152.0 ± 47.4	165.7 ± 35.4	179.9 ± 42.3	170.2 ± 36.1	189.5 ± 46.5
CD, mmol/l	61.9 (54.1–84.3)***	57.3 (53.4–68.7)*	63.8 (55.3–94.2)*	54.1 (41.3–63.6)	53.3 (33.8–63.6)	57.5 (42.9–68.1)
cTRAP, µmol/l	823 (766–877)**	875 (816–909)$^{+++}$	785 (732–835)**	773 (691–820)	809 (768–865)$^{+++}$	701 (655–776)

GPX1 = glutathione peroxidase 1; GR = glutathione reductase; GSH = reduced glutathione; CAT = catalase; CuZnSOD = CuZn-superoxide dismutase; PON1 = paraoxonase1 – arylesterase activity; CD = conjugated dienes in precipitated LDL; cTRAP = calculated total peroxyl radical trapping – calculation: [0.63 (albumin) + 1.02 (uric acid) + 1.50 (bilirubin)]; Met = metabolic syndrome; Data presented as mean ± standard deviation (S.D.) for parametric and median (IQR) for non-parametric variables.

MetS versus controls: *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Female versus male: $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$.

Table 3. Spearman correlation coefficients for components of the metabolic syndrome and parameters of oxidative stress in the combined group (metabolic syndrome plus controls) (N = 80)

	SBP	TG	HDL-C	Glucose	HOMA-IR	MetSC	CD	PON1	GR	GPX1	CAT	CuZnSOD
Waist	0.313	0.533^{+++}	−0.602^{+++}	0.402^{++}	0.570^{+++}	0.717^{+++}	0.336	−0.103	0.377^{+}	−0.160	−0.115	0.049
SBP	–	0.270	−0.147	0.141	0.103	0.405^{++}	0.338	−0.039	0.129	0.076	−0.108	−0.097
TG	-	–	−0.631^{+++}	0.396^{+}	0.453^{++}	0.736^{+++}	0.571^{+++}	−0.170	0.219	−0.067	−0.182	0.017
HDL-C	-	-	–	−0.357^{+}	−0.405^{+}	−0.681^{+++}	−0.374^{+}	0.321	−0.148	0.086	0.133	−0.015
Glucose	–	-	-	–	0.555^{+++}	0.540^{+++}	0.019	−0.103	0.127	−0.286	−0.081	−0.118
HOMA-IR	–	-	-	-	–	0.493^{+++}	0.099	−0.088	0.216	0.025	−0.066	−0.073
MetSC	-	-	-	-	–	–	0.442^{++}	−0.193	0.261	−0.097	−0.249	−0.115

SBP = Systolic blood pressure; TG = triglycerides; HDL-C = high density lipoprotein; HOMA-IR = homeostasis model assessment of insulin resistance; Met = metabolic syndrome; MetSC = number of components of the MetS (N = 1–5; waist circumference, glucose, triglycerides, HDL-C, SBP); GPX1 = glutathione peroxidase 1; GR = glutathione reductase; CAT = catalase; CuZnSOD = CuZn-superoxide dismutase; PON1 = paraoxonase-1-arylesterase activity; CD = conjugated dienes in precipitated LDL. $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; after Bonferroni adjustment.

patients. In contrast, activities of CAT (–7.3%; $p < 0.05$) and PON1 (–11.7%; $p < 0.05$) as well as serum concentration of GSH (–61%; $p < 0.05$) were significantly decreased. The HOMA-IR demonstrated evidence of a significantly increased insulin resistance in subjects with MetS.

Under resting physiological conditions, biologic systems generate only small amounts of the superoxide anion. Its overproduction can result from mitochondrial electron leakage in hyperglycemia [15]. Other causes of superoxide overproduction are increased activities of NAD(P)H oxidases [16], xanthine oxidase, lipoxygenase, and cyclooxygenase as well as an imbalance in the thioredoxin system [17]. Large amounts of superoxide and other RONS arise in the accumulated fat, mainly due to increased activities of NAD(P)H oxidases and a decreased expression of antioxidant enzymes [18]. Adipose tissue is an important generator of oxidative stress and inflammation, contributing to the production of pro-inflammatory cytokines

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(TNFα, IL-1, IL-6 etc.). Oxidative stress is supposed to worsen the inflammatory state in MetS via activation of redox-sensitive transcription factors (particularly NFκB) by RONS, inducing the expression of TNFα and IL-6. These cytokines increased CRP synthesis. However, in our study, we did not find a statistically significant difference in CRP levels between MetS patients and controls. This could be caused by the method used for CRP measurement. The method used in our study lacks the sensitivity to differ between low-grade inflammation in MetS (CRP between 1.0 and 3 mg/l) and subjects without MetS (CRP < 1.0 mg/l).

The raised CuZnSOD activities in the erythrocytes of patients with MetS found in our study may be compared with the results of Mitrijevic-Sreckovic et al. [19], who described slightly increased CuZnSOD activities in children with MetS in comparison with obese children without MetS. Studies on serum CuZnSOD activities did not show consistent results [20, 21]. Increased CuZnSOD activity results in raised amounts of H_2O_2 which becomes toxic when activity of CAT is normal or decreased. Induction of one enzyme (CAT or CuZnSOD) does not necessarily lead to the induction of the other one [22]. Another source of H_2O_2 is its passage through the erythrocyte membrane [23]. The elevated production of ROS in the endothelium could thus lead to increased levels of ROS also in erythrocytes.

In our study, we have found a significantly decreased activity of CAT. Because of the increased activity of CuZnSOD in our study, elevated levels of H_2O_2 have to be expected. According to study of Kirkman et al. [24], during lengthy exposure of CAT to H_2O_2, the CAT-bound NADPH became oxidized to $NADP^+$ and activity of CAT fell to about one third of the initial activity. Consequently, the cause of the decrease of CAT activity could be the damage of erythrocyte CAT by H_2O_2. Contrary to our study, Cardona et al. [20, 21] found increased activities of CAT in patients with hypertriglyceridemia (concentration of TG > 1.7 mmol/l) apart from the presence of MetS, and these activities were further increased after fat overload. Decreased activities of CAT were described in patients bearing only individual components of MetS – obesity [25], hypertension [26], or insulin resistance [27]. Decreased activity of CAT implies stressed condition of erythrocytes when complete removal of H_2O_2 is not possible [28]. Low activities of CAT were associated with an increased risk of diabetes mellitus and its complications [5, 29].

The GPX1 activity in our study was not altered in MetS patients. This result is in accordance with the study of Mitrijevic-Sreckovic [19]. On the contrary, Cardona et al. [20, 21] found lower activities of GPX1 in a group of subjects with hypertriglyceridemia, a part of MetS presence, and the drop of its activity was almost to 75% of that of the control group. Bougoulia et al. [30] showed decreased activity of GPX1 in obese subjects as well as an increase after weight reduction.

As expected, concentrations of GSH were significantly decreased and activities of GR increased in our group of subjects with MetS. Decreased concentrations of GSH with opposite changes in GSSG levels were also found in MetS subjects in the study of Cardona et al. [20]. On the other hand, Cardona et al. [21] registered a significant drop in GR activity in MetS subjects. Increased activity of GR could be attributed to a compensatory protective mechanism of the cells against ROS. Furthermore, our expected increase in the GSSG/GSH ratio due to lower levels of GSH may stimulate compensatory increase in GR activity in blood to reduce higher levels of GSSG into GSH [31].

The finding of decreased arylesterase activities of PON1 in our subjects with MetS is in accordance with other studies [32, 33]. Because it was shown [34] that there is a strong positive correlation between arylesterase and paraoxonase activity of PON1, we could therefore discuss arylesterase and paraoxonase activity of PON1 together. Low activities of PON1 have been shown to be associated with oxidative stress, hypercholesterolemia, diabetes mellitus, cardiovascular diseases, and sepsis [34, 35].

In the present study, we found significantly higher concentrations of CD-LDL in subjects with MetS. This test was shown to be the most sensitive indicator of lipid peroxidation and can be regarded as a global marker of systemic oxidative stress [36]. In this study, several

anthropometric and biochemical characteristics of MetS correlated significantly with increased concentrations of CD-LDL, which reflect oxidation of the lipid component of LDL. This finding is in agreement with the results of our studies concerning the severity of MetS, oxidative stress, hypertriglyceridemia, and fatty acid metabolism [37, 38]. The important role of lipid peroxidation in the pathogenesis of MetS has been proven in many experimental and clinical studies [39].

Conclusion

In the present study, we estimated a wide variety of antioxidant enzymes, and activities of several enzymes were changed in subjects with MetS. Enzyme activities were assessed in the erythrocytes where the concentration of enzymes remain stable throughout the life span and reflect adaptive changes in their expression in erythroid precursors. According to our results, alterations of antioxidant enzymes related to MetS are not uniform. While activities of CuZnSOD and GR were higher in the MetS group than in healthy subjects, a decrease in CAT and PON1 as well as the absence of the expected increase in GPX1 indicate a disorder in antioxidant defense mechanisms. Our results could be interpreted that the erythrocytes and their GSH levels and activities of GR and GPX1 protect against oxidative stress in MetS. The severity of MetS, as assessed by the number of its components, significantly correlated with the concentrations of CD-LDL.

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Disclosure Statement

We hereby state that there is no conflict of interest.

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References

1 Grundy SM: Metabolic syndrome: a multiplex cardiovascular risk factor. J Clin Endocrinol Metabol 2007;92:399–404.
2 Roberts CK, Sindhu KK: Oxidative stress and metabolic syndrome. Life Sci 2009;84:705–712.
3 Duracková Z: Some current insights into oxidative stress. Physiol Res 2010;59:459–469.
4 Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J: Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44–84.
5 Halliwell B, Gutteridge JMC: Free Radicals in Biology and Medicine, 4th ed. Oxford, Oxford University Press, 2008.
6 Surh YJ: Transcriptional regulation of cellular antioxidant defense mechanism; in Surh YJ, Packer L (eds): Oxidative Stress, Inflammation and Health. Boca Raton, CRC Press, 2005, pp21–40.
7 Soran H, Younis NN, Charlton-Menys V, Durrington P: Variation in paraoxonase-1 activity and atherosclerosis. Curr Opin Lipidol 2009;20:265–274.
8 Harris ED: Regulation of antioxidant enzymes. FASEB J 1992;6:2675–2683.
9 Alberti KG, Zimmet P, Shaw J: The metabolic syndrome – a new worldwide definition. Lancet 2005;366:1059–1062.
10 Kodydková J, Vávrová L, Zeman M, Jirák R, Macásek J, Stanková B, Tvrzická E, Zák A: Antioxidative enzymes and increased oxidative stress in depressive women. Clin Biochem 2009;42:1368–1374.
11 Griffith OW: Glutathione and glutathione disulphide; in Bergemeyer HU (ed): Methods of Enzymatic Analysis, Weinheim, VCH 1985, pp 521–529.

12 Wieland H, Seidel D: A simple specific method for precipitation of low density lipoproteins. J Lipid Res 1983; 24:904–909.
13 Vogeser M, König D, Frey I, Predel HG, Parhofer KG, Berg A: Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. Clin Biochem 2007;40:964–968.
14 Roth E, Manhart N, Wessner B: Assessing the antioxidative status in critically ill patients. Curr Opin Clin Nutr Metab Care 2004;7:161–168.
15 Yamagishi SI, Edelstein D, Du XL, Brownlee M: Hyperglycemia potentiates collagen-induced platelet activation through mitochondrial superoxide overproduction. Diabetes 2001;50:1491–1494.
16 Paravicini TM, Touyz RM: NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. Diabetes Care 2008;31:S170–S180.
17 Nordberg J, Arnér ES: Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 2001;31:1287–1312.
18 Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I: Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–1761.
19 Dimitrijevic-Sreckovic V, Colak E, Djordjevic P, Gostiljac D, Sreckovic B, Popovic S, Canovic F, Ilic M, Obrenovic R, Vukcevic V, Nikolic D, Nisic T, Milic G, Pejcic G: Prothrombogenic factors and reduced antioxidative defense in children and adolescents with pre-metabolic and metabolic syndrome. Clin Chem Lab Med 2007;45:1140–1144.
20 Cardona F, Túnez I, Tasset I, Montilla P, Collantes E, Tinahones FJ: Fat overload aggravates oxidative stress in patients with the metabolic syndrome. Eur J Clin Invest 2008;38:510–515.
21 Cardona F, Tunez I, Tasset I, Murri M, Tinahones FJ: Similar increase in oxidative stress after fat overload in persons with baseline hypertriglyceridaemia with or without the metabolic syndrome. Clin Biochem 2008;41: 701–705.
22 Amstad P, Peskin A, Shah G, Mirault ME, Moret R, Zbinden I, Cerutti P: The balance between Cu,Zn-superoxide dismutase and catalase affects the sensitivity of mouse epidermal cells to oxidative stress. Biochemistry 1991; 30:9305–9313.
23 Low FM, Hampton MB, Winterbourn CC: Peroxiredoxin 2 and peroxide metabolism in the erythrocyte. Antioxid Redox Signal 2008;10:1621–30.
24 Kirkman HN, Galiano S, Gaetani GF: The function of catalase-bound NADPH. J Biol Chem 1987;262:660–666.
25 Viroonudomphol D, Pongpaew P, Tungtrongchitr R, Phonrat B, Supawan V, Vudhivai N, Schelp FP: Erythrocyte antioxidant enzymes and blood pressure in relation to overweight and obese Thai in Bangkok. Southeast Asian J Trop Med Public Health 2000;31:325–334.
26 Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bächler JP: Relationship between oxidative stress and essential hypertension. Hypertens Res 2007;30:1159–1167.
27 Shin MJ, Park E: Contribution of insulin resistance to reduced antioxidant enzymes and vitamins in nonobese Korean children. Clin Chim Acta 2006;365:200–205.
28 Nandeesha H, Sathiyapriya V, Bobby Z, Pavithran P, Agrawal A, Selvaraj N: Altered oxidant-antioxidant status in non-obese men with moderate essential hypertension. Indian J Med Sci 2007;61:326–331.
29 Chistiakov DA, Zotova EV, Savost'anov KV, Bursa TR, Galeev IV, Strokov IA, Nosikov VV: The 262T>C promoter polymorphism of the catalase gene is associated with diabetic neuropathy in type 1 diabetic Russian patients. Diabetes Metab 2006;32:63–68.
30 Bougoulia M, Triantos A, Kolioakos G: Plasma interleukin-6 levels, glutathione peroxidase and isoprostane in obese women before and after weight loss. Association with cardiovascular risk factors. Hormones (Athens) 2006;5:192–199.
31 Paşaoğlu H, Muhtaroğlu S, Güneş M, Utaş C. The role of the oxidative state of glutathione and glutathione-related enzymes in anemia of hemodialysis patients. Clin Biochem1996:29:567–72.
32 Garin MC, Kalix B, Morabia A, James RW: Small, dense lipoprotein particles and reduced paraoxonase-1 in patients with the metabolic syndrome. J Clin Endocrinol Metab 2005;90:2264–2269.
33 Rizos E, Tambaki AP, Gazi I, Tselepis AD, Elisaf M: Lipoprotein-associated PAF-acetylhydrolase activity in subjects with the metabolic syndrome. Prostaglandins Leukot Essent Fatty Acids 2005;72:203–209.
34 Novak F, Vavrova L, Kodydkova J, Novak F Sr, Hynkova M, Zak A, Novakova O: Decreased paraoxonase activity in critically ill patients with sepsis. Clin Exp Med 2010;10:21–25.
35 Rosenblat M, Aviram M: Paraoxonases role in the prevention of cardiovascular diseases. Biofactors 2009;35: 98–104.
36 Güzel S, Seven A, Satman I, Burçak G: Comparison of oxidative stress indicators in plasma of recent-onset and long-term type 1 diabetic patients. J Toxicol Environ Health A 2000;59:7–14.
37 Zák A, Tvrzická E, Vecka M, Jáchymová M, Duffková L, Stanková B, Vávrová L, Kodydková J, Zeman M: Severity of metabolic syndrome unfavorably influences oxidative stress and fatty acid metabolism in men. Tohoku J Exp Med 2007;212:359–371.
38 Zeman M, Zák A, Vecka M, Tvrzická E, Romaniv S, Konárková M: Treatment of hypertriglyceridaemia with fenofibrate, fatty acid composition of plasma and LDL, and their relations to parameters of lipoperoxidation of LDL. Ann N Y Acad Sci 2002;967:336–341.
39 Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR Jr: Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA 2008;299:2287–2293.

Increased inflammatory cytokines together with impaired antioxidant status persist long after clinical recovery from severe sepsis: correlation with HDL-cholesterol and albumin

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ABSTRACT

Objective: To observe markers of oxidative stress and antioxidant status in relation to inflammatory mediators in septic patients at onset of systemic inflammatory response syndrome (SIRS), one week later and one week after the clinical recovery from sepsis.

Design: The prospective study.

Setting: Multidisciplinary adult intensive care unit (11 beds).

Patients: 30 adult patients in severe sepsis and septic shock (SP); 19 SP completed 3 samplings (S1: enrolled within 24 hours after the onset of sepsis, S7: 7 days after S1, R7: 7 days after the recovery).

Interventions: None

Measurements: C-reactive protein, procalcitonin, interleukins (IL-1β, IL-6, IL-10), tumor necrosis factor α, oxidized-LDL (ox-LDL, conjugated dienes (CD), nitrites, nitrotyrosine, paraoxonase 1 activity, HDL cholesterol, apoprotein A1, serum amyloid, cofactors of antioxidant enzymes, non-enzymatic antioxidants and antioxidant enzyme activities (CuZn-superoxide dismutase, catalase, glutathione peroxidase 1, glutathione reductase).

Main Results: Comparing SP with healthy controls (HC), the enhanced concentrations of C-reactive protein, procalcitonin and bilirubin in serum as well CuZnSOD activity in erythrocytes was found in S1 only. The serum levels of ox-LDL, CD, nitrites and nitrotyrosine were increased in S1, culminated in S7 and reverted nearly to the HC level in R7. The reduction in CAT activity and increased concentration of SAA observed in S1 endured till S7. The increase in IL-6, IL-10 and TNFα accompanied by the decrease in the PON1, GPX1, apo-A1, HDL-C, Se, Zn and albumin appeared in S1 and persisted until R7. The increased TNFα in R7 was in the close negative correlation with HDL-C and albumin concentrations.

Conclusions: Increased level of cytokines, lasting after cessation of clinical signs of severe sepsis, was accompanied by significant depletion of antioxidant capacity and persistence of inflammatory activity. At this critical period of recovery, the patients should be dealt as high risk population thus carefully followed up and considered for special antioxidant, nutritional and physiotherapeutic interventions.

Key words: sepsis, oxidative stress, antioxidant enzymes, cytokines, reactants of acute phase, paraoxonase 1

INTRODUCTION

Sepsis is defined as a systemic inflammatory response syndrome (SIRS) in the presence of infection progressing with different degree of severity (1;2). Patients with severe sepsis and septic shock show deregulation of inflammatory process that corresponds to extensive exhaustion of individual functional reserves and development of organ dysfunction. These patients require intensive care in order to improve survival (3). Nevertheless, many of them who survive beyond intensive care and are clinically recovered still possess subclinical impairments and thus remain susceptible to secondary complications with negative impact for their long-term prognosis. With this respect, using appropriate markers for the identification of these patients at risk would enable the follow up care to concentrate the effort and resources on sufficient functional recovery.

Sepsis arises through the activation of an innate immune response, with changes in the expression and activity of many endogenous mediators of pro- and anti-inflammatory processes (4-6) interplaying in order to eliminate the insult and establish new homeostasis (7;8). SIRS, typically present in early sepsis and lasting 3-5days, is characterized by tachycardia, tachypnoe and abnormal body temperature or white blood count. This predominately pro-inflammatory period is usually followed by the development of so called Compensatory Anti-inflammatory Response Syndrome (CARS), a complex but incompletely defined pattern of immunologic responses to attenuate pro-inflammatory reaction of host that when unbalanced under severe infection can result in energy and immunosuppression with increased susceptibility to the development of a new infection (9-11). From this point of view, pro- and anti-inflammatory cytokines facilitating and modulating the response to the inflammatory stimulus seem to serve as an important prognostic marker of the subsequent patient outcome (12;13). Moreover recent clinical studies have shown that the increased levels of IL-6, TNF-α and IL-10 persisting after clinical recovery from sepsis, rather than their initial

peak, are more characteristic of those patients who ultimately have further complications or die (14;15) **.**

The activation of leukocytes and release of mediators in sepsis is indispensably accompanied by an increased production of reactive oxygen and nitrogen species (RONS) (16). RONS are well recognised for playing a dual role as both deleterious and/or beneficial species. Beneficial effects occur at low/moderate concentrations of RONS and involve physiological role in cellular responses as for example in defence against infectious agents and in the function of a number of cellular signalling pathways. Under physiological conditions, the balance is established between RONS production and antioxidant defence capacity. This balance can be disturbed through variable extent of increased RONS production and/or impaired antioxidant defence. The pro-anti-oxidant imbalance, in favour of the former, is known as oxidative stress (17). Overproduction of RONS is a deleterious process that can be an important mediator of damage to cell structures under pathological conditions (18). The oxidative modification of molecules occurring in adult and paediatric sepsis is probably an important promoter of sepsis progression toward shock and organ dysfunction (16;19).

The idea of the study was to describe inflammatory process of severe sepsis/septic shock in SIRS, CARS and 7 days after the clinical recovery in carefully selected group of ICU patients. The analysis of inflammatory mediators together with oxidative stress markers and antioxidant status would help to confirm clinical stages of sepsis emphasizing the persistence of risk after the recovery (usually after discharge from ICU or hospital) that should be addressed in standard follow up measures to determine the patient status and prognosis as well the choice of appropriate interventions. To our knowledge, studies of this completeness have not been published so far.

PATIENTS AND METHODS

This prospective study was carried out in medical adult intensive care unit (ICU) of the University Teaching Hospital. The study protocol was approved by the institutional review board and the Ethics Committee of the General Teaching Hospital in Prague. Written informed consent was obtained from all participants.

Patients: The population under study consisted of two groups: 30 septic patients (SP) and 30 age and sex matched healthy controls (HC). The sepsis was defined according to the Society of Critical Care Medicine/American College of Chest Physicians (SCCM/ACCP) definitions (2). SP had to fulfil the following inclusion criteria: APACHE II score > 10 and C-reactive protein in serum > 20 mg/l. Exclusion criteria for SP were: antioxidant therapy, chronic dialysis, history of diabetes, generalized tumours, immunosupressive therapy and chemotheraphy. Sepsis was treated according to guidelines (5). HC were defined as individuals without known major disease.

Data collection: Samples from SP were collected three times: during the first 24 hours after ICU admission (S1), 7 days after S1 (S7) and recovery (R7), e. g. 7 days after the cessation of septic clinical sings, CRP < 20 mg/l and temperature < 37 °C. Samples from HC group were obtained once. From the group of 30 SP 8 patients died because of sepsis and 3 SP were lost from follow up because they never fully recovered from sepsis thus all three samplings were available from 19 patients. These SP were compared with group of 19 sex and age matched HC. The main source of sepsis was lung, in 13 cases. In all study participants the medical history and the intake of any medicaments were documented at the study entry. The first seven days after ICU admission, the SOFA score (20;21) was calculated from laboratory and clinical parameters in SP. Blood was taken after overnight fasting from an arterial line (SP) or by puncturing a peripheral vein (HC).

The concentration of C-reactive protein (CRP), procalcitonin (PCT), interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor α (TNFα), serum amyloid A (SAA), oxidized LDL (ox-LDL), albumin, bilirubin, uric acid, Cu, Zn, Fe, Se, vitamins A and E and lipid parameters, as well as PON1 activity were measured in serum. Serum was prepared (after coagulation in vacutainer tubes) by centrifugation at 3500 rpm at 4 °C for 10 min. Conjugated dienes (CD) were measured in precipitated LDL. Activities of antioxidant enzymes were measured in haemolysed erythrocytes. The samples were stored at -80 °C until assay. All samples were marked with unique identification numbers, merging data only after assays had been completed.

Laboratory measurements: The routine biochemical tests were measured in Central Biochemical Laboratory of General Teaching Hospital in Prague.

Concentration of CRP was measured with immunoturbidimetric method using K-ASSAY CRP kit (Kamiya Biomedical Company, USA) on analyzer Hitachi Modular (Japan). Concentration of PCT was measured with immunoluminometric assay (ILMA) using BRAHMS PCT LIA-Kit (Brahms Diagnostica GmbH; catalogue number 54.1, Berlin, Deutschland). Cytokines: IL6, IL10 and TNFα were analyzed using Fluorokine MAP kits (R&D Systems, USA) and Luminex®100 analyzer. Fluorokine MAP kits are composed of a Base kit and a panel of Analyte kits. Each kit contains antibody-coated microparticles and biotinylated detection antibodies. SAA concentration was analysed by a solid phase sandwich ELISA kit (Invitrogen Corporation, USA). The arylesterase activity of PON1 was measured according to the method as previously described by Eckerson et al. using phenylacetate as a substrate (22). The rate of phenol generation was monitored spectrophotometrically at 270 nm. Arylesterase activity of PON1 was calculated using the molar extinction coefficient of the produced phenol (1310 $M^{-1}cm^{-1}$) and expressed as U/ml of serum. Oxidized-LDL measurement was performed by Oxidized LDL ELISA kit (Mercodia, Sweden). Activities of

antioxidant enzymes were determined by spectrofotometric kinetic methods and concentration of CD/LDL was measured as previously described by Kodydková et al. (23). Concentration of nitrotyrosine was measured by a solid phase sandwich ELISA kit (Biovendor**,** Czech Republic). The concentration of nitrites and nitrates in serum was assessed by the Griess reaction according to method of Guevara et al. (24). The total peroxyl radical trapping was calculated according to the formula: [0.63 (albumin) + 1.02 (uric acid) + 1.50 (bilirubin)](25).

Statistical analysis: Data are expressed as mean ± S.D. for parametric and median as median (25^{th}-75^{th} percentiles) for nonparametric variables. Normality of data distribution was tested with Shapiro-Wilks W test. Differences between SP and HC were tested with one-way ANOVA with Dunnettpost test. For nonparametric analysis Kruskal-Wallis ANOVA was used. Friedman ANOVA was used for dependent analysis. All statistical analyses were performed using version 8.0 of StatSoft software Statistica (2007, CZ). $P < 0.05$ was considered to be statistically significant.

RESULTS

Basic characteristics: Table 1 summarizes the demographic and clinical characteristics of 19 SP in all three samplings and 19 sex and age matched HC.

Acute-phase response markers: The serum PCT and CRP concentrations increased in S1 but no significant difference was observed, in S7compared to HC. The increased concentrations of interleukins (IL-6, IL-10, TNF-α) persisted from S1 till R7 and SOFA gradually decreased from S1 till S7 (Figure 1).

Serum markers of oxidative stress: The levels of ox-LDL, CD and nitrotyrosine increased in S1, culminated in S7 and returned to the HC values in R7. Enhanced serum concentration of nitrites/nitrates was observed only in S7 (Figure 2).

Antioxidant capacity: CuZnSOD activity was increased in S1 and returned to the HC value already in S7. The decline in CAT activity found in S1 and S7, returned to the HC level in R7 while the decrease in GPX1 activity persisted in all three samplings. No significant difference in GR activity between HC and individual SP samplings was found (Figure 3).

Table 2 presents non-enzymatic antioxidants and cofactors of antioxidant enzymes. The decrease in concentrations of vitamin A, vitamin E and bilirubin was found in S1 only, however, the decrease in Zn was observed in both S1 and S7. The significant decline of uric acid and the rise in Cu was observed only in S7 compared to HC. Nevertheless all these changes returned nearly to the HC values in R7. On the other hand, the substantial decrease in transferrin, Fe, Se and albumin as well the increase in the ferritin concentrations and Calculated TRAP observed already in S1, persisted still 7 days after recovery (R7) and never reached the HC levels. Marked fall in PON1 activity appeared at the onset (S1) and persisted until recovery (R7). The decline in the PON1 activity was closely followed by decreased HDL-C and ApoA1 concentrations. SAA concentration was significantly increased in S1 and in S7 reaching nearly HC level in R7 (Figure 4). We also measured TC (mmol/l): S1 = 3.3 (2.5-3.5), S7 = 3.7 (2.8-4.3), R7 = 4.4 (4.0-5.2), HC = 5.7 (4.8-6.7); LDL-C (mmol/l): S1 = 1.8 (1.2-2.2), S7 = 2.2 (1.1-2.4), R7 = 2.9 (2.2-3.2), HC = 3.7 (3.0-4.3) and TAG (mmol/l): S1 = 1.3 (0.8-1.9), S7 = 1.8 (1.1-2.4), R7 = 1.5 (1.1-2.1), HC = 1.5 (1.0-1.7).

DISCUSSION

The design of this study emerged from the recent clinical trials monitoring the basic pro-inflammatory (IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines as innate immunity markers on greater population of patients with severe sepsis together with their clinical outcomes (14). These studies have concluded that despite clinical recovery, the patients leaving hospital with increased level of cytokines are exposed to increased risk of death

during next year (15). Our relatively small but carefully selected group of patients in early severe sepsis/septic shock diagnostic category, allowed us to analyse broader set of parameters characteristic for different stages and aspects of inflammatory process in the similar clinical setting and corresponding (similar) mortality rate (14). Nineteen patients were available for three samplings. The first sampling was done within 24 hours after onset of sepsis, the time for second sampling was chosen 7 days later when the signs of SIRS are usually over and organ function is restored. In accordance, we present that the SOFA score was improved by day 7 in this study. The third sampling, 7 days after cessation of all clinical sings of inflammation, reflected the time difference of illness progress in individual subjects. This timing enabled us to catch patients in the similar stage of recovery regardless of the sepsis duration and subsequent inflammatory complications occurrence. The hospital discharge as the time for last sampling, used in the study cited above (26), we considered as inappropriate due to bias caused by organisational aspects of health care system such as accessibility of follow up care. Despite of this difference in timing, we confirmed the persistence of increased levels of cytokines after the cessation of sepsis in R7. Many studies have evidenced a significant correlation between the level of individual cytokines and other markers of SIRS/sepsis together with its severity and patient outcome. TNF-α and IL-6 are known to mediate mainly pro-inflammatory SIRS while IL-10 is the most important in CARS response.

In our group of patients a significant decrease in IL-10/TNF- α ratio was caused mainly by the decrease of serum IL-10 level, whereas TNF- α level declined between S1 and S7 and remained practically unchanged after. As for the main acute-phase response markers (CRP, PCT), the enhanced concentrations were observed only in S1 that corresponds with other studies showing particularly PCT as a typical marker of early sepsis (27-29).

We hypothesized that increased levels of cytokines in monitored times would be reflected by the concomitant rearrangement of red-ox status that inspired us to analyse the markers of oxidative damage together with the levels of enzymatic and non-enzymatic antioxidants. As for lipid peroxidation markers, ox-LDL and CD were elevated in S1, persisted till S7 and both returned nearly to the values of HC range in R7. In line, the study of Behnes et al. also presented the increased concentration of ox-LDL in patients with severe sepsis during the first week of illness (30). Similarly, the endotoxin administration caused a sharp rise in plasma levels of CD in the porcine model of burn and sepsis (31). Another study showed increased TBARS and protein carbonyls as markers of lipid peroxidation and protein oxidation, respectively. While TBARS normalized during 7 days of sepsis, increased protein carbonyls persisted still three months after the onset of sepsis, probably due to the slow protein turnover (32). In accordance with other studies (33-35), we present decreased serum concentrations of vitamins E and A in S1. These vitamins are lipid phase antioxidants, crucial for prevention of lipid peroxidation(36). The increased level of the nitrotyrosine appeared already in S1, persisted till S7 while the nitrites/nitrates were increased just in S7 however both parameters were normalized after recovery in R7. The rise in these nitrogen compounds is in accordance with previous studies on septic shock patients indicating enhanced NO and RNS formation during the generalized inflammatory response(32). The observed shift between starting of growth in nitrotyrosine and nitrites/nitrates is in line with results of Strand et al. who showed that peak of nitrotyrosine need not coincide with the peak of nitrites/nitrates concentration in septic shock (37).

The important findings of our study reveal that whereas increased concentrations of peroxidation products are accompanied by diminished antioxidant capacity in the course of sepsis (SIRS and CARS), lowered antioxidant capacity is still persisting after the recovery (R7) while peroxidation products are nearly normalized with the close negative correlation of

ox-LDL to GPX1 and albumin (R = -0.528 and -0.519 respectively). Normal levels of lipid markers of peroxidation in R7 are accompanied with low level of antioxidant capacity

We confirmed reduced antioxidant defence capacity in septic critically ill patients (16) and we have found that reduction of some its components even lasted in R7. In our study, increased CuZnSOD and decreased CAT and GPX1 activities in erythrocytes were found in S1. While CuZnSOD normalization was observed already in S7, the decrease in GPX1 and the trend to the decline in CAT activities persisted still in R7. In line, Warner et al.(38) also found the increased activity of CuZnSOD in erythrocytes at the onset of sepsis. Similarly in pediatric sepsis there was also observed apparent trend towards the increase of CuZnSOD activity in erythrocytes (39). CuZnSOD is one of the most important antioxidant enzymes responsible for the decomposition of superoxide radical while producing H_2O_2 that is further transformed to H_2O by the CAT and GPX1 action. It is necessary to note that the increase in CuZnSOD activity observed in the early stage of sepsis cannot be, by principle, the result of the rise in protein amount because mature erythrocytes do not possess any transcriptional apparatus but it is the result of the activity stimulation (40). We propose that the increase of CuZnSOD in combination with simultaneous decrease in CAT and GPX1 activities may intensify the H_2O_2 accumulation with subsequent spontaneous formation of highly reactive hydroxyl radicals causing escalation of oxidative damage. Therefore, the increased CuZnSOD activity in S1 may act predominantly as a pro-oxidant (41) . Published results on erythrocyte CAT in sepsis are rather controversial to our study. Warner et al.(38) and Leff et al.(42) published increased activity of CAT in both erythrocytes and plasma of SP. The decrease in CAT activity observed in our group of SP could possibly be explained by the results of the *in vitro* study published by Kirkman et al.(43) where human erythrocyte CAT was exposed (for 12-24hr) to H_2O_2. The catalase-bound NADPH, important for its activity, became oxidized to $NADP^+$ causing subsequent CAT activity fell down to about one-third of the initial value (43).

We have found decreased activity of GPX1 during the sepsis and after recovery. The main reason could be low level of GSH as well decline in Se concentration observed in sepsis (35;44;45). Reduced glutathione (GSH) acts as a reducing substrate of GPX. and Se, bound in the active site of the enzyme in the form of one selenocysteine residue, is essential for its activity (46). In accordance suppressed activity of GPX1was accompanied by the decrease in the Se concentration till R7. Supplementation with Se has been shown to improve antioxidant capacity as demonstrated by increased GPX activity (47). As for the decrease of GPX1 activity in R7, we have also to consider relatively long regeneration of the enzyme due to the slow turnover of mature erythrocytes. The enzyme was shown to protect red blood cells against haemoglobin oxidation and haemolysis (48) that is why the diminished antioxidant capacity of erythrocytes could impact on the patient outcome in the case of secondary insult.

Serum PON1 is considered as further antioxidant enzyme playing important role in defence against oxidative stress (49;50). We confirmed our pilot study presenting the decline of PON1 activity in sepsis (51) and on larger set of patients we have shown that this decrease persisted till R7. Simultaneously another authors published the decrease of PON1activity in patients at the onset of sepsis compared to HC (52;53). It was found that antioxidative effect of HDL on LDL oxidative modifications is mediated by HDL-bound PON1. The inactivation of PON1 by ox-LDL involves the interaction of oxidized lipids with its free sulfhydryl group. Thus the ability of PON1 to protect LDL against oxidation is together accompanied by inactivation of the enzyme (54).

In this study, the decrease in PON1, HDL-C and apo-A1concentrations was closely followed by a marked increase of SAA persisting until R7. It is known that during inflammation SAA replaces Apo-A1 and displaces PON1 from the association with HDL, accompanied by the decrease in its activity (55). Our finding of decreased PON1 activity in SP is consistent with the aforementioned parallels and therefore this enzyme activity should

be classified among the negative acute phase parameters. Together with the PON1 decrease and in accordance with others, we observed the fall down of total cholesterol (TC) which just as PON1 and HDL-C did not normalized in R7. Similar decrease of HDL-C, in the course of severe sepsis, was also observed in the study of van Leeuwen et al.(56)**.** The fall in HDL-C negatively correlated with persisting increase in TNF-α.

We have measured decreased values of TC, LDL-C and HDL-C in SP in all three samplings. Similarly to serum lipids, the decrease in serum albumin, Apo-A1, transferrin and Fe in all three samplings was also observed. In line with our results Gordon *et al*. showed that in critically ill patients, the mean high-density lipoprotein cholesterol (HDL-C) concentration was significantly lower in patients with an infection compared to patients without infection (57).

We have seen a good positive correlation of HDL-C with albumin and to a lesser extent with CRP (58) and the correlation with HDL-C found in this study points towards HDL-C as an acute phase reactant. Changes in acute-phase protein synthesis are mediated by cytokines produced in response to a variety of stimuli in multiple cell types that include macrophages, monocytes, T lymphocytes, endothelial and parenchymal cells (59). Several clinical and experimental studies suggest that high circulating levels of different cytokines may be responsible for the cholesterol decrease in acute illness (60).

We have seen a good correlation of HDL-C with albumin and, to a lesser extent, with CRP. Albumin and CRP are well known as acute phase proteins (49) and the correlation with HDL-C found in this study points towards HDL-C as an acute phase reactant. Changes in acute-phase protein synthesis are mediated by cytokines produced in response to a variety of stimuli in multiple cell types that include macrophages, monocytes, T lymphocytes, endothelial and parenchymal cells (49). Several clinical and experimental studies suggest that high circulating levels of different cytokines may be responsible for the cholesterol decrease

in acute illness (60-62). The correlation of HDL-C and IL-6 found in this study strengthens the association of HDL-C with the acute phase response. We observed also a correlation between HDL-C and procalcitonin. Clinical and laboratory parallels with low grade inflammatory process in atherosclerosis – the higher markers of inflammation the higher probability of complications (ischemia, infections etc.). Moreover in the well-functioning elderly subjects, preinfection systemic levels of TNF- α. and IL-6 were associated with higher risk of subsequent infection (15).

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Reference List

(1) Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 2003; 31(4):1250-1256.

(2) American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992; 20(6):864-874.

(3) Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29(7):1303-1310.

(4) Calvano SE, Xiao W, Richards DR, Felciano RM, Baker HV, Cho RJ et al. A network-based analysis of systemic inflammation in humans. Nature 2005; 437(7061):1032-1037.

(5) Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008; 36(1):296-327.

(6) Pierrakos C, Vincent JL. Sepsis biomarkers: a review. Crit Care 2010; 14(1):R15.

(7) Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348(2):138-150.

(8) Gullo A, Bianco N, Berlot G. Management of severe sepsis and septic shock: challenges and recommendations. Crit Care Clin 2006; 22(3):489-501, ix.

(9) Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). Ann Intern Med 1996; 125(8):680-687.

(10) Adib-Conquy M, Cavaillon JM. Compensatory anti-inflammatory response syndrome. Thromb Haemost 2009; 101(1):36-47.

(11) Ward NS, Casserly B, Ayala A. The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. Clin Chest Med 2008; 29(4):617-25, viii.

(12) Rodriguez-Gaspar M, Santolaria F, Jarque-Lopez A, Gonzalez-Reimers E, Milena A, de la Vega MJ et al. Prognostic value of cytokines in SIRS general medical patients. Cytokine 2001; 15(4):232-236.

(13) Bozza FA, Salluh JI, Japiassu AM, Soares M, Assis EF, Gomes RN et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. Crit Care 2007; 11(2):R49.

(14) Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med 2007; 167(15):1655-1663.

(15) Yende S, D'Angelo G, Kellum JA, Weissfeld L, Fine J, Welch RD et al. Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. Am J Respir Crit Care Med 2008; 177(11):1242-1247.

(16) Crimi E, Sica V, Williams-Ignarro S, Zhang H, Slutsky AS, Ignarro LJ et al. The role of oxidative stress in adult critical care. Free Radic Biol Med 2006; 40(3):398-406.

(17) Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82(1):47-95.

(18) Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39(1):44-84.

(19) von DB, Bongain J, Molina V, Quilodran J, Castillo R, Rodrigo R. Oxidative stress as a novel target in pediatric sepsis management. J Crit Care 2011; 26(1):103-107.

(20) Ferreira FL, Bota DP, Bross A, Melot C, Vincent JL. Serial evaluation of the SOFA score to predict outcome in critically ill patients. JAMA 2001; 286(14):1754-1758.

(21) Vincent JL, de MA, Cantraine F, Moreno R, Takala J, Suter PM et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998; 26(11):1793-1800.

(22) Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet 1983; 35(6):1126-1138.

(23) Kodydkova J, Vavrova L, Zeman M, Jirak R, Macasek J, Stankova B et al. Antioxidative enzymes and increased oxidative stress in depressive women. Clin Biochem 2009; 42(13-14):1368-1374.

(24) Guevara I, Iwanejko J, Dembinska-Kiec A, Pankiewicz J, Wanat A, Anna P et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clin Chim Acta 1998; 274(2):177-188.

(25) Roth E, Manhart N, Wessner B. Assessing the antioxidative status in critically ill patients. Curr Opin Clin Nutr Metab Care 2004; 7(2):161-168.

(26) Friedman G, Silva E, Vincent JL. Has the mortality of septic shock changed with time. Crit Care Med 1998; 26(12):2078-2086.

(27) Kibe S, Adams K, Barlow G. Diagnostic and prognostic biomarkers of sepsis in critical care. J Antimicrob Chemother 2011; 66 Suppl 2:ii33-ii40.

(28) Luzzani A, Polati E, Dorizzi R, Rungatscher A, Pavan R, Merlini A. Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med 2003; 31(6):1737-1741.

(29) Uzzan B, Cohen R, Nicolas P, Cucherat M, Perret GY. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 2006; 34(7):1996-2003.

(30) Behnes M, Brueckmann M, Liebe V, Liebetrau C, Lang S, Putensen C et al. Levels of oxidized low-density lipoproteins are increased in patients with severe sepsis. J Crit Care 2008; 23(4):537-541.

(31) Baron P, Traber LD, Traber DL, Nguyen T, Hollyoak M, Heggers JP et al. Gut failure and translocation following burn and sepsis. J Surg Res 1994; 57(1):197-204.

(32) Andresen M, Regueira T, Bruhn A, Perez D, Strobel P, Dougnac A et al. Lipoperoxidation and protein oxidative damage exhibit different kinetics during septic shock. Mediators Inflamm 2008; 2008:168652.

(33) Ogilvie AC, Groeneveld AB, Straub JP, Thijs LG. Plasma lipid peroxides and antioxidants in human septic shock. Intensive Care Med 1991; 17(1):40-44.

(34) Goode HF, Cowley HC, Walker BE, Howdle PD, Webster NR. Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. Crit Care Med 1995; 23(4):646-651.

(35) Weber SU, Lehmann LE, Schewe JC, Thiele JT, Schroder S, Book M et al. Low serum alpha-tocopherol and selenium are associated with accelerated apoptosis in severe sepsis. Biofactors 2008; 33(2):107-119.

(36) Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001; 54(3):176-186.

(37) Strand OA, Leone A, Giercksky KE, Kirkeboen KA. Nitric oxide indices in human septic shock. Crit Care Med 2000; 28(8):2779-2785.

(38) Warner A, Bencosme A, Healy D, Verme C. Prognostic role of antioxidant enzymes in sepsis: preliminary assessment. Clin Chem 1995; 41(6 Pt 1):867-871.

(39) Cherian S, Jameson S, Rajarajeswari C, Helena V, Latha L, Anu Rekha MR et al. Oxidative stress in sepsis in children. Indian J Med Res 2007; 125(2):143-148.

(40) Taylor DE, Piantadosi CA. Oxidative metabolism in sepsis and sepsis syndrome. J Crit Care 1995; 10(3):122-135.

(41) Bulger EM, Maier RV. Antioxidants in critical illness. Arch Surg 2001; 136(10):1201-1207.

(42) Leff JA, Parsons PE, Day CE, Moore EE, Moore FA, Oppegard MA et al. Increased serum catalase activity in septic patients with the adult respiratory distress syndrome. Am Rev Respir Dis 1992; 146(4):985-989.

(43) Kirkman HN, Galiano S, Gaetani GF. The function of catalase-bound NADPH. J Biol Chem 1987; 262(2):660-666.

(44) Forceville X, Vitoux D, Gauzit R, Combes A, Lahilaire P, Chappuis P. Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients. Crit Care Med 1998; 26(9):1536-1544.

(45) Sakr Y, Reinhart K, Bloos F, Marx G, Russwurm S, Bauer M et al. Time course and relationship between plasma selenium concentrations, systemic inflammatory response, sepsis, and multiorgan failure. Br J Anaesth 2007; 98(6):775-784.

(46) Ding L, Liu Z, Zhu Z, Luo G, Zhao D, Ni J. Biochemical characterization of selenium-containing catalytic antibody as a cytosolic glutathione peroxidase mimic. Biochem J 1998; 332 (Pt 1):251-255.

(47) Mishra V, Baines M, Perry SE, McLaughlin PJ, Carson J, Wenstone R et al. Effect of selenium supplementation on biochemical markers and outcome in critically ill patients. Clin Nutr 2007; 26(1):41-50.

(48) Mills GC. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J Biol Chem 1957; 229(1):189-197.

(49) Mackness B, Hine D, McElduff P, Mackness M. High C-reactive protein and low paraoxonase1 in diabetes as risk factors for coronary heart disease. Atherosclerosis 2006; 186(2):396-401.

(50) Precourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E et al. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis 2011; 214(1):20-36.

(51) Novak F, Vavrova L, Kodydkova J, Novak F, Sr., Hynkova M, Zak A et al. Decreased paraoxonase activity in critically ill patients with sepsis. Clin Exp Med 2010; 10(1):21-25.

(52) Kedage V, Muttigi MS, Shetty MS, Suvarna R, Rao SS, Joshi C et al. Serum paraoxonase 1 activity status in patients with liver disorders. Saudi J Gastroenterol 2010; 16(2):79-83.

(53) Draganov D, Teiber J, Watson C, Bisgaier C, Nemzek J, Remick D et al. PON1 and oxidative stress in human sepsis and an animal model of sepsis. Adv Exp Med Biol 2010; 660:89-97.

(54) Aviram M, Rosenblat M, Billecke S, Erogul J, Sorenson R, Bisgaier CL et al. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med 1999; 26(7-8):892-904.

(55) James RW, Deakin SP. The importance of high-density lipoproteins for paraoxonase-1 secretion, stability, and activity. Free Radic Biol Med 2004; 37(12):1986-1994.

(56) van Leeuwen HJ, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. Crit Care Med 2003; 31(5):1359-1366.

(57) Gordon BR, Parker TS, Levine DM, Saal SD, Wang JC, Sloan BJ et al. Low lipid concentrations in critical illness: implications for preventing and treating endotoxemia. Crit Care Med 1996; 24(4):584-589.

(58) Luthold S, Berneis K, Bady P, Muller B. Effects of infectious disease on plasma lipids and their diagnostic significance in critical illness. Eur J Clin Invest 2007; 37(7):573-579.

(59) Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999; 340(6):448-454.

(60) Gordon BR, Parker TS, Levine DM, Saal SD, Wang JC, Sloan BJ et al. Relationship of hypolipidemia to cytokine concentrations and outcomes in critically ill surgical patients. Crit Care Med 2001; 29(8):1563-1568.

(61) Akgun S, Ertel NH, Mosenthal A, Oser W. Postsurgical reduction of serum lipoproteins: interleukin-6 and the acute-phase response. J Lab Clin Med 1998; 131(1):103-108.

(62) Fraunberger P, Schaefer S, Werdan K, Walli AK, Seidel D. Reduction of circulating cholesterol and apolipoprotein levels during sepsis. Clin Chem Lab Med 1999; 37(3):357-362.

Table 1. Clinical characteristics of studied groups

	SP			HC
	S1	**S7**	**R7**	
N (M/F)	10/9			10/9
AGE (years)	74 (56-79)			71 (56-78)
APACHE II	16.0 (13.0-23.0)	-	-	-
Diagnosis (medical/surgical)	11/8			-
Source of sepsis (lungs/others)	12/7			-
Day of sampling	1	7	22.0 (14.0-34)	-
ICU hospitalization (days)	20.0 (9-53)			-
Hospitalization (days)	24.0 (16.0-61)			-
Duration of sepsis (days)	14.0 (6.0-26)			-
SOFA	7.0 (2.5-10.0)	3.0 (1.5-9.0)	-	-
APV (number/percent)	7 (36.8 %)	7 (36.8 %)	1 (5.3 %)	-
CRRT (number/percent)	0	3 (15.8 %)	-	-

SP: septic patients; S1: SP enrolled within 24 hours after the onset of sepsis, S7: septic patients 7 days after S1 and R7: septic patient one week after the recovery from sepsis, HC: healthy controls; SOFA: Sequential Organ Failure Assessment, APV: Artificial Pulmonary Respiration, CRRT: Continuous Renal Replacement Therapy; data presented as median and interquartile range (25^{th}-75^{th} percentile).

Table 2. Non-enzymatic antioxidants, cofactors of antioxidant enzymes and other parameters of antioxidant capacity

	SP			HC
	S1 (n = 19)	**S7 (n = 19)**	**R7 (n = 19)**	**(n = 19)**
Vitamin E (mg/l)	12.2 ± 4.6[a,c]	14.5 ± 4.55	16.4 ± 5.0	18.2 ± 8.6
Vitamin A (mg/l)	0.52 ± 0.20[a,b,c]	0.81 ± 0.28	0.96 ± 0.44	0.97 ± 0.27
Fe (µmol/l)	2.8 (2.0-3.3)[a,b,c]	7.1 (4.8-10.0)[a,c]	11.6 (7.5-13.6)[a]	20.0 (15.6-27.3)
Ferritin (µg/l)	452 (240-1436)[a]	356 (222-1347)[a]	278 (194-646)[a]	84 (67-161.3)
Transferin (g/l)	1.58 (1.13-1.91)[a,b,c]	1.86 (1.55-2.18)[a,c]	2.19 (2.05-2.35)[a]	2.65 (2.45-3.09)
Ceruloplasmin (g/l)	0.43 ± 0.08	0.47 ± 0.12	0.45 ± 0.10	0.40 ± 0.07
Cu (µmol/l)	20.3 ± 3.7	22.5 ± 5.1[a]	21.6 ± 4.7	18.5 ± 3.2
Zn (µmol/l)	8.9 ± 2.9[a,b,c]	11.8 ± 2.6[a,c]	14.1 ± 3.6	15.1 ± 1.7
Se (µg/l)	33.3 ± 13.3[a,c]	46.5 ± 28.4[a]	53.7 ± 24.3[a]	72.5 ± 13.8
Albumin (µmol /l)	437 ± 95[a,c]	438 ± 118[a,c]	548 ± 944[a]	707 ± 63
Bilirubin (µmol/l)	14.8 (9.4-25.9)[c]	12.5 (6.1-21.4)	7.7 (6.7-17.0)	10.3 (7.3-14.5)
Uric acid (µmol/l)	270 ± 103	224 ± 106[a,c]	293 ± 122	331 ± 90
cTRAP (µmol/l)	585 ± 143[a,c]	535 ± 157[a,c]	669 ± 143[a]	781 ± 132

S1: patients enrolled within 24 hours after the onset of sepsis, S7: patients 7 days after S1 and R7: one week after the recovery, HC: healthy controls; cTRAP: calculated total peroxyl radical trapping - calculation: [0.63 (albumin) + 1.02 (uric acid) + 1.50 (bilirubin)]; data presented as mean ± S.D. for parametric and median (25^{th}-75^{th} percentile) for nonparametric variables; [a] septic patients (all samplings) vs. healthy controls, [b] S1 vs. S7, [c] S1 or S7 vs. R7; $p < 0.05$.

Table 3. Correlations of inflammatory markers, albumin, HDL-C and ox-LDL

		Albumin	HDL-C	Ox-LDL/LDL-C
CRP	**S1**	-0,556**	-0,384	0,398
	S7	-0,743***	-0,546***	0,464*
	R7	-0.306	-0.018	-0.070
	HC	-0.512*	-0,242	0,117
PCT	**S1**	-0,324	-0,472*	0,405
	S7	-0,563*	-0,680**	0,548*
	R7	-0.341	-0.140	0.511*
	HC	-0,173	0,051	0,075
IL-6	**S1**	-0,448	-0,123	-0,116
	S7	-0,641**	-0,351	0,465*
	R7	-0.120	-0.169	0.049
	HC	-0,637**	-0,247	-0,009
IL-10	**S1**	0,172	-0,052	-0,093
	S7	-0,456*	-0,387	0,349
	R7	-0.712***	-0.302	0.144
	HC	-0,380	0,026	-0,043
TNF	**S1**	0,060	-0,299	0,116
	S7	-0,503*	-0,523*	0,552*
	R7	-0.775***	-0.456*	0.464*
	HC	0,234	0,669**	0,526*

S1: patients enrolled within 24 hours after the onset of sepsis, S7: patients 7 days after S1 and R7: one week after the recovery, HC: healthy controls; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

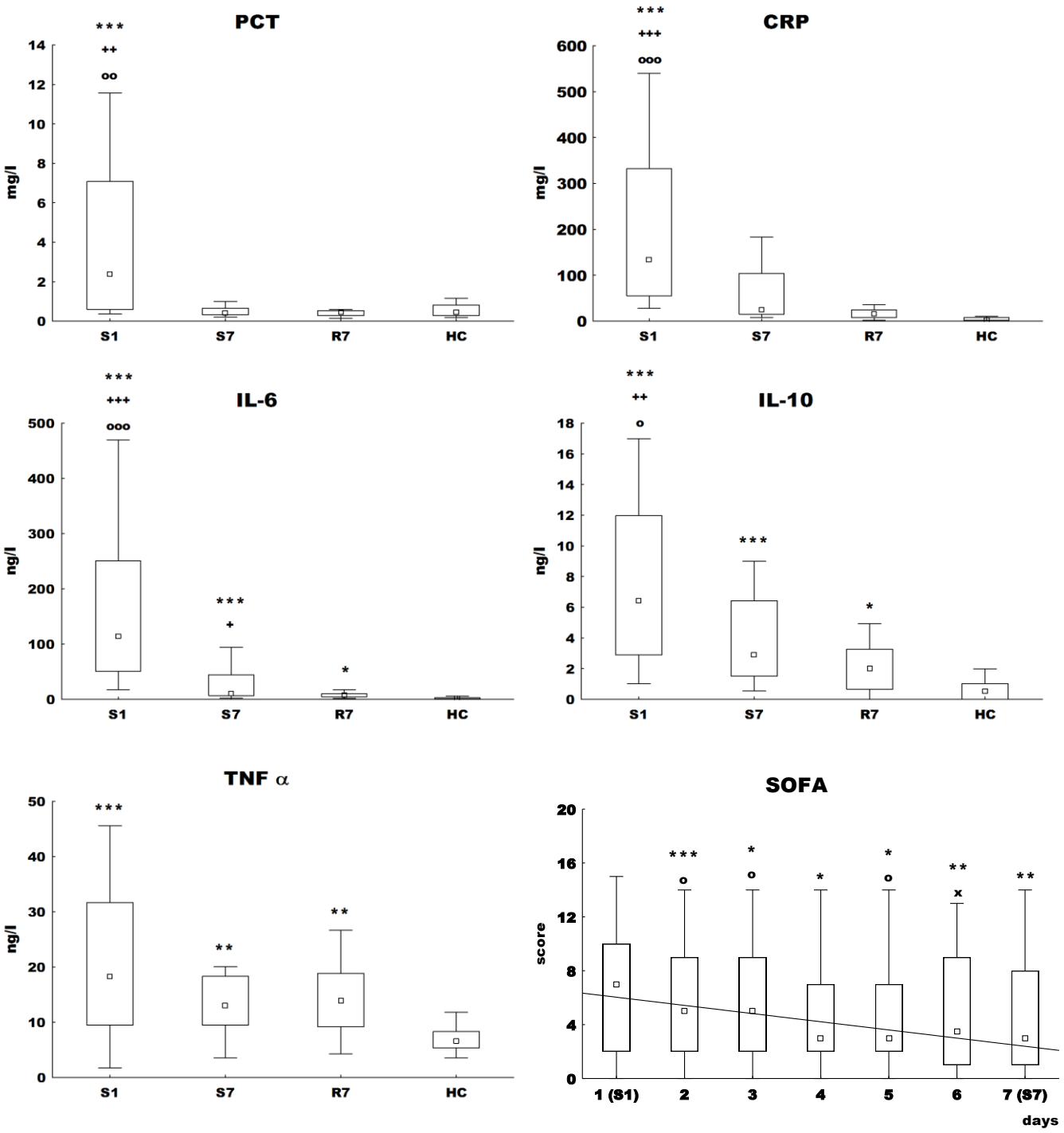

Figure 1. Changes in inflammation markers and SOFA in the course of sepsis.

S1: septic patients enrolled within 24 hours after the onset of sepsis, S7: septic patients 7 days after S1 and R7: septic patients one week after the recovery, HC: healthy controls; PCT: procalcitonin, TNF-α: tumor necrosis factor α, IL-6: interleukin-6, IL-10: interleukin-10; Data

presented as median (quartile, range), * S1 or S7 or SR7 vs. HC; $^{+}$ S1 or S7 vs. R7; ^{b} S1 vs. S7; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

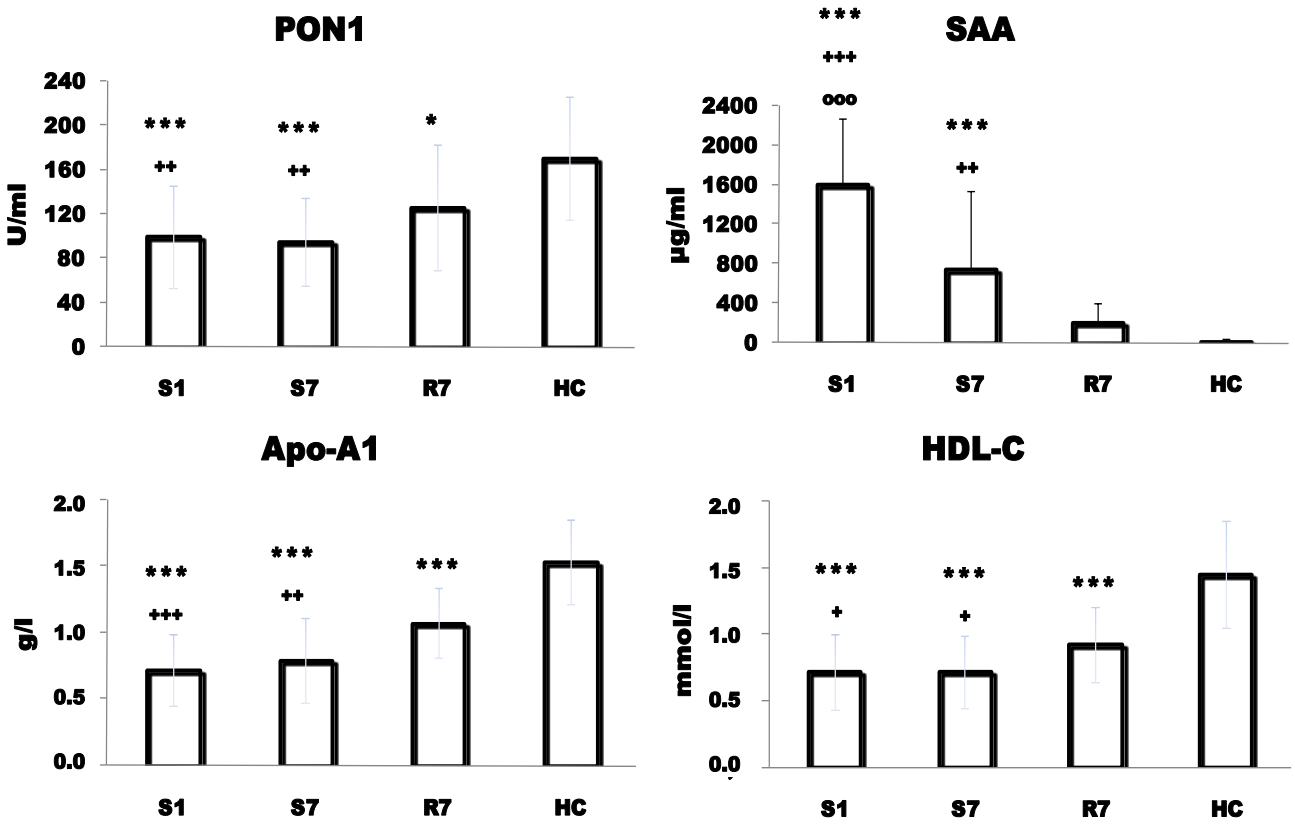

Figure 2. Changes in PON1 activity and associated parameters in the course of sepsis

S1: septic patients enrolled within 24 hours after the onset of sepsis (n = 19), S7: septic patients 7 days after S1 (n = 19) and R7: septic patients one week after the recovery (n = 19), HC: healthy controls (n = 19); PON1: enzyme paraoxonase1 – arylesterase activity, SAA: serum amyloid A, Apo-A1: apolipoprotein A1, HDL-C: high density lipoprotein cholesterol, data presented as mean ± S.D., * S1or S7 or R7 vs. HC; $^{+}$ S1 or S7 vs. R7; ^{b} S1 vs. S7; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

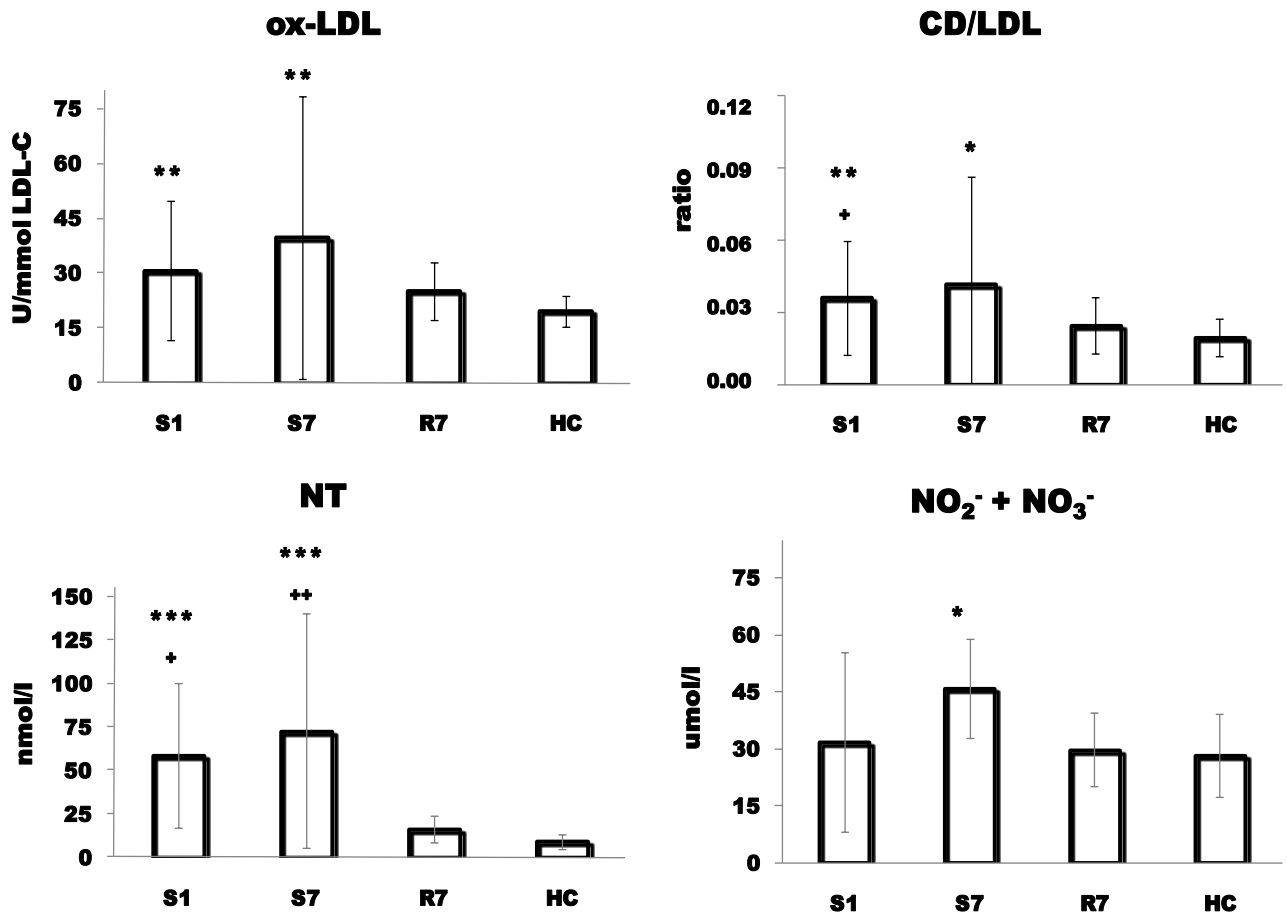

Figure 3. Changes in activities of oxidative stress parameters in the course of sepsis

S1: septic patients enrolled within 24 hours after the onset of sepsis (n = 19), S7: septic patients 7 days after S1 (n = 19) and R7: septic patients one week after the recovery (n = 19), HC: healthy controls (n = 19); Ox-LDL: oxidized low density lipoproteins, CD: conjugated dienes in precipitated LDL, LDL-C: low density lipoprotein cholesterol, NT: 3-nitrotyrosine; data presented as mean ± S.D., * S1or S7 or R7 vs. HC; $^{+}$ S1 or S7 vs. R7; ^{b} S1 vs. S7; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

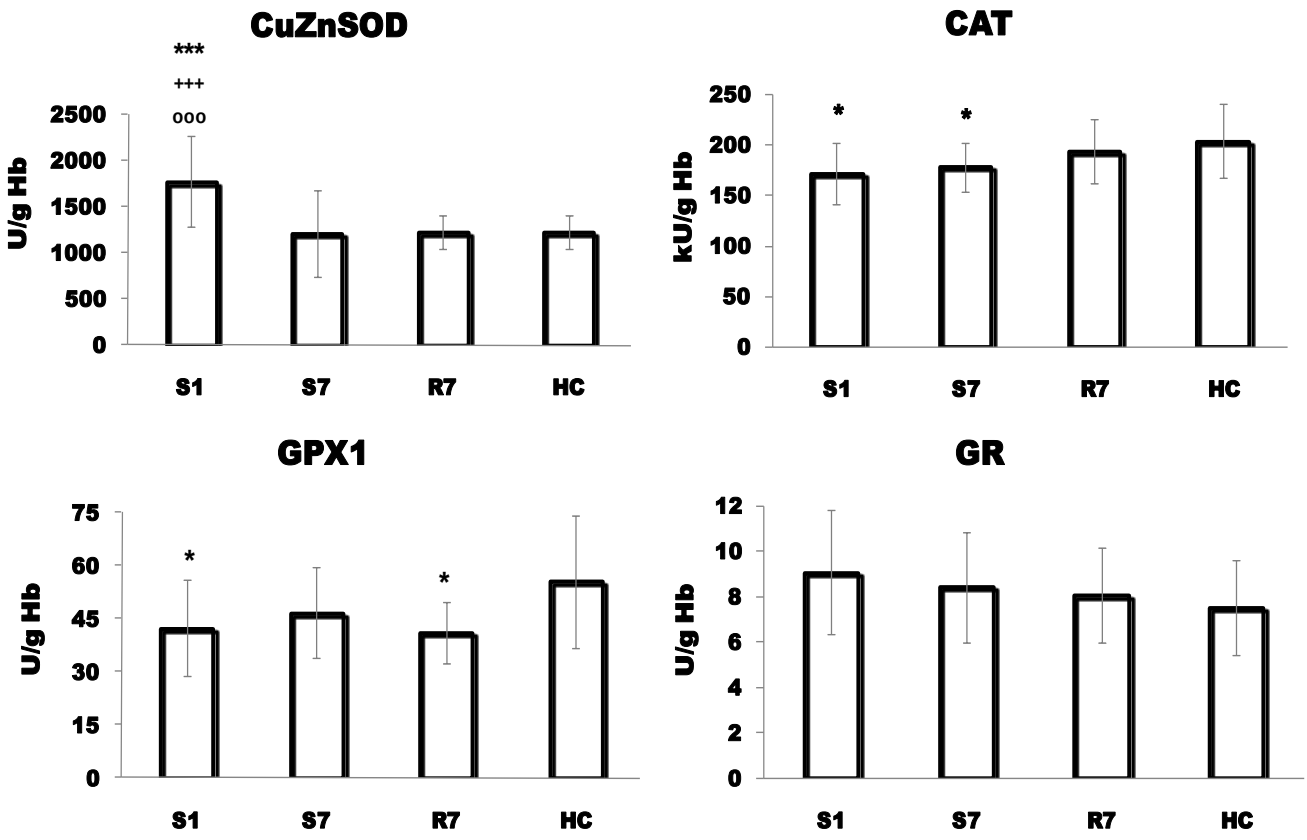

Figure 4. Changes in activities of antioxidant enzymes in the course of sepsis

S1: patients enrolled within 24 hours after the onset of sepsis (n = 19), S7: patients 7 days after S1 (n = 19) and R7: one week after the recovery (n = 19), HC: healthy controls (n = 19); CuZnSOD: superoxide dismutase, CAT: catalase, GPX1: glutathione peroxidase1, GR: glutathione reductase; data presented as mean ± S.D., * S1or S7 or R7 vs. HC; $^{+}$ S1 or S7 vs. R7; ^{b} S1 vs. S7; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

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Oxidační stres v průběhu akutní pankreatitidy

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SOUHRN
Cíl studie: stanovení parametrů oxidačního stresu a statutu antioxidačního systému v průběhu akutní pankreatitidy
Typ studie: observační, strukturálně vyvážená studie případů a kontrol
Materiál a metody: Do studie bylo zařazeno 13 pacientů s akutní pankreatitidou (AP) a dále na základě věku a pohlaví spárované dvě kontrolní skupiny, a to skupina zdravých osob (KON) a osob, které prodělaly v minulých 2-3 letech akutní pankreatitidu (PAP). Pacientům s AP byly odebírány vzorky celkem 4, nejprve během prvních 24 hodin od objevení příznaků, poté po 72 hodinách, třetí odběr byl prováděn 5. den a poslední odběr 10. den onemocnění. U všech pacientů byly stanovovány kromě základních klinických a biochemických parametrů aktivity antioxidačních enzymů, koncentrace některých antioxidantů (redukovaný glutation (GSH), vitamin A a E) a parametry oxidačního stresu (konjugované dieny v precipitovaných LDL (CD/LDL) a oxidované LDL(ox-LDL)). Ke statistickému zpracování výsledků byl použit program STATISTICA (Stat Soft, CZ).
Výsledky: Výsledky naší studie potvrzují zvýšený oxidační stres u pacientů s AP, a to zvýšenými hladinami CD/LDL u všech odběrů AP ve srovnání s CON ($p < 0,05$) a vzrůstajícími hladinami ox-LDL v průběhu AP s maximem 5. den AP. Pozorovali jsme rovněž změny v antioxidačním systému u AP pacientů; u těchto pacientů jsme zjistili snížené aktivity glutationperoxidázy a arylesterázové i laktonázové paraoxonázy během všech odběrů a dále pak snížené hladiny sérových antioxidantů – albuminu, vitaminu A a vitaminu E při porovnání s kontrolní skupinou.
Závěr: Ve studii byl pozorován zvýšený oxidační stres a porušený antioxidační systém v časné fázi AP s gradací mezi třetím a pátým dnem AP.
Klíčová slova: akutní pankreatitida, oxidační stres, antioxidační enzymy

SUMMARY
Vávrová L., Kodydková J., Macášek J., Ulrych J., Žák A.: Oxidative stress in the course of acute pancreatitis
Objective: to assess oxidative stress and antioxidant status in acute pancreatitis and their natural course over the 10-day period.
Design: observation, matched case-control study
Material and methods: Into our study 13 patients with acute pancreatitis (AP) were included together with 13 sex- and age-healthy controls (CON) and 13 sex- and age- matched controls enrolled from persons that suffered from AP 2 – 3 years ago (PAP). We observed the antioxidant status of AP patients during the disease and the samplings were taken four times – on the first 24 hours of disease (AP1), after 72 hours from disease onset (AP3), on the 5th (AP5) and on the 10th day (AP10). In all studied groups markers of oxidative stress (level of conjugated dienes in precipitated LDL, oxidized LDL) and levels of antioxidants were assessed. We measured activities of superoxide dismutase (CuZnSOD), catalase (CAT), glutathione peroxidase 1 (GPX1) and glutathione reductase (GR) in erythrocytes and arylesterase (PON1-A) and lactonase (PON1-L) activities of paraoxonase in serum and concentrations of reduced glutathione (GSH) in erythrocytes and concentrations of vitamins E and A in serum.
Results: In our study we confirmed increased oxidative stress in AP, with higher levels of CD/LDL in all AP samplings compared to CON ($p < 0.05$) and with increasing levels of ox-LDL during the AP with the maximum on the 5th day. We have shown altered status of antioxidant system; the activities of both PON1 activities as well as activity of GPX1 were depressed in all AP samplings in comparison to CON. We have also observed decreased levels of serum antioxidants – albumin, vitamin A and vitamin E in AP
Conclusion: High oxidative stress and impaired antioxidant status was observed during early phase of AP with the gradation between 3rd and 5th day of AP.
Key words: acute pancreatitis, oxidative stress, antioxidant enzymes

Úvod

V patogenezi všech akutních zánětlivých procesů hrají důležitou roli reaktivní formy kyslíku (ROS), které se uplatňují v časné fázi zánětu, jako vysoce aktivní metabolity vedoucí k poruše buněčné homeostázy, k poškození DNA a k peroxidaci membránových lipidů s následným zvýšením permeability a k buněčné smrti [1]. Udržení oxidační rovnováhy organismů zajišťuje antioxidační systém, tvořený antioxidačními enzymy – superoxiddismutáza (SOD), kataláza (CAT), glutationperoxidáza (GPX), glutationreduktáza GR) a paraoxonáza (PON) – a neenzymovými antioxidanty, kde nejdůležitějším je redukovaný glutation (GSH) [2].

Cílem naší práce bylo sledovat změny antioxidačního systému v průběhu akutní pankreatitidy (AP), která představuje rychle se rozvíjející zánětlivý proces spojený s významnými metabolickými změnami a významnou klinickou odezvou. Klíčovými patogenetickými pochody, které probíhají během rozvoje AP, jsou autodiges-

ce, patologická stimulace zánětlivých buněk, ischemie, reperfuze a hemoragie. Významným faktorem, který se uplatňuje v patogenezi AP je oxidační stres (OS), [1]. Mezi nejčastější etiologické faktory vedoucí k rozvoji AP se řadí alkohol a cholelitiáza [3].

Materiál a metody

Do pilotní observační studie bylo celkem zařazeno 13 pacientů s AP a dále pak na základě věku a pohlaví spárované dvě kontrolní skupiny – skupina 13 zdravých osob (CON) a skupina 13 osob, jež během 2-3 let před odběrem prodělaly akutní pankreatitidu a v době studie byly bez obtíží (PAP). U pacientů s AP byly prováděny celkem 4 náběry krevních vzorků: první náběr byl proveden během prvních 24 hodin od objevení prvních příznaků (AP1), druhý odběr po 72 hodinách (AP3), třetí náběr byl uskutečněn 5. den (AP5) a poslední náběr pak 10. den onemocnění (AP10). Pacienti s AP byli vybíráni na JIP IV. Interní kliniky a JIP I. chirurgické kliniky hrudní, břišní a úrazové chirurgie 1. LF UK a VFN v Praze. U těchto pacientů probíhala diagnostika a zařazení do studie na základě následujících kritérií: aktivita AMS, APACHE II skóre, Ransonova kriteria, koncentrace C-reaktivního proteinu (CRP), CTSI skóre, kontrastního CT vyšetření. Na základě nové klasifikace závažnosti AP dle Petrova et al. (2010) [4] se v jednom případě jednalo o kritickou AP (pacient v průběhu studie zemřel), ve 4 případech o středně těžkou a v 8 případech o lehkou formu AP. U 8 pacientů byla AP biliárního původu, u 2 pacientů se jednalo o etylickou AP a u 2 o idiopatickou AP, v jednom případě byla AP vyvolána endoskopickou retrográdní cholangio-pankreatografií (ERCP).

Do kontrolní skupiny CON byli zařazeni zdraví dobrovolníci, do druhé kontrolní skupiny PAP byli zařazeni dobrovolníci vybíraní z pacientů, kteří byli před 2-3 roky hospitalizováni na IV. Interní klinice s diagnózou akutní pankreatitidy a v době studie netrpěli žádným chronickým onemocněním pankreatu. Z těchto 13 osob, 6 prodělalo v minulosti těžkou formu AP a 7 lehkou formu AP, v 5 případech se jednalo o biliární, ve 4 případech o etylickou a ve 3 případech o idiopatickou pankreatitidu, v jednom případě byla AP vyvolána vyšetřením ERCP. Pro všechny osoby platila stejná vylučovací kritéria: zavedená terapie antioxidanty (farmakologické dávky vitaminu C a E, allopurinol, N-acetylcystein), chronická dialýza, imunosuprese, manifestní diabetes mellitus, generalizace tumoru a chemoterapie. Studie byla schválena Etickou komisí VFN Praha. Všechny osoby zařazené do studie podepsaly informovaný souhlas.

U všech osob zařazených do studie byly prováděny odběry krevních vzorků po celonočním lačnění (min. 10 hodin). Odebrané krevní vzorky byly zpracovány do 1 hodiny od náběru a materiál pro další analýzy byl uchováván při -80°C. U pacientů byly sledovány základní klinické, antropometrické a biochemické parametry, dále pak byly stanovovány aktivity antioxidačních enzymů CAT, GPX1, GR, CuZnSOD v erythrocytech a arylesterázové a laktonázové aktivity PON1 v séru, koncentrace antioxidantů jako je redukovaný glutation (GSH) v erythrocytech, či vitaminy E a A, albumin a bilirubin v séru. Jako parametr oxidačního stresu byla měřena koncentrace konjugovaných dienů v precipitovaných LDL (CD/LDL) a hladina oxidovaných LDL (ox-LDL) v séru. Speciální vyšetření (hladiny antioxidantů, markery oxidačního stresu) byla prováděna v laboratořích IV. Interní kliniky, rutinní biochemické parametry a stanovení hladin vitaminů bylo provedeno v Ústavu lékařské biochemie a laboratorní diagnostiky VFN Praha. Metody ke stanovení aktivity antioxidačních enzymů a koncentrací GSH a CD/LDL byly podrobně popsány v publikaci Kodydkové et al. (2009) [5], ke stanovení ox-LDL byl využit komerčně dodávaný ELISA kit od firmy Mercodia. Ke stanovení hladin selenu byla využita atomová absorpční spektrometrie s elektrotermickou atomizací (ETAAS) na Varian Spectra A220 FS. Koncentrace vitaminů A a E byla stanovena pomocí diagnostických kitů (Radanal s. r. o., ČR) a metody vysokoúčinné kapalinové chromatografie (HPLC) s UV detektorem (Ecom).

Výsledky jsou vyjádřeny jako průměr ± S. D. pro parametrické veličiny a jako medián (0,25-0,75 percentil) pro neparametrické veličiny. Normalita byla testována prostřednictvím Shapiro-Wilkova W testu. Rozdíly mezi jednotlivými skupinami AP vs. kontrolní soubory byly zkoumány pomocí jedno-faktorové ANOVY s Neuman-Keulsovým post-testem. Pro neparametrickou analýzu byla použita Kruskal-Wallisova ANOVA. Při testování rozdílů mezi jednotlivými odběry pacientů s AP byla použita ANOVA pro závislé vzorky. Pro všechny statistické analýzy byl používán program STATISTICA 10.0 (Stat Soft, CZ). Za statisticky signifikantní byly považovány výsledky s $p < 0,05$.

Výsledky

Do studie bylo zařazeno celkem 13 pacientů s diagnostikovanou AP s průměrným APACHE II skóre (APACHE II = 5,7 ± 3,8) při vstupu do studie. Základní biochemické charakteristiky jednotlivých skupin jsou shrnuty v Tabulce 1.

Hlavními sledovanými parametry byly antioxidanty a markery OS. Jako markery OS byly měřeny hladiny CD/LDL a ox-LDL. V koncentraci CD/LDL nebyly zjištěny žádné signifikantní rozdíly mezi jednotlivými odběry AP, ale vyšší hladiny CD/LDL byly pozorovány u pacientů s AP během všech odběrů ve srovnání s CON ($p < 0,05$). Hladina ox-LDL se v průběhu AP zvyšovala a svého maxima dosáhla 5. den onemocnění (obr. 1).

Ze sledovaných antioxidačních enzymů docházelo k největším změnám aktivit v průběhu AP u obou sledovaných aktivit PON1. Obě PON1 aktivity byly ve všech odběrech AP signifikantně snížené při srovnání s CON. Nejnižší aktivita u PON1-A byla pozorována 5. den AP (obr. 2). V aktivitách GPX1, GR a CuZnSOD nebyly pozorovány žádné rozdíly mezi jednotlivými odběry u AP. Aktivita CAT byla signifikantně zvýšená v AP1 oproti AP10 (231,7 ± 21,2 vs. 219,8 ± 26,0; $p < 0,05$).

Při srovnání aktivit těchto enzymů u AP s kontrolními skupinami, byla pozorována snížená aktivita

Table 1: Basic biochemical characteristics of the studied groups

	AP1	PAP	CON
N (M/F)	13 (9/4)	13 (9/4)	13 (9/4)
Age (years)	56.1 ± 21.5	54.8 ± 20.8	55.8 ± 19.4
Glucose (mmol/l)	6.6 ± 2.9**	6.1 ± 1.4**	5.2 ± 0.4
TC (mM)	4.9 ± 3.3	4.9 ± 1.3	5.2 ± 1.2
α-AMS (μkat/l)	10.5 (7.0 – 19.4)***+++	0.4 (0.3 - 0.4)	0.5 (0.3 - 0.6)
ALT (μkat/l)	1.7 (0.7 – 4.6)***+	0.4 (0.3 - 0.6)	0.5 (0.4 - 0.6)
AST (μkat/l)	1.8 (0.7 – 3.9)***+	0.5 (0.4 - 0.6)	0.4 (0.4 - 0.5)
GGT (μkat/l)	4.3 (1.9 – 8.5)***+++	0.6 (0.4 - 0.7)	0.4 (0.3 - 0.5)
WBC (*10⁹/l)	13,2 ± 5,5***+++	6.6 ± 1.0	6.6 ± 1.5
PCT (μg/l)	0.16 (0.13 – 0.84)***+	0.05 (0.05 - 0.05)*	0.03 (0.02 - 0.03)
Albumin (g/l)	36.5 ± 7.8***+++	48.4 ± 4.1	47.1 ± 3.1

AP1: acute pankreatitis- first sampling, CON: healthy controls, PAP: controls 2-3 years after AP; M: male, F: female, TC: total cholesterol, TG: triacylglycerols, α-AMS: panceatic α-amylase, ALT: alanin-amino-transferase, AST: Aspartat-amino-transferase, GGT: γ-glutamyl-transferase, PCT: procalcitonin, WBC: white blood cells; * AP or PAP vs. CON, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; + AP vs. PAP, + $p < 0.05$; ++ $p < 0.01$, +++ $p < 0.001$

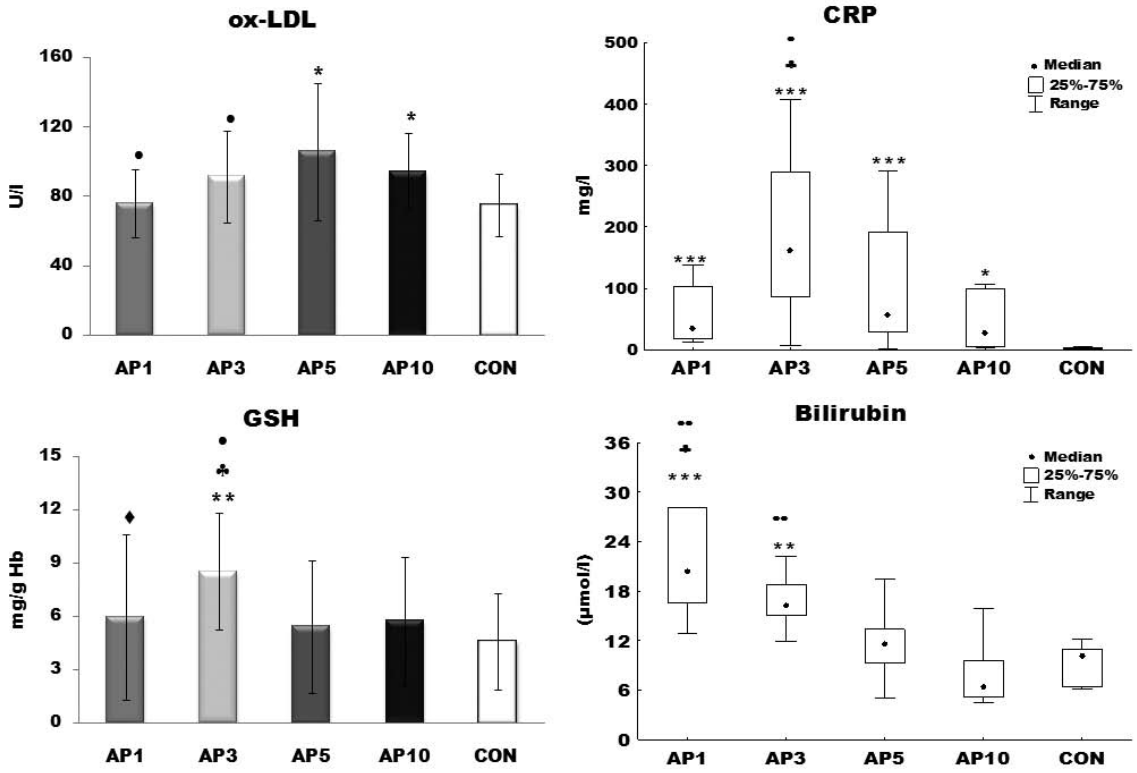

Fig. 1. Parameters of oxidative stress and antioxidant status in course of acute pancreatitis

ox-LDL: oxidized LDL, CRP: C-reactive protein, GSH: reduced glutathione; AP: patients with acute pancreatitis (1, 3, 5, 10: days of sampling), CON: healthy controls; * AP group vs. CON, * $p < 0.05$, ** $p < 0.01$; ♣ AP1 or AP3 or AP5 vs. AP10, ♣ $p < 0.05$; ♣♣ $p < 0.01$ • AP1 or AP3 vs. AP5, •$p < 0.05$, ••$p < 0.01$; ♦ AP1 vs. AP3, ♦ $p < 0.05$, ♦♦ $p < 0.01$;

GPX1 během všech AP odběrů v porovnání s CON, a dále pak snížená hodnota GPX1 u PAP ku CON (obr. 3). U CAT byla pozorována zvýšená aktivita u pacientů s AP během 1., 3. a 5. dne při srovnání s PAP ($p < 0,05$). Aktivita CAT při AP10 se již signifikantně nelišila od PAP, ale zato byl pozorován trend ke sníženým hodnotám vůči CON ($p = 0,06$). Při ostatních odběrech byla CAT u AP srovnatelná s hodnotami CON. Pro aktivity GR a CuZnSOD nebyly zjištěny žádné rozdíly mezi kontrolními skupinami a AP.

Z neenzymatických antioxidantů byla sledována koncentrace GSH, která byla signifikantně nejvyšší 3. den AP (obr. 1) a hladiny sérového albuminu (Tabulka 1) a bilirubinu. Hladiny albuminu byly u všech AP odběrů signifikantně snížené oproti oběma kontrolním skupinám a mezi sebou se nelišily. Koncentrace bilirubinu byly nejvyšší při záchytu AP a postupně docházelo k jejich poklesu (obr. 1). Dále pak byla stanovována koncentrace vitaminů E a A při AP1 a srovnávána s oběma kontrolními skupinami (obr. 3), koncentrace obou vitaminů byla snížená u AP1 ve srovnání s CON.

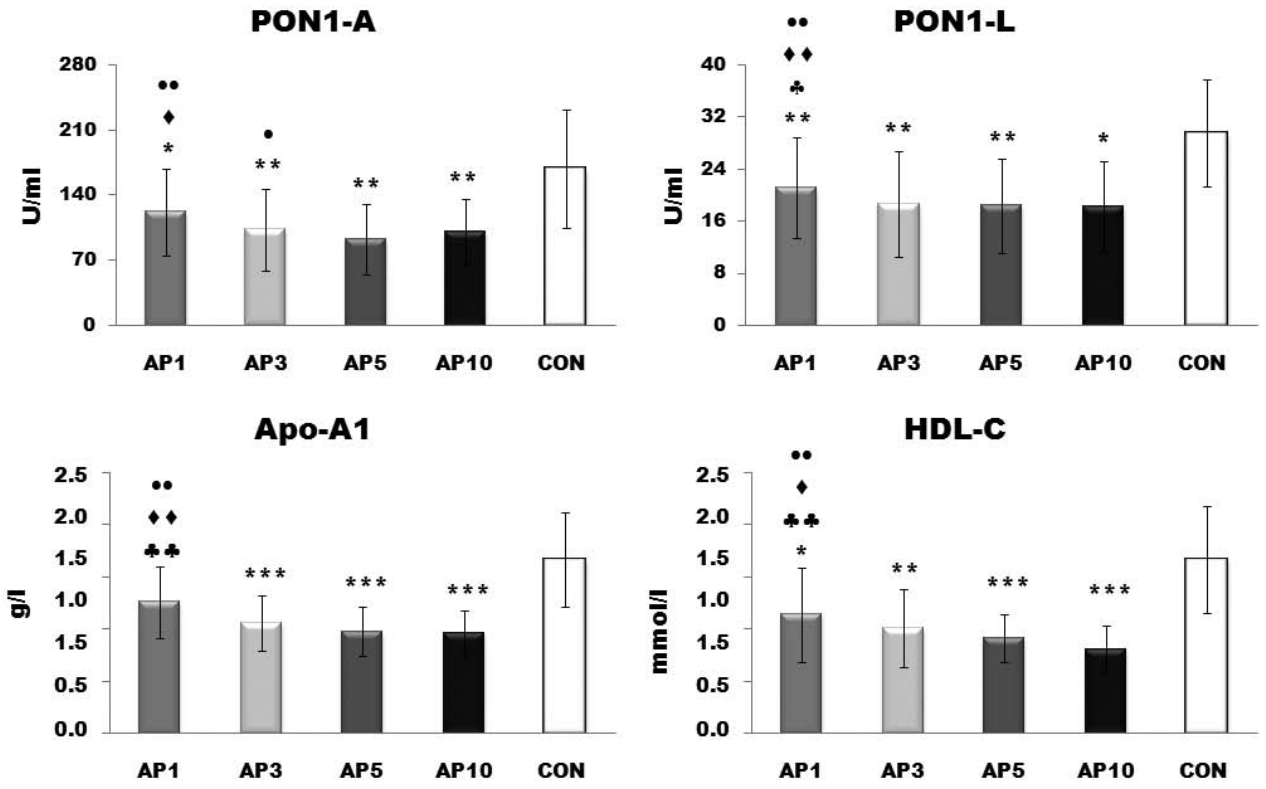

Fig. 2. Paraoxonase and its associate parameters in course of acute pancreatitis

PON1-A: arylesterase activity of paraoxonase 1, PON1-L: lactonase activity of paraoxonase 1, HDL-C: high density lipoprotein, Apo-A1: apolipoprotein A1; AP: patients with acute pancreatitis (1, 3, 5, 10: days of sampling), CON: healthy controls; * AP group vs. CON, * $p < 0.05$, ** $p < 0.01$; ♣ AP1 or AP3 or AP5 vs. AP10, ♣ $p < 0.05$; ♣♣ $p < 0.01$ • AP1 or AP3 vs. AP5, •$p < 0.05$, ••$p < 0.01$; ♦ AP1 vs. AP3, ♦ $p < 0.05$, ♦♦ $p < 0.01$;

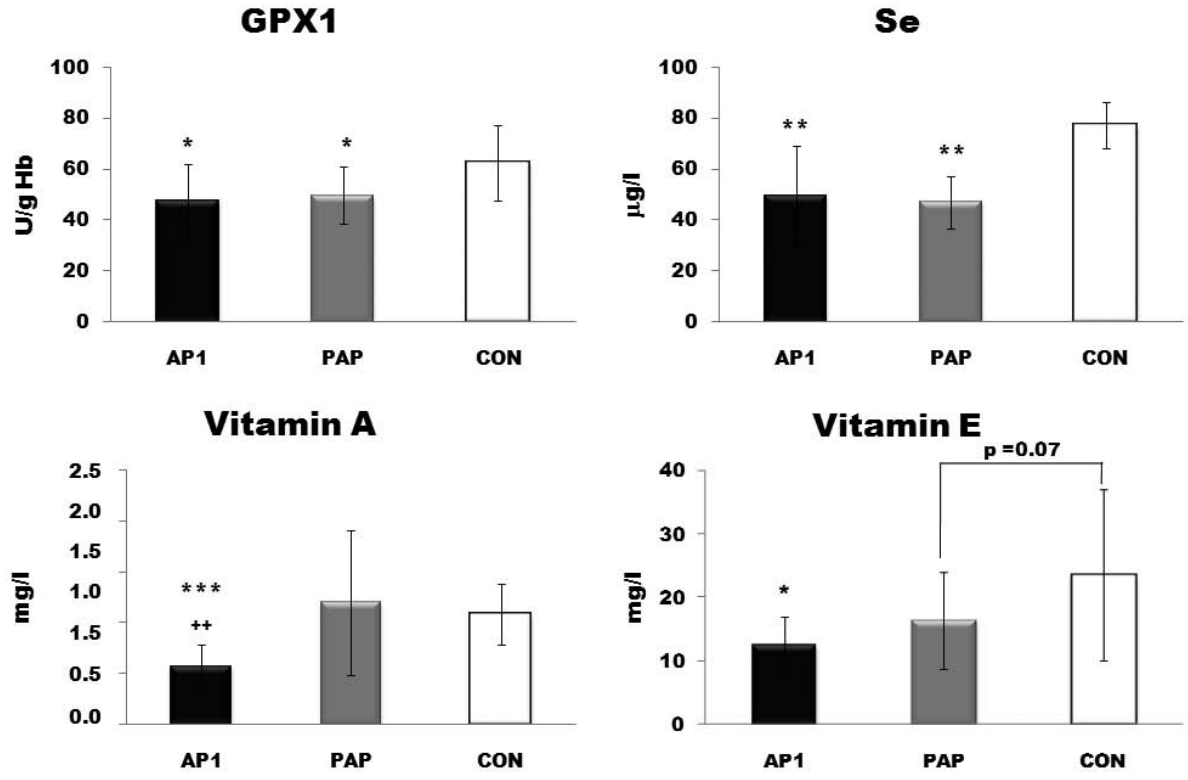

Fig. 3. Antioxidants in acute pancreatitis

GPX1: glutathione peroxidase 1, AP1: patients with acute pancreatitis, CON: healthy controls, PAP controls 2-3 years after AP; * AP or PAP vs. CON, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $^{+}$ AP or R vs. CON, $^{++}$ $p < 0.01$

Diskuse

V naší studii jsme se zaměřili na sledování jednotlivých komponent antioxidačního systému a měření markerů peroxidace v průběhu AP. Naše výsledky ukazují na zvýšený oxidační stres u tohoto onemocnění, který je doprovázen změnou ve fungování některých složek antioxidačního systému. Největší změny je možno pozorovat v arylesterázové a laktonázové aktivitě PON1 a v aktivitě GPX1, dále pak v koncentraci vitaminů A a E.

U některých antioxidačních enzymů jsme však nepozorovali žádné změny spojené s onemocněním AP. K těmto enzymům se řadí CuZnSOD, u které byly hodnoty aktivit téměř konstantní v průběhu AP. Nepozorovali jsme ani rozdíl mezi aktivitou CuZnSOD u AP a u kontrolních skupin. Dosud publikované výsledky aktivit CuZnSOD v erytrocytech u pacientů s AP jsou nejednotné. Byly publikovány jak snížené [6, 7], tak zvýšené [8] aktivity CuZnSOD u pacientů s těžkou i lehkou formou AP. Zvýšená aktivita extracelulární SOD (EC-SOD) byla pozorována v průběhu AP (1., 3., 7.den) v porovnání s kontrolami [9], kdy 1. den byla signifikantně vyšší než 3. a 7. den. Zvýšenou EC-SOD u AP při srovnání s kontrolami pozorovali ve svých studiích i Góth (1982, 1989) [10, 11] a Szuster-Czielska (2001a) [12]. CuZnSOD má v organismu za úkol odbourávat superoxidový radikál, ze kterého při této reakci vzniká peroxid vodíku, za jehož degradaci jsou zodpovědné CAT, GPX1 a peroxiredoxiny. Při nízkých – fyziologických – koncentracích je H_2O_2 odbouráván GPX1 a peroxiredoxiny, naopak při zvýšeném oxidačním stresu a vyšších koncentracích je za odbourávání odpovědná CAT [13].

V naší studii byly aktivity CAT 1., 3. a 5. den srovnatelné s hodnotami zdravých kontrol, ale signifikantně se lišily od hodnot získaných u skupiny osob, které AP prodělaly před 2-3 lety. Při odběru prováděném desátý den (AP10) byl pozorován signifikantní pokles v aktivitách CAT ve srovnání s AP1 a i se zdravými kontrolami, i když zde je možno mluvit pouze o trendu. Tyto výsledky ukazují, že při dlouhodobém vystavení CAT působení oxidačního stresu, může dojít k poklesu její aktivity. Kirkman a Gaetani (1987) ve své studii ukázali, že dlouhodobé vystavení CAT působení H_2O_2 může vést k oxidaci NADPH na $NADP^+$ a následnému snížení aktivity CAT až na 1/3 její původní aktivity [14]. Ve studii, která se zabývala erythrocytární aktivitou CAT nebyly pozorovány žádné významné rozdíly mezi pacienty s AP a kontrolami [8]. Doposud získané výsledky aktivity CAT v séru ukazují zvýšené aktivity u pacientů s AP ve srovnání s kontrolní skupinou [10 – 12, 15].

Degradace H_2O_2 není jedinou funkcí GPX1, dále je také zodpovědná za odbourávání lipidových peroxidů. Glutationperoxidáza 1 je selenoprotein, jehož aktivita je závislá nejen na dostatku selenu, ale ke své funkci potřebuje GSH jako druhý substrát. U pacientů s AP jsme pozorovali snížené koncentrace selenu a snížené aktivity GPX1 ve srovnání s CON. Aktivita GPX1 byla snížená u všech odběrů AP a také u skupiny PAP. Ve studii, kde se zabývali aktivitou GPX1 v erythrocytech v průběhu AP, pozorovali sníženou hladinu GPX1 u AP až při odběru 9. den AP [16]. V séru byly pozorovány snížené hladiny GPX1 u pacientů s AP vzhledem ke kontrolám již v několika dřívějších studiích [17 – 19], i když existuje i studie, kde nenašli žádný rozdíl mezi pacienty a kontrolami [12]. U koncentrací GPX1 v séru nebyl nalezen rozdíl mezi AP a ambulantními kontrolami [20]. Také snížené koncentrace Se v séru u pacientů s AP byly již dříve publikovány [16, 19], i když opět ne ve všech pracech [21].

Koncentrace GSH byla u našich pacientů s AP srovnatelná s koncentracemi u CON, pouze při odběru 3. den nemoci (AP3) bylo pozorováno zvýšení koncetrace GSH oproti ostatním odběrům AP i oproti CON. Na rozdíl od naší studie Rahman et al. (2004, 2009) [22, 23] ve svých studiích pozoroval snížené hladniny GSH v erytrocytech u lehké i těžké formy AP ve srovnání s kontrolní skupinou, stejně tak pro GSH v séru byly pozorovány signifikantně snížené koncentrace u AP v porovnání s CON [17]. Možným vysvětlením zvýšených hladin GSH u AP3 je obranná reakce organismu na aktuálně vzniklý oxidační stres, ale i možná desynchronizace aktivit GPX1 a GR v období 2. odběru (AP3).

S GPX1 spolupracuje v organismu GR, která udržuje hladinu GSH zpětnou redukcí oxidovaného glutationu vzniklého působením GPX1. V naší studii jsme nepozorovali žádné signifikantní změny v aktivitě GR v průběhu akutní pankreatitidy a nezjistili jsme ani žádný rozdíl při srovnání pacientů s AP s kontrolními skupinami, tento výsledek je ve shodě s již dříve publikovanou studií [17].

Dalšími sledovanými antioxidanty byly vitaminy A a E. Koncentrace obou vitaminů byla signifikantně snížená u pacientů s AP ve srovnání s CON. Snížené hladiny vitaminu A byly pozorovány též ve studii Musil et al. (2005) [12], zatímco u koncentrace vitaminu E nebyl nalezen žádný rozdíl [12, 21].

Posledním sledovaným antioxidačním enzymem byla s HDL asociovaná paraoxonáza, u níž byly měřeny dvě její různé aktivity, a to arylesterázová a laktonázová. Obě tyto aktivity byly v celém průběhu AP signifikantně snížené oproti zdravým kontrolám. U obou aktivit též došlo k dalšímu snížení v rámci odběrů AP3 a AP5, kdy arylesterázová aktivita dosáhla svého minima u odběru 5. den AP. V tento den byly naměřeny též nejvyšší koncentrace oxidovaných-LDL, jako markeru lipidové peroxidace. Kinetika změn aktivit PON v průněhu AP odpovídá změnám aktivit PON1, které byly pozorovány v průběhu sepse a během jejího zotavování, a které mají zřejmě obecnější zákonitosti [24].

Literatura

1. **Špičák, J.,** Kyslíkové radikály v patogenezi akutní a chronické pankreatitidy. In Štípek, S. a kol., *Antioxidanty a volné radikály ve zdraví a v nemoci.* Grada, Praha, 2000; p. 159-163.
2. **Racek, J., Holeček, V.,** Vznik volných radikálů a enzymy. *Klin. Biochem. Metab.*, 1999, 7, p. 158-163.
3. **Špičák, J.,** *Akutní pankreatitida*. Grada, Praha, 2005, 216S, ISBN: 80-247-0942-2.

4. **Petrov, M. S., Windsor, J. A.,** Classification of the severity of acute pancreatitis: how many categories make sense? *Am. J. Gastroenterol.*, 2010, 105, p. 74–76.
5. **Kodydková, J., Vávrová, L., Zeman, M. et al.,** Antioxidative enzymes and increased oxidative stress in depressive women. *Clin. Biochem.* 2009, 42, p. 1368-1374.
6. **Abu-Hilal, M., McPhail, M. J., Marchand, L., Johnson, C. D.,** Malondialdehyde and superoxide dismutase as potential markers of severity in acute pancreatitis. *JOP*, 2006, 7(2), p. 185-192.
7. **Park, B. K., Chung, J. B., Lee, J. H. et al.,** Role of oxygen free radicals in patients with acute pancreatitis. *World J. Gastroenterol*, 2003, 9(10), p. 2266-2269.
8. **Chmiel, B., Grabowska-Bochenek, R. et al.,** Red blood cells deformability and oxidative stress in acute pancreatitis. *Clin. Hemorheol. Microcirc.*, 2002, 27(3-4), p. 155-62.
9. **Thareja, S., Bhardwaj, P., Sateesh, J., Saraya, A.,** Variations in the levels of oxidative stress and antioxidants during early acute pancreatitis. *Trop. Gastroenterol.*, 2009, 30(1), p. 26-31.
10. **Góth, L., Mészáros, I., Németh, H.,** Serum catalase enzyme activity in acute pancreatitis. *Clin. Chem.*, 1982, 28(9), p. 1999-2000.
11. **Góth, L.,** Origin of serum catalase activity in acute pancreatitis. *Clin. Chim. Acta*, 1989, 186(1), p. 39-44.
12. **Szuster-Ciesielska, A., Daniluk, J., Kandefer-Szerszeń, M.,** Oxidative stress in bloodofpatientswithalcohol-relatedpancreatitis. *Pancreas*, 2001a; 22, p. 261-266.
13. **Halliwell, B., Gutteridge, J. M. C.,** Free radicals in biology and medicine. 4th ed. *Oxford University Press*, 2008.
14. **Kirkman, H. N., Galiano, S., Gaetani, G. F.,** The function of Catalase-bound NADPH. *J. biol. Chem.*, 1987, 262(2), p. 660-666.
15. **Fukui, M., Kanoh, M., Takamatsu, Y., Arakawa, Y.,** Analysis of serum catalase activities in pancreatic diseases. *J. Gastroenterol.* 2004, 39, p. 469-474.
16. **Musil, F., Zadák, Z., Solichová, D., Hyspler, R., Kaska, M., Sobotka, L., Manák, J.,** Dynamics ofantioxidants in patientswithacutepancreatitis and in patients operated for colorectal cancer: a clinical study. *Nutrition*, 2005, 21(2), p. 118-124.
17. **Czeczot, H., Majewska, M., Skrzycki, M. et al.,** Activity of GSH-dependent enzymes in blood serum of patients with acute and chronic pancreatitis. *Wiad. Lek.*, 2009, 62, p. 87-92.
18. **Modzelewski, B.,** Serum anti-oxidative barrier in acute pancreatitis. *Pol. Merkur Lekarski*. 2005, 18(106), p. 418-420.
19. **Wereszczynska-Siemiatkowska, U., Mroczko, B., Siemiatkowski, A., Szmitkowski, M., Borawska, M., Kosel, J.,** The importance of interleukin 18, glutathione peroxidase, and selenium concentration changes in acute pancreatitis. *Dig. Dis. Sci.*, 2004, 49, p. 642-650.
20. **Szuster-Ciesielska, A., Daniluk, J., Kandefer-Szerszeń, M.,** Alcohol-related cirrhosis with pancreatitis. The role of oxidative stress in the progression of the disease. *Arch. Immunol. Ther. Exp.*, 2001b, 49(2), p. 139-146.
21. **Morris-Stiff, G. J., Bowrey, D. J., Oleesky, D., Davies, M., Clark, G. W., Puntis, M.C.,** The antioxidant profiles of patients with recurrentacute and chronic pancreatitis. *Am. J. Gastroenterol.*, 1999, 94(8), p. 2135-2140.
22. **Rahman, S. H., Ibrahim, K., Larvin, M., Kingsnorth, A., McMahon, M. J.,** Association of antioxidant enzyme gene polymorphisms and glutathione status with severe acute pancreatitis. *Gastroenterology*, 2004, 126(5), p. 1312-1322.
23. **Rahman, S. H., Srinivasan, A. R., Nicolaou, A.,** Trans sulfuration pathway defects and increased glutathione degradation in severe acute pancreatitis. *Dig. Dis. Sci.*, 2009, 54(3), p. 675-682.
24. **Novak, F., Vavrova, L., Kodydkova, J. et al.,** Decreased paraoxonase activity in critically ill patients with sepsis. *Clin. Exp. Med.*, 2010, 10(1), p. 21-25.

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Antioxidant Status and Oxidative Stress Markers in Pancreatic Cancer and Chronic Pancreatitis

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Objectives: Oxidative stress has been implicated in the pathogenesis of chronic pancreatitis (CP) and pancreatic cancer (PC). The study aim was to assess the oxidative stress markers and antioxidant defense system in patients with CP and those with PC.

Methods: Activities of superoxide dismutase 1 (SOD1), catalase (CAT), glutathione peroxidase 1 (GPX1), glutathione reductase (GR), arylesterase (PON1-A) and lactonase (PON1-L) activities of paraoxonase 1 (PON1) and concentrations of reduced glutathione, conjugated dienes in low-density lipoprotein (CD/LDL) and oxidized LDL (ox-LDL/LDL) were assessed in 50 PC and 50 CP patients and 50 age and sex-matched controls.

Results: Comparison of PC and CP groups to controls found the following changes: glutathione peroxidase 1 (GPX1) (−20.2%, −25.5%; $P < 0.001$), glutathione reductase (GR) (−9.5%, −11.9%; $P < 0.05$), SOD1 (+22.9%; $P < 0.01$), CAT (−10.6%; $P < 0.05$), PON1-A (−34.3%, −16.0%; $P < 0.001$), PON1-L (−44.2%; −17.0%; $P < 0.01$), conjugated dienes in LDL (CD/LDL) (+20%, +33.3%; $P < 0.05$) and ox-LDL/LDL (+42.2%, +14.4%; $P < 0.05$). The patients with PC had changed activities and levels of SOD1 (+24.2%), CAT (−10.4); $P < 0.01$), PON1-A (−21.7%), PON1-L (−32.9%), and ox-LDL/LDL (+24.3%); (all $P < 0.01$) compared with the patients with CP.

Conclusions: Reduced antioxidant defense system capacity and increased markers of oxidative stress were found in PC and CP. PON1-L and CAT activities, along with ox-LDL/LDL levels, were the independent factors differentiating the patients with PC from the patients with CP.

Key Words: oxidative stress, oxidative stress markers, antioxidant enzymes, chronic pancreatitis, pancreatic cancer, discriminant analysis

Abbreviations: CAT – catalase, CD - conjugated dienes, CP - chronic pancreatitis, CT - computed tomography, EUS – endoscopic ultrasonography, GPX1 - glutathione peroxidase 1, GR - glutathione reductase, GSH - reduced glutathione, HDL - high-density lipoprotein, HOMA-IR - homeostasis model assessment of insulin resistance, LDL - low-density lipoprotein, MDA - multivariate discriminant analysis, MRCP - magnetic resonance cholangiopancreatography, NRI – Nutritional Risk Index, NT – nitrotyrosine, ox-LDL – oxidized LDL, PC - pancreatic carcinoma, PON1 - paraoxonase 1, PON1-A - PON1 arylesterase, PON1-L – PON1 lactonase, RONS – reactive oxygen and nitrogen species, ROS - reactive oxygen species, SAA - serum amyloid A, SOD1 - Cu-Zn superoxide dismutase

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Overproduction of reactive oxygen and nitrogen species (RONS) and oxidative stress have been implicated in the pathogenesis of pancreatitis, both in its acute and chronic form, as well as in the pathogenesis of pancreatic cancer (PC).[1] Chronic pancreatitis (CP) shares risks with PC such as smoking and alcohol abuse as well as being a risk factor per se for PC.[1–3] Among them, cigarette smoking, alcohol abuse, diabetes mellitus, and other insulin resistance (IR) states are connected with increased RONS formation and oxidative stress.[1] Chronic pancreatitis is a progressive inflammatory disease with irreversible damage to the pancreas and the destruction of exocrine and endocrine tissue.[4] The underlying causes of CP seem to be multifaceted, including environmental as well as genetic factors, but its pathogenesis to date has not been completely understood. Although most cases of CP have been attributed to alcohol abuse and/or genetic predisposition, other etiologic risk factors such as enhanced oxidative stress could play an important role.[5,6]

Reactive oxygen and nitrogen species are generated during endogenous oxidative stress that is linked to the pancreatic renin-angiotensin system[7] or exogenous oxidative stress caused by environmental or lifestyle-related xenobiotics, which is connected with the detoxification system.[3] It has been proposed that local oxidative stress and reactive oxygen species (ROS) generation, caused by overexpression of membrane nonmitochondrial nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, is connected with pancreatic (patho)biology such as cell growth regulation and apoptosis, acinar cell inflammation, fibrosis, and disturbed islet microcirculation.[7,8] The inflammatory process is associated with increased production of RONS resulting in local or systemic oxidative stress.[9,10] A number of experimental and clinical studies have demonstrated impaired antioxidant status that may be a contributing factor for increasing oxidative stress in CP. The involvement of oxidative stress in CP has been described both in experimental and clinical studies.[1,3]

Oxidative stress could not only be the cause of CP (and PC) but also a consequence of the underlying disease (CP or PC, respectively). Moreover, increased RONS production and oxidative stress seem to be independent from the etiology of CP.[1,11]

In patients with CP, decreased levels of antioxidant thiols (cysteine, glutathione, and cysteinylglycine), decreased total antioxidant capacity, along with increased carbonylated proteins, thiobarbituric acid–reactive substances, malondialdehyde and 4-hydroxynonenal levels were found.[10,12–14] Similarly, in patients with CP (both alcoholic and tropical), decreased concentrations of glutathione, vitamin C, and zinc in erythrocytes were connected with elevated thiobarbituric acid–reactive substances.[15]

Levels of conjugated dienes (CD) are the most sensitive indicator of lipid peroxidation and can be regarded as a global marker of systemic oxidative stress[16] and also are a marker of minimally oxidatively modified low-density lipoprotein (LDL). On the contrary, oxidized LDL (ox-LDL) reflects concentration of malondialdehyde and 4-hydroxynonenal, the highly reactive

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end products of lipid peroxidation that are bound, as adducts, to ε-amino group of lysine in molecule of apolipoprotein B.[17] Increased susceptibility of LDL to oxidation (LDL oxidability) was observed in CP.[18] Concentration of ox-LDL has not yet been studied in human PC.

Among enzymes that regulate RONS, glutathione peroxidase (GPX) and catalase (CAT) play an important role by the reduction of hydrogen peroxide, which is generated by superoxide dismutase (SOD) in the dismutation of superoxide. The main ROS scavenger in the pancreas is supposed to be reduced glutathione (GSH), which is recycled back to its reduced form by glutathione reductase (GR).[1,19] The enzyme paraoxonase 1 (PON1) as high-density lipoprotein (HDL)-associated enzyme is implicated in the anti-inflammatory and antioxidant activities of HDL and impedes oxidative modification of LDL, protects cell membranes from the damage caused by products of lipoperoxidation, and eliminates carcinogenic lipid-soluble radicals.[20–22]

The activities of some antioxidant enzymes in CP were already studied: SOD activity in the studies by Girish et al,[15] Quillot et al,[18] and Szuster-Ciesielska et al[23] and GPX1 activity in the studies by Quillot et al[18] and Szuster-Ciesielska et al.[18,23] These studies show lowered antioxidant capacity in CP.

The aim of the study was to ascertain the importance of lipoperoxidation markers (CD and ox-LDL) in relation to the group of main antioxidant enzymes, such as SOD, CAT, GPX1, GR, and PON1 in patients with CP and PC. Because there are intercorrelations between oxidative stress markers and antioxidant enzymes activities, both in CP and PC groups, we used the multivariate discriminant analysis to differentiate PC from CP as well as to evaluate the discriminative power of different oxidative stress markers and antioxidant enzymes.

MATERIALS AND METHODS

This prospective study was carried out at the Fourth Department of Internal Medicine of General University Hospital from January 2009 to September 2011. The study protocol was approved by the institutional review board and the Ethics Committee of the General University Hospital in Prague. Written informed consent was obtained from all participants.

Patients

The study population consisted of 3 groups: 50 patients with PC, 50 patients with CP, and 50 healthy controls. All groups are age and sex matched.

Diagnosis of PC was confirmed in all of the patients (based on histological examination of pancreatic resection or endoscopic ultrasonography-guided aspiration cytology). The tumor staging was evaluated by the combination of criteria issued by the Union Internationale Contre le Cancer and the American Joint Committee on Cancer (UICC/AJCC 2002).[24] The clinical diagnosis of CP was based on clinical features (abdominal pain, nausea and/or vomiting, anorexia and/or malnutrition, and steatorrhea) confirmed by 2 or more imaging methods (abdominal ultrasonography [USG], contrast-enhanced computed tomography [CT]), endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography (MRCP), and endoscopic ultrasonography (EUS). Only patients with definite CP were included. The grade of CP (mild, moderate, or severe) was assessed according to the M-ANNHEIM pancreatic imaging criteria[25] (M-ANNHEIM stands for M, multiple risk factor classification; A, alcohol consumption; N, nicotine consumption; N, nutritional factors; H, hereditary factors; E, efferent pancreatic duct factors; I, Immunological factors; M, miscellaneous and metabolic factors). All the patients were assessed by the combination of EUS and other imaging methods (CT, or USG, or MRCP) because EUS does not differentiate between the moderate and severe grades, and other methods (CT, or USG, or MRCP) cannot differentiate between mild and moderate changes.[25]

Exclusion criteria for all the 3 groups were the following: current antioxidant therapy (eg, vitamin C, vitamin E, allopurinol, *N*-acetylcysteine, supplementation with n-3 polyunsaturated fatty acids), kidney disease (creatinine >150 μmol/L), clinically manifest proteinuria (urinary protein >500 mg/L), and liver cirrhosis, decompensate diabetes mellitus, concomitant malignancies, chronic, immunosuppressive, and anti-inflammatory therapy, as well as chemotherapy. Further criteria for exclusion were the following: endocrine disease, acute pancreatitis, or acute relapse of CP; unstable angina pectoris, stage within 1 year after acute myocardial infarction; coronary aortic bypass grafting, or percutaneous coronary intervention, and stroke. Persons who were operated on in the upper gastrointestinal tract (in the previous year) and subjects after systemic inflammation in the previous 6 months were also excluded. Patients with CP enrolled into the study were reexamined after 2 years to exclude the development of PC and thus to avoid enrollment of patients with initial stages of PC into the study.

Among our 50 patients with PC, there were 22 patients with diabetes. In this group were the following stage distributions: 9 patients with stage II (2 patients with stage IIA and 7 patients with stage IIB) disease, 24 patients with stage III disease, and 17 patients with stage IV disease. Alcoholic CP was diagnosed in 38 patients, obstructive CP in 5 subjects, and idiopathic CP in 7 patients. Among the 50 patients with CP, there were 30 patients with severe grade, 17 patients with moderate, and 3 patients with mild grade changes in pancreatic morphology. Severe exocrine dysfunction (concentration of pancreatic stool elastase 1 <200 ng/g) was found in 29 patients with CP. Complications (ascites, bleeding, obstruction/or stricture ductus choledochus, pancreatic fistula, duodenal stenosis, splenic and/or portal vein thrombosis, and segmental portal hypertension) were found in 28 patients with CP.

Data Collection

Samples from all participants were obtained after overnight fast (at least 10 hours). All study participants' medical history and intake of any medications were documented at study entry. Blood was taken by puncturing a peripheral vein. Concentrations of C-reactive protein (CRP), conjugated dienes (CD/LDL) in precipitated LDL, serum amyloid A (SAA), 3-nitrotyrosine (NT), tumor markers (CA 19-9, CA 72-4, and CEA), albumin, bilirubin, uric acid, calcium, copper, zinc, iron, selenium, vitamins A and E, and lipid parameters, as well as activity of routine biochemical tests (pancreatic amylase, alanine transaminase, aspartate aminotransferase, γ-glutamyltransferase, cholinesterase alkaline phosphatase [data not shown]), PON1 arylesterase (PON1-A), PON1 lactonase (PON1-L), and oxidized-LDL (ox-LDL) were measured in serum. Serum was prepared after coagulation in vacutainer tubes by centrifugation at 3500 rpm at 4°C for 10 minutes. Activities of antioxidant enzymes CAT, GPX1, GR, and Cu-Zn superoxide dismutase (SOD1), as well as the concentration of GSH were measured in hemolyzed erythrocytes. The samples were stored at −80°C until assay. All samples were marked with unique identification numbers made anonymous, and the data were merged only after the assays had been completed.

Laboratory Measurements

Activities of antioxidant enzymes were determined by spectrophotometric kinetic methods, and the concentration of

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GSH were assessed spectrophotometrically as previously described by Kodydková et al.[26] The lactonase activity of PON1 was measured according to the modified method described earlier[27] using dihydrocoumarin (final concentration, 1 mmol/L) as a substrate. The increase in absorbance at 270 nm was monitored for 2 minutes. The enzyme activity was calculated from the molar extinct coefficient of the reaction product [3-(2-hydroxyphenyl)-propionate (ε = 1295 dm^3 mol^{-1} cm^{-1})] estimated during the linear phase of reaction.[28] The concentration of CD in precipitated LDL was determined by the Wieland modified spectrophotometric method at 234 nm.[29,30]

The levels of SAA, 3-NT, and ox-LDL were established using sandwich enzyme-linked immunosorbent assay kits (Invitrogen, Camarillo, Calif; Biovendor, Brno, Czech Republic, Czech Republic; and Mercodia, Upsala, Sweden; respectively).

All routine clinical tests were measured at the Institute for Clinical Biochemistry and Laboratory Diagnostics of General University Hospital in Prague. Concentration of CRP was measured by the immunoturbidimetric method using a K-ASSAY CRP kit (Kamiya Biomedical Company, Seattle, Wash) on a Hitachi Modular analyzer (Tokyo, Japan). Tumor markers (CEA, CA 19–9, and CA 72–4) were measured by chemiluminescence assay on ADVIA Centaur analyzer, Siemens (Tarrytown, NY). Selenium, copper, and zinc were measured using atomic absorption spectrometry. Concentrations of total cholesterol and triglycerides were measured by enzymatic-colorimetric methods. High-density lipoprotein cholesterol was determined in the supernatant after precipitation of lipoproteins B by phosphotungstic acid/Mg^{2+} (Boehringer Mannheim, Germany); LDL cholesterol was calculated according to the Friedewald formula. Apolipoprotein B and apolipoprotein A1 were measured by the Laurell rocket electroimmunoassay using standards and specific antibodies (Behringwerke Marburg, Germany). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as HOMA-IR = [fasting serum glucose (mmol/L) × fasting serum insulin (μU/mL)] / 22.5.[31] Malnutrition was categorized into the mild, moderate, and severe forms according to the Nutritional Risk Index (NRI).[32] The NRI was calculated according to the following formula: NRI = (1.519 × albumin + 41.7 × current body weight/usual body weight), and the classification was as follows: normal nutrition, NRI > 100; mild malnutrition, NRI = 97.5–100; moderate malnutrition, NRI = 83.0–97.4; severe malnutrition, NRI < 83.0.

Statistical Analysis

Data are expressed as mean ± SD for parametric and as median and interquartile range (25th–75th percentiles) for nonparametric data. Normality of data distribution was tested with the Shapiro-Wilks *W* test. Differences between the compared groups (PC, CP, and controls) were tested with the one-way analysis of variance with Scheffé and Newman-Keuls post-tests. For nonparametric analysis, the Kruskal-Wallis analysis of variance was used. For correlation analysis, the Spearmen coefficient was used. All previously described statistical analyses were performed using StatSoft Statistica version 9.0 software (2007, Czech Republic). $P < 0.05$ was considered statistically significant.

The multivariate discriminant analysis (MDA) was carried out by using Statistical Analysis System (SAS, Cary, NC), JMP version 9 software. The process of MDA is carried out in a stepwise manner using the minimum Wilks λ (within-groups sum of squares–total sum of squares ratio) as a measure of group discrimination. At each step in the process, the variable, which contains the most discriminating power, is identified and its coefficient is determined. The relative importance of each variable is indicating by so-called approximate F statistic. This is a transformation of Wilks λ, which can be compared with F distribution. The process is stopped when the remaining variables are determined to lack significant discriminating power ($P > 0.05$). We used discriminant models for classification into the groups of PC and CP. Only variables with an appropriate final F statistic ($P < 0.05$) were included in our discriminant functions. The oxidative stress and inflammatory markers (CD/LDL, ox-LDL/LDL, and SAA), the antioxidant enzymes (SOD1, CAT, GPX1, GR, PON1-A, PON1-L), and their combinations as variables were subjected into the discriminant functions.

RESULTS

This study was focused on the antioxidant status in patients with PC and CP. Fifty patients with PC and 50 patients with CP were enrolled in the study. The basic clinical and biochemical data of the studied groups are summarized in Table 1.

As markers of oxidative stress, the levels of CD/LDL, ox-LDL/LDL, and NT were determined. The levels of CD/LDL and ox-LDL/LDL were significantly increased in both the patients with PC and those with CP compared with the controls (Figs. 1A, B). Furthermore, the patients with PC had higher levels of ox-LDL/LDL than the patients with CP ($P < 0.001$). There was an increase in the concentration of 3-NT only in the patients with CP ($P < 0.01$) in comparison with the controls (Fig. 1C).

Erythrocyte activity of SOD1 was increased and CAT activity was decreased in the patients with PC compared with the patients with CP and the controls (Figs. 2A, B). In addition, the serum concentration of SOD1 cofactor Cu was observed as elevated; and the serum concentration of Fe, the CAT cofactor, was decreased in the patients with PC in comparison with the controls (Table 2). The concentration of Zn was increased in the patients with CP compared with the controls. Conversely, decreased erythrocyte activities of GPX1 and GR were found in patients with PC and those with CP as compared with the controls (Figs. 2C, D). The decreased GPX1 activities in both the PC and CP groups were accompanied with lower serum selenium levels compared with the controls (Table 2). The concentration of GSH in erythrocytes in the patients with PC differed from that of the subjects with CP and controls, respectively (Fig. 1D). We have found that serum concentrations of Zn negatively correlate with activity of SOD1 in the entire group ($r = -0.312$; $P < 0.001$), in the patients with PC ($r = -0.357$; $P < 0.05$) and also in the patients with CP ($r = -0.458$; $P < 0.001$). There was a significant correlation between Se and GPX1 in the entire group ($r = 0.319$; $P < 0.01$) and also in the patients with CP ($r = 0.470$; $P < 0.01$).

The PON1-A and PON1-L activities in serum were decreased in the patients with PC and those with CP in comparison with the controls (Figs. 2E, F). Furthermore, decreased activities of these enzymes in the patients with PC compared with the patients with CP (both $P < 0.001$) were observed. Both PON1 activities were significantly correlated in the PC ($r = 0.711$; $P < 0.001$) and CP ($r = 0.811$; $P < 0.001$) groups and in the controls ($r = 0.687$; $P < 0.001$) as well as in all the studied groups ($r = 0.806$; $P < 0.001$). The levels of both PON1 activities correlated negatively with ox-LDL/LDL ($r = -0.309$; $P < 0.001$; and $r = -0.358$; $P < 0.001$; respectively) in the entire group.

Serum amyloid A concentrations were higher in the patients with PC than in the patients with CP and in the controls (both $P < 0.001$; Table 1). The studied groups did not differ in concentrations of PON1 cofactor—calcium. Additionally, we

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TABLE 1. Basal Clinical and Biochemical Characteristics

	PC	CP	CON
No. patients (M/F)	50 (40/10)	50 (40/10)	50 (40/10)
Age (range), yrs	63 (56–68)	59 (53–65)	60 (55–65)
Smokers, n (%)	34 (68)	15 (30)	13 (26)
DM, no. patients (M/F)	28 (24/4)	30 (23/7)	–
NRI, kg/m^2	96.4 ± 12.3*	109.1 ± 7.3	–
CRP, mg/L	10.9 (5.8–54.8)***; $^{+++}$	4.5 (2.0–10.3)*	2.1 (1.0–4.9)
SAA, μg/mL	49.0 (21.7–134.2)***; $^{+++}$	14.5 (7.2–49.8)	12.7 (4.6–25.6)
CEA, μg/L	2.75 (1.65–6.45) ***	2.37 (1.44–3.42)***	0.71 (0.5–1.45)
CA 19–9, kU/L	105.2 (24–2301.3)***; $^{+++}$	14.6 (8.5–26.5)*	8.7 (6.1–10.7)
CA 72–4, kU/L	2.32 (1.31–9.70)*; $^{++}$	1.45 (0.97–2.23)	1.44 (1.02–3.86)
Glucose, mmol/L	6.5 (5.2–8.9) ***	6.6 (5.8–7.6)***	5.1 (4.9–5.3)
Glycated hemoglobin, mmol/mol	5.46 ± 1.85***	5.11 ± 1.49***	3.91 ± 0.34
TC, mmol/L	4.7 (3.7–6.6)*	4.9 (4.4–6.0)	5.4 (4.6–5.9)
TG, mmol/L	1.67 (1.26–2.12) ***	1.48 (1.10–2.01)***	1.06 (0.79–1.34)
HDL-C, mmol/L	0.92 (0.73–1.05)***; $^{+++}$	1.42 (1.21–1.59)	1.5 (1.27–1.73)
LDL-C, mmol/L	2.76 (2.12–3.24)*	2.73 (2.34–3.17)*	3.33 (2.72–3.73)
Apo A1, g/L	0.88 ± 0.32***; $^{+++}$	1.61 ± 0.44	1.53 ± 0.30
NEFA, mmol/L	0.71 ± 0.35**	0.62 ± 0.40*	0.48 ± 0.24

Data are expressed as mean ± SD for parametric variables, and as median and interquartile range (IQR, 25th–75th percentile) for nonparametric variables;

PC or CP versus CON: ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; PC versus CP: $^{+++}P < 0.001$, $^{+}P < 0.05$ (one-way analysis of variance (ANOVA) with Newman-Keuls posttest), and $^{++}P < 0.01$.

Apo-A1, apolipoprotein A1; CA, carbohydrate antigen; CEA, carcinoembryonic antigen; CON, control subjects; NEFA, nonesterified fatty acids; TC, total cholesterol; TG, triglycerides.

have observed statistically decreased concentrations of vitamin A, albumin, and uric acid and higher levels of bilirubin in the patients with PC compared with the patients with CP and the controls (Table 2).

Discriminant models for classification into the groups of PC and CP are shown in Table 3. The concentrations of ox-LDL and CD/LDL were the best discriminators (model A) when only oxidative stress and inflammatory markers were entered into the

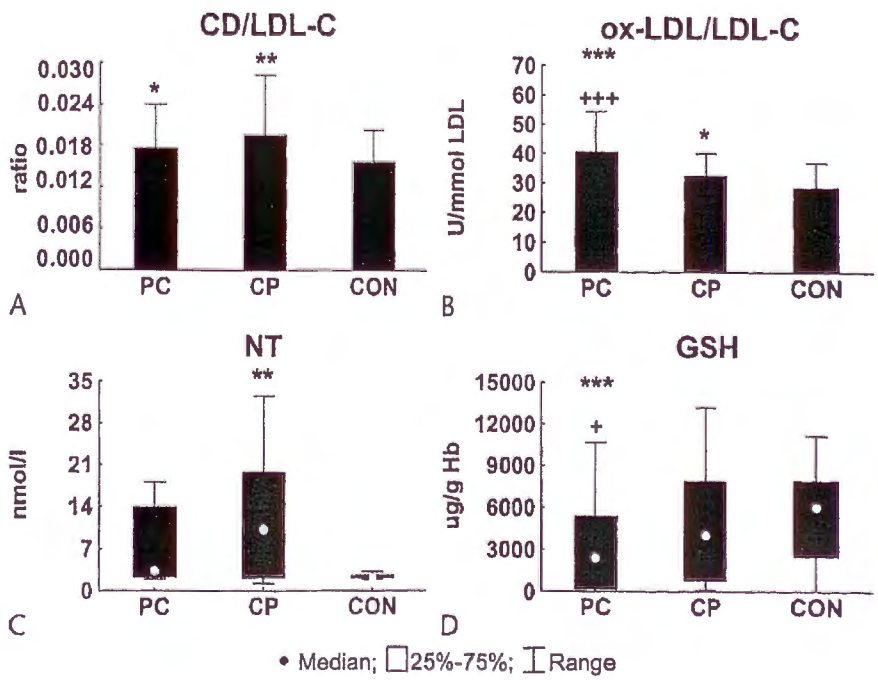

FIGURE 1. Serum concentration of oxidative stress markers and reduced glutathione. Data are expressed as mean ± SD for parametric variables and as median and IQR (25th–75th percentile) for nonparametric variables. PC or CP versus CON: ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; PC versus CP: $^{+++}P < 0.001$ and $^{+}P < 0.05$ (one-way ANOVA with Newman-Keuls posttest or Kruskal-Wallis ANOVA).

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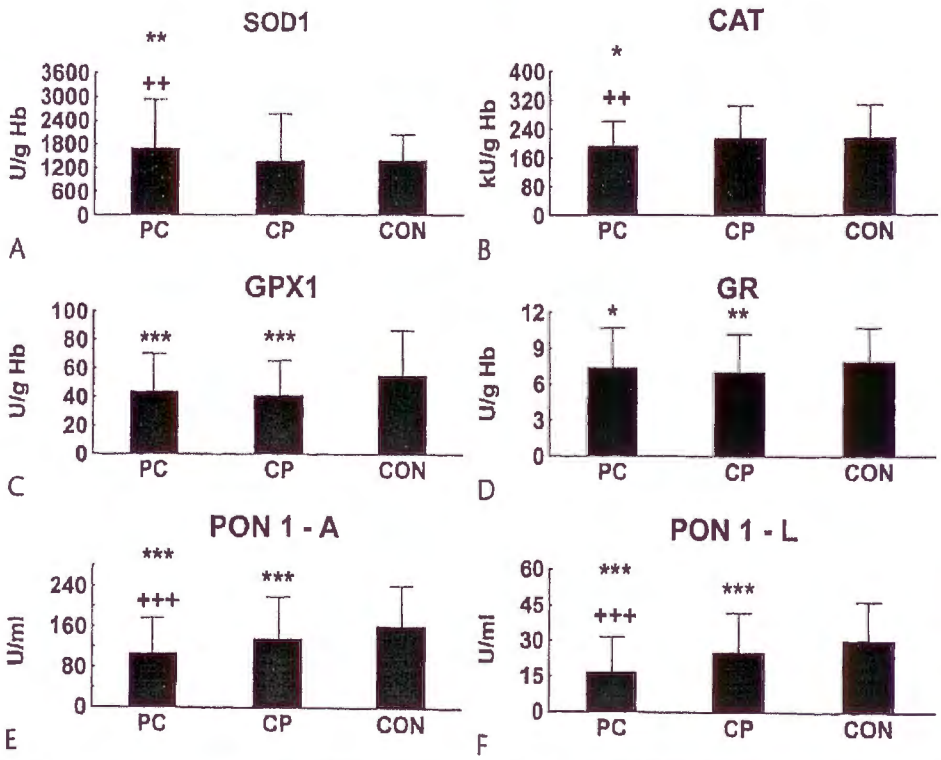

FIGURE 2. Activities of antioxidant enzymes. Data are expressed as mean ± SD; PC or CP versus CON: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$; PC versus CP: $^{++}P < 0.01$ (one way ANOVA, Newman-Keuls posttest) and $^{+++}$ and $^{***}P < 0.001$ $P < 0.001$.

MDA. Using only antioxidant enzymes as discriminating variables resulted in 73% of the final correct classification. Variables subjected into the analysis were PON1-L, SOD1, and CAT (model B). A combination of both models resulted in 83% of the final correct classification. The activity of PON1-L, the levels of ox-LDL/LDL, and the activity of CAT, in that order, were found to be the best set of independent factors discriminating PC and CP, with higher values for PC (model C).

Table 2 presents data of nonenzymatic antioxidants and of cofactors of antioxidant enzymes.

DISCUSSION

The present study demonstrates contemporary changes in the levels of selected inflammatory and oxidative stress markers as well as a set of the antioxidant defense system (both enzymatic and nonenzymatic) in the patients with PC and those with CP in comparison with age- and sex-matched controls. The reduced capacity of the antioxidant defense system and an increased oxidative stress in patients with PC and those with CP was confirmed in our study. The antioxidant system seems to be more affected in the patients with PC compared with the patients with CP.

The groups of CP and PC differed statistically significantly in many oxidative stress and antioxidant variables using univariate analysis. Moreover, there were intercorrelations between oxidative stress markers and activities of antioxidant enzymes. Therefore, multivariate discriminant analysis was performed to determine the set of independent oxidative stress

TABLE 2. Nonenzymatic Antioxidants, Cofactors of Antioxidant Enzymes, and Other Parameters of Antioxidant Capacity

	PC	CP	CON
Vitamin A, mg/L	0.51 ± 0.24*; +	0.80 ± 0.41	0.83 ± 0.35
Vitamin E, mg/L	16.0 ± 7.7	12.3 ± 5.0	14.4 ± 6.1
Fe, μmol/L	13.5 ± 8.3***	16.4 ± 7.2	19.2 ± 7.9
Ca, mmol/L	2.26 ± 0.16	2.24 ± 0.13	2.27 ± 0.13
Cu, μmol/L	21.9 ± 6.3***; +++	18.8 ± 4.5	15.6 ± 3.1
Zn, μmol/L	19.2 ± 4.7	19.9 ± 3.9*	17.8 ± 2.6
Zn/Cu	0.86 ± 0.34***; +++	1.13 ± 0.38	1.15 ± 0.40
Se, μg/L	31.3 ± 10.9***	43.1 ± 21.5*	58.9 ± 26.0
Albumin, g/L	41.4 (37.7–45.5)***; +++	45.7 (42.5–46.9)	47.2 (44.9–48.6)
Bilirubin, μmol/L	18.7 (11.0–64.1)***; +++	10.2 (7.2–14.2)	11.0 (8.0–15.3)
Uric acid, μmol/L	257 ± 108**; ++	324 ± 90	310 ± 76

Data are expressed as mean ± SD for parametric variables and as median and IQR (25th –75th percentile) for nonparametric variables;
PC or CP versus CON: $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$; PC versus CP: $^{+++}P < 0.001$, $^{++}P < 0.01$, and $^{+}P < 0.05$ (one-way ANOVA with Newman-Keuls posttest).

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TABLE 3. Discriminant Models for Classification of CP and PC

	F-Statistic (df)	Final Correct Classification	Percent
Model A			
ox-LDL/LDL	13.80 (1, 98)	CP	74.0
ox-LDL/LDL + CD/LDL	11.32 (2, 97)	PC	72.0
		Total	73.0
Model B			
PON1-L	31.77 (1, 98)	CP	68.0
PON1-L + SOD1	19.33 (2, 97)	PC	74.0
PON1-L + SOD1 + CAT	14.83 (3, 96)	Total	71.0
Model C			
PON1-L	31.77 (1, 98)	CP	80.0
PON1-L + ox-LDL/LDL	27.72 (2, 97)	PC	86.0
PON1-L + ox-LDL/LDL + CAT	21.32 (3, 96)	Total	83.0

Model A: Only oxidative stress and inflammatory markers included in the analysis (CD/LDL, Ox-LDL/LDL, and SAA). Model B: Only activities of antioxidant enzymes included in the analysis (SOD1, CAT, GPX1, GR, PON1-A, and PON1-L). Model C: combination of the model A and the model B.

df, degrees of freedom;

F statistic (so-called an approximate F statistic), transformation of Wilks λ.

and antioxidant variables giving the most discrimination power to separate CP and PC.

Multivariate discriminant analysis indicated that the activities of PON1-L and CAT, along with ox-LDL/LDL levels (in the order of PON1-L, ox-LDL/LDL, and CAT), are the independent factors discriminating the patients with PC and those with CP.

Antioxidant enzymes play an important role in the defense of cells against RONS and thus may protect the pancreas against development of CP, which is a risk factor of PC. The first scavenger of ROS is SOD, which converts superoxide radical to H_2O_2, which should be later removed by GPX1 and CAT. Our study found increased SOD1 activity in erythrocytes of the patients with PC in comparison with the patients with CP and the controls. Some experimental studies have described relationships between the expression of SOD, its activity, and PC cell growth in vitro, on the one hand, and tumor growth and survival in nude mice, on the other.[8] The insignificant differences in the erythrocyte activities of SOD1 in the patients with CP and controls found in our study were consistent with the study of Quillot et al.[18] On the other hand, decreased SOD1 activity in the patients with CP was found in the study of Girish et al.[15] Inconsistent results concerning serum SOD activities in hereditary and alcohol-related pancreatitis have been published. Some reports have described increased[23,33] serum SOD activity, and in some studies, no differences in serum SOD activities were found.[18,34] It could be supposed that discordance in elevated activity of SOD1 without an appropriate change in the GPX1 and/or CAT activities in the patients with PC resulted in the increased production of H_2O_2, which cannot be detoxified by the action of GPX1 and CAT. Accumulation of H_2O_2 can thus participate in a Haber-Weiss reaction and generate hydroxyl radicals.[35,36] Catalase and glutathione peroxidase are both able to detoxify H_2O_2. Under physiologic conditions, H_2O_2 is mainly removed by GPX1. The activity of CAT is involved in the degradation of H_2O_2 in severe oxidative stress connected with higher H_2O_2 concentrations.[37] It was previously shown that long-term exposure of CAT to H_2O_2 leads to the oxidation of the catalase-bound NADPH to $NADP^+$ and to a decrease in the activity of CAT to approximately 30% of the initial activity.[38] Because our patients with PC had the highest level of oxidative stress markers associated with decreased erythrocyte CAT activity, our results implicated that under stressed conditions, erythrocyte CAT is unable to detoxify H_2O_2. In contrast, no changes in serum CAT activity in the patients with PC were found.[39] The insignificant differences in CAT activities in erythrocytes of the patients with CP observed in our study were consistent with the results of Fukui et al,[39] dealing with serum CAT activities in patients with CP. In the contrast, other authors described increased serum CAT[23,40] or decreased serum and erythrocyte CAT[18] activities in patients with CP.

Glutathione peroxidases use GSH to metabolize H_2O_2 and lipid hydroperoxides to water/related alcohols.[1] We have found decreased activity of erythrocyte GPX1 in the patients with PC and those with CP compared with the controls. The decreased erythrocyte GPX1 in CP was also found in the study of Girish[15] but also no differences in erythrocyte GPX1 activity in patients with CP and the controls were observed.[18,33] Published results in serum and plasma GPX activities are inconsistent.[18,23,33,34,40] Decreased GPX1 activity may be explained by the lowered serum levels of selenium in both groups and/or decreased concentrations of reduced glutathione in erythrocytes found in the patients with PC. Selenium is bound as the selenocysteine at the active site of GPX1, and it is essential for its activity. Moreover, selenium deficiency leads to decreased GPX1 activities.[35]

Glutathione reductase is a NADPH-dependent enzyme that catalyzes the regeneration of GSH from oxidized glutathione (GSSG) and thus maintains a constant supply of GSH for GPX.[41] In the present study, a decrease in GR erythrocyte activity was observed in the patients with PC and those with CP and decreased erythrocyte levels of GSH in PC but not in the patients with CP in comparison with the controls. It is supposed that depletion in GSH concentration may be caused by accumulation of GSSG as a result of impaired GR (pentose-phosphate pathway may limit NADPH supply). Formed GSSG could react with the sulfhydryl group, via mixed disulphide reactions, or could be secreted out of the cell. It is supposed that the abovementioned processes may lead to GSH depletion.[42] In

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contrast to our study, decreased levels of reduced glutathione were observed in the patients with CP.[43]

We found a decrease in serum activities of arylesterase as well as lactonase activity of PON1 in the patients with PC and those with CP. The lowest PON1 activities were observed in the patients with PC. At present, the decreased PON1 activity in patients with PC has been described only in one study,[44] and there is no study dealing with PON1 activities in CP. A number of studies have shown decreased serum arylesterase and/or paraoxonase activities of PON1 in different malignancies.[45]

Under conditions of systemic inflammation and/or oxidative stress, several mechanisms are implicated in a drop of PON1 activities. Among them, displacement of PON1 from its linkage to apolipoprotein A1 in HDL by SAA,[46] down-regulation of liver PON1 lipopolysaccharides and cytokines (tumor necrosis factor α and interleukin-1) via IL-6,[20] and inhibition of PON1 activity by oxidized phospholipids[47] are the most important. All the abovementioned mechanisms can be related to cancer-related decrease in PON1 activities. Using MDA, the PON1-L activity had the most discriminating power to differentiate PC from PC.

The finding of increased SAA levels in the patients with PC in our study is consistent with the results of other studies.[48,49] Serum amyloid A is implicated in carcinogenesis, and it was associated with tumor progression and its metastasizing.[50] Some authors considered SAA as a tumor marker for PC. However, SAA did not reach appropriate specificity and sensitivity as tumor marker for PC diagnostics.[48,49]

Human serum PON1 should contribute to the detoxification of organophosphorus compounds and carcinogenic lipid-soluble radicals from lipid peroxidation and, moreover, should impede oxidative modification of LDLs.[22] In this study, we found a negative correlation between PON1 activities and ox-LDL/LDL levels. The reduced PON1 activities in the patients with PC and those with CP could lead to the increase in ox-LDL/LDL levels. Oxidatively modified LDL represents heavily oxidized LDL characterized by oxidative altered both the lipid and the apolipoprotein B, moiety of particle. Low-density lipoprotein is supposed to be oxidize not only within the artery wall (by endothelial cells, smooth muscle cells, and monocyte/macrophages) but also at peripheral tissues altered by inflammation (by neutrophils and fibroblasts).[51]

In our study, increased concentrations of CD/LDL in the patients with PC and those with CP compared with the controls were found. Concentrations of CD/LDL are partly considered as a marker of systemic oxidative stress and partly reflect minimally modified LDL, in which only the lipid component is oxidatively modified.[29,52] Currently, serum concentration of CD in CP patients was described only in the study of Santini et al,[53] where the levels of CD and lipid hydroperoxides did not differ between patients with CP and controls. However, the patients with CP, in comparison with the controls, had increased levels of CD and lipid hydroperoxides in pancreatic juice after secretin stimulation. These results indicated local enhanced oxidative stress in pancreas without systemic oxidative stress response. An increase of lipid peroxidation connected with PC and CP were observed in many studies using products of lipid peroxidation (such as thiobarbiturate-reactive substances, malondialdehyde, 4-hydroxynonenal, lipid hydroperoxides).[1,3] The importance of ox-LDL/LDL and CD/LDL was pointed out in discriminating PC from CP using MDA.

In conclusion, our study demonstrates the persisting oxidative stress in patients with CP and those with PC, which is associated with the reduced capacity of the antioxidant defense system. The oxidative stress defense system seems to be more affected in patients with PC compared with those with CP. Multivariate discriminant analysis indicates the importance of PON1-L and CAT activities, along with ox-LDL/LDL levels, as the independent factors discriminating patients with PC and those with CP. Further studies concerning antioxidant defense systems and oxidative stress are warranted, especially with respect to potential diagnostic and therapeutic implications.

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REFERENCES

1. Leung PS, Chan XC. Role of oxidative stress in pancreatic inflammation. *Antioxid Redox Signal.* 2009;11:135–165.
2. Stevens T, Conwell DL, Zuccaro G. Pathogenesis of chronic pancreatitis: an evidence-base review of past theories and recent developments. *Am J Gastroenterol.* 2004;99:2256–2270.
3. Tandon RK, Garg PK. Oxidative stress in chronic pancreatitis: pathophysiological relevance and management. *Antioxid Redox Signal.* 2011;15:2757–2766.
4. Witt H, Apte MV, Keim V, et al. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology.* 2007;132:1557–1573.
5. Etemad B, Whitcomp DC. Chronic pancreatitis. Diagnosis, classifications and new genetic developments. *Gastroenterology.* 2001;120:682–707.
6. Grigsby B, Rodriguez-Rilo H, Khan K. Antioxidants and chronic pancreatitis: theory of oxidative stress and trials of antioxidant therapy. *Dig Dis Sci.* 2012;57:835–841.
7. Skipworth JRA, Szabadkai G, Olde Damink SWM, et al. Review article: pancreatic renin-angiotensin systems in health and disease. *Aliment Pharmacol Ther.* 2011;34:840–852.
8. Teoh ML, Sun W, Smith BJ, et al Modulation of reactive oxygen species in pancreatic cancer. *Clin Cancer Res.* 2007;13:7441–7450.
9. Schoenberg MH, Birk D, Beger HG. Oxidative stress in acute and chronic pancreatitis. *Am J Clin Nutr.* 1995b;62:1306S–1314S.
10. Verlaan M, Roelofs HM, van-Schaik A, et al. Assessment of oxidative stress in chronic pancreatitis patients. *World J Gastroenterol.* 2006;12:5705–5710.
11. Petrov M. Therapeutic implications of oxidative stress in acute and chronic pancreatitis. *Curr Opin Clin Nutr Metab Care.* 2010;13:562–568.
12. Podborska M, Sevcikova A, Trna J, et al. Increased markers of oxidative stress in plasma of patients with chronic pancreatitis. *Neuroendocrinol Lett.* 2009;30(suppl 1):116–120.
13. Sajewicz W, Milnerowicz S, Nabzdyk S. Blood plasma defense in patients with pancreatitis. *Pancreas.* 2006;32:139–144.
14. Vinokurova LV, Berezina OI, Drozdov VN, et al. Nitric oxide and indicators of oxidative stress in patients with exacerbation of chronic pancreatitis. *Eksp Klin Gastroenterol.* 2011;2:57–81.
15. Girish BN, Rajesh G, Vaidyanathan K, et al. Assessment of oxidative status in chronic pancreatitis and its relation with zinc status. *Indian J Gastroenterol.* 2011;30:84–88.
16. Güzel S, Seven A, Satman I, et al. Comparison of oxidative stress indicators in plasma of recent-onset and long-term type 1 diabetic patients. *J Toxicol Environ Health A.* 2000;59:7–14.
17. Miller YI, Tsimikas S. Lipoprotein oxidation and modification. In: Ballantyne CM, ed. *Clinical Lipidology: A Companion to Braunwalds's Heart Disease.* Philadelphia, PA: Elsevier; 2009:93–110.
18. Quillot D, Walters E, Bontze JP, et al. Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis. *Am J Clin Nutr.* 2005;81:1117–1125.
19. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet.* 1994;344:721–724.

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20. Deakin SP, James RW. Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. *Clin Sci.* 2004;107:435–447.
21. Précourt LP, Amre D, Denis MC, et al. The three-gene paraoxonase family: physiologic roles, action and regulation. *Atherosclerosis.* 2011;214:20–36.
22. Soran H, Younis NN, Charlton-Menys V, et al. Variation in paraoxonase-1 activity and atherosclerosis. *Curr Opin Lipidol.* 2009;20:265–274.
23. Szuster-Ciesielska A, Daniluk J, Kandefer-Szerszeń M. Oxidative stress in blood of patients with alcohol-related pancreatitis. *Pancreas.* 2001a;22:261–266.
24. Greene FL, Page DL, Fleming ID, et al, eds. *AJCC Cancer Staging Manual.* 6th ed. New York, NY: Springer-Verlag; 2002.
25. Schneider A., Löhr JM, Winter MV. The M-ANNHEIM classification of chronic pancreatitis: introduction of a unifying classification system based on a review of previous classification of the disease. *J Gastroenterol.* 2007;42:101–119.
26. Kodydková J, Vávrová L, Zeman M, et al. Antioxidative enzymes and increased oxidative stress in depressive women. *Clin Biochem.* 2009;42:1368–1374.
27. Draganov DI, Stetson PL, Watson CE, et al. Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protect slow density lipoprotein against oxidation. *J Biol Chem.* 2000;275:33435–33442.
28. Rainwater DL, Rutherford S, Dyer TD, et al. Determinants of variation in human serum paraoxonase activity. *Heredity (Edinb).* 2009;102:147–154.
29. Ahotupa M, Ruutu M, Mantyla E. Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. *Clin Biochem.* 1996;29:139–144.
30. Wieland H, Seidel D. A simple specific method for precipitation of low-density lipoproteins. *J Lipid Res.* 1983;24:904–909.
31. Vogeser M, König D, Frey I, et al. Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of life style interventions in obese persons. *Clin Biochem.* 2007;40:964–968.
32. McMillan DC. Systemic inflammation, nutritional status and in patients with cancer. *Curr Opin Clin Nutr Metab Care.* 2009;12:223–226.
33. Mathew P, Wyllie R, Van Lente F, et al. Antioxidants in hereditary pancreatitis. *Am J Gastroenterol.* 1996;91:1558–1562.
34. Quilliot D, Dousset B, Guerci B, et al. Evidence that diabetes mellitus favors impaired metabolism of zinc, copper, and selenium in chronic pancreatitis. *Pancreas.* 2001;22:299–306.
35. Michiels C, Raes M, Toussaint O, et al. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. *Free Rad Biol Med.* 1994;17:235–248.
36. Li S, Yan T, Yang JQ, et al. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. *Cancer Res.* 2000;60:3927–3939.
37. Halliwell B, Gutteridge J. *Free Radicals in Biology and Medicine.* 4th Ed. New York, NY: Oxford University Press; 2007.
38. Kirkman HN, Galiano S, Gaetani GF. The function of catalase-bound NADPH. *J Biol Chem.* 1987;262:660–666.
39. Fukui M, Kanoh M, Takamatsu Y, et al. Analysis of serum catalase activities in pancreatic diseases. *J Gastroenterol.* 2004;39:469–474.
40. Szuster-Ciesielska A, Daniluk J, Kandefer-Szerszeń M. Alcohol-related cirrhosis with pancreatitis. The role of oxidative stress in the progression of the disease. *Arch Immunol Ther Exp.* 2001b;49:139–146.
41. Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci.* 2000;57:1825–1835.
42. Deneke SM, Fanburg BL. Regulation of cellular glutathione. *Am J Physiol.* 1989;257:L163–L173.
43. Schoenberg MH, Büchler M, Pietrzyk C, et al. Lipid peroxidation and glutathione metabolism in chronic pancreatitis. *Pancreas.* 1995a;10:36–43.
44. Akçay MN, Polat MF, Yilmaz I, et al. Serum paraoxonase levels in pancreatic cancer. *Hepatogastroenterology.* 2003;50(suppl 2): ccxxv–ccxxvii.
45. Goswami B, Tayal D, Gusta N, et al. Paraoxonase: a multifaceted biomolecule. *Clin Chim Acta.* 2009;410:1–12.
46. James RW, Deakin SP. The importance of high-density lipoproteins for paraoxonase-1 secretion, stability, and activity. *Free Radic Biol Med.* 2004;37:1986–1994.
47. Tavori H, Aviram M, Khatib S, et al. Human carotid lesion linoleic acid hydroperoxide inhibits paraoxonase 1 (PON1) activity via reaction with PON1 free sulfhydryl cysteine 284. *Free Radic Biol Med.* 2011;50:148–156.
48. Yokoi K, Shih LC, Kobayashi R, et al. Serum amyloid A as a tumor marker in sera of nude mice with orthotopic human pancreatic cancer and in plasma of patients with pancreatic cancer. *Int J Oncol.* 2005;27:1361–1369.
49. Firpo MA, Gay DZ, Granger SR, et al. Improved diagnosis of pancreatic adenocarcinoma using haptoglobin and serum amyloid A in a panel screen. *World J Surg.* 2009;33:716–722.
50. Malle E, Sodin-Semrl S, Kovacevic A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. *Cell Mol Life Sci.* 2009;66:9–26.
51. Liao F, Andalibi A, Qiao JH, et al. Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice. *J Clin Invest.* 1994;94:877–884.
52. Esterbauer H, Gebicki J, Puh H, et al. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic Biol Med.* 1992;13:341–390.
53. Santini SA, Spada C, Bononi F, et al. Liver, pancreas and biliary tract enhanced lipoperoxidation products in pure pancreatic juice: evidence for organ-specific oxidative stress in chronic pancreatitis. *Dig Liver Dis.* 2003;35:888–892.

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Přehledový článek

Reaktivní kyslíkové a dusíkové sloučeniny v klinické medicíně

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SOUHRN

V poslední době dochází k rychlému růstu poznatků o reaktivních kyslíkových a dusíkových sloučeninách (RONS, reactive oxygen and nitrogen species) v klinické medicíně. Jejich významná úloha byla popsána v patogenezi mnoha chorob včetně těch, které značně zatěžují zdravotnické systémy vyspělých států. Výzkumu reaktivních kyslíkových a dusíkových radikálů je proto věnováno velké úsilí. Jedná se o nestabilní částice ochotně reagující s biomolekulami v organismech. Tyto reakce se řetězově propagují a vedou k mnohočetnému poškození buněčných systémů, což se uplatňuje v patogenezi mnoha chorob. Chemickou podstatou těchto částic je přítomnost nespárovaného elektronu v zevním orbitalu. Patří sem také sloučeniny snadno oxidující jiné molekuly. Volné kyslíkové radikály vznikají během fyziologických procesů, jako jsou oxidativní fosforylace v mitochondriích, fagocytóza či při metabolismu purinů. Při nadměrné tvorbě ROS během těchto procesů může dojít k poškození tkáně. Dusíkaté radikály vznikají především při metabolismu oxidu dusnatého, který reguluje mnoho procesů v organismu, rozpřažením jeho syntézy působením např. asymetrického dimetylargininu. Při vzniku radikálů či oxidačně působících látek hrají roli mnohé enzymy jako peroxizomální oxidázy, NAD(P)H oxidáza, xanthinoxidáza, syntáza NO, myeloperoxidáza, lipooxygenáza a mnoho dalších. RONS svůj negativní účinek zprostředkovávají chemickou modifikací DNA, proteinů a lipidů, čímž zasahují do základních biochemických a molekulárně biologických dějů buněk. Proti působení RONS zasahují antioxidační systémy, které se dělí na enzymatické a neenzymatické. RONS se uplatňují v rozvoji mnoha chorobných stavů, z nichž jmenujme aterosklerózu a její kardiovaskulární komplikace, diabetes mellitus, hyperlipidémii, neurodegenerativní či psychiatrická onemocnění.

Klíčová slova: RONS, radikály, superoxidový anion, radikál oxidu dusnatého, antioxidanty, ateroskleróza, diabetes mellitus, neurodegenerativní a psychiatrická onemocnění.

SUMMARY

Macášek J, Zeman M, Vecka M, Vávrová L, Kodydková J, Tvrzická E, Žák A. Reactive oxygen and nitrogen species in the clinical medicine

Vast knowledge has accumulated recently on the role of reactive oxygen and nitrogen species (RONS) in clinical medicine. Strong evidence was disclosed on their important role in the pathogenesis of several diseases. Free radicals have unpaired electron and this is the reason for extreme reactivity causing propagation reactions that lead to the multiple damage to cells. Oxidizing agents belong to the family of reactive species. Reactive oxygen species are produced during biochemical processes such as oxidative phosphorylation, phagocytosis and metabolism of purins. Overproduction of reactive oxygen species can cause the tissue damage. Reactive nitrogen species are produced by inhibition of nitric oxide synthase by the action of asymmetric dimethylarginine. Peroxisomal oxidases, NAD(P) oxidase, xanthinoxidase, nitric oxide synthase, myeloperoxidase and lipooxygenase catalyze biochemical reactions producing reactive oxygen and nitrogen species. Biochemical and molecular processes in cells are negatively influenced by chemical modification of DNA, proteins and lipids caused by the action of reactive oxygen and nitrogen species. Antioxidant metabolites and enzymes work together to stop and to prevent oxidative modification of biomolecules. Reactive oxygen and nitrogen species play an important role in the pathogenesis of many diseases such as atherosclerosis, diabetes, hyperlipidaemia and neurodegenerative diseases.

Key words: RONS, radicals, superoxide anion, nitric oxide radical, antioxidants, atherosclerosis, diabetes mellitus, neurodegenerative and psychiatric diseases. *Ma.*

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ÚVOD

V klinické medicíně je dnes věnována velká pozornost pochodům spojeným s oxidačním stresem a působením reaktivních sloučenin kyslíku a dusíku (RONS – reactive oxygen and nitrogen species). Mezi tyto látky patří nejen volné radikály, ale i sloučeniny, které nejsou v chemickém slova smyslu radikály, ale snadno oxidují jiné látky, nebo se na radikály mění. Většina chemických sloučenin obsahuje v zevních orbitalech spárované elektrony. Tzv. volné radikály obsahují v zevním orbitalu jeden nepárový elektron, což je pro ně energeticky nevýhodné, a tudíž se snaží spárovat elektron vazbou s jiným atomem či molekulou. Získáním elektronu od jiného atomu či molekuly (redukce) nebo jeho odevzdáním na jiný atom či molekulu (oxidace) přejde atom či molekula do energeticky stabilnějšího stavu s nižší reaktivitou. Mezi radikály v organismu patří například superoxidový anion $^{\cdot}O_2^-$, oxid dusnatý $^{\cdot}NO$, nebo hydroxylový radikál $^{\cdot}OH$. Mezi neradikálové reaktivní substance patří například peroxid vodíku (H_2O_2), ky-

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Tab. 1. Přehled reaktivní sloučenin kyslíku a dusíku

Reaktivní formy kyslíku	**volné radikály**	tripletový kyslík $^3O_2$ [a]
		superoxidový anion $O_2^{\cdot-}$
		hydroxylový radikál $HO^{\cdot}$
		alkoxyl $RO^{\cdot}$
		hydroperoxyl $HO_2^{\cdot}$
		peroxyl $ROO^{\cdot}$
	látky, které nejsou volnými radikály	peroxid vodíku H_2O_2
		kyselina chlorná HClO
		ozon O_3
		singletový kyslík $^1O_2$
Reaktivní formy dusíku	**volné radikály**	oxid dusnatý $NO^{\cdot}$
		oxid dusičitý $NO_2^{\cdot}$
		nitroxylový anion $NO^{\cdot-}$
	látky, které nejsou volnými radikály	nitrosonium NO^+
		dimer oxidu dusičitého N_2O_4
		kyselina dusitá HNO_2
		oxid dusitý N_2O_3
		nitronium NO_2^+
		peroxynitrit $ONOO^-$
		alkylperoxynitrit ROONO

[a] Kyslík v základním energetickém stavu ($^3S_g^-$) je vlastně biradikál; jeho reaktivita je díky tomu, že reakce tripletové molekuly se singletovou (většina molekul) je spinově zakázána, relativně nízká. Protože je ale molekula kyslíku hojně rozšířena, v přehledu ji uvádíme.

selina chlorná (HClO) a peroxynitrit (ONOO-). Příklady nejdůležitějších reaktivních látek jsou uvedeny v tabulce 1.

Reaktivní formy kyslíku i dusíku vznikají v průběhu metabolických pochodů u všech aerobních organismů. Na jejich vzniku se podílejí i vnější vlivy, jako je ionizační záření, xenobiotika, toxiny či léky. Buňky a tkáně živých organismů jsou před poškozením těmito látkami chráněny antioxidačními ochrannými systémy (enzymatickými i neenzymatickými). V organismu však RONS nepůsobí pouze jako patogeny, ale podílejí se také na obraně vůči infekčním agens a v přiměřených koncentracích ovlivňují signální transdukci a genovou transkripci, přičemž oxid dusnatý ($NO^{\cdot}$) je jednou z nejvýznamnějších signálních molekul v lidském organismu (1).

Nadměrná tvorba a/nebo nedostatečné odstraňování RONS, resp. zvýšený poměr prooxidační k antioxidační aktivitě je označována pojmem **oxidační stres** (OS) (2). V důsledku OS může dojít k: 1. adaptaci buňky nebo organismu se zvýšením aktivity obranných systémů, 2. poškození buňky s oxidativní modifikací lipidů, DNA, proteinů, sacharidů atd., 3. buněčné smrti (3). Oxidační stres podle současných názorů hraje roli zejména u onemocnění, v jejichž patogenezi se uplatňuje zánět, který je s OS spojen. Jde o řadu rozšířených chorob, jako je ateroskleróza a její komplikace (ischemická choroba srdeční – ICHS), ischemická kolitida, ischemické cévní mozkové příhody, arteriální hypertenze, diabetes mellitus, neurodegenerativní neurologická onemocnění (Alzheimerova nemoc, roztroušená skleróza, Parkinsonova nemoc), psychiatrická onemocnění (schizofrenie, deprese) i zhoubné nádory (4).

ZDROJE A VZNIK RONS V LIDSKÉM ORGANISMU

Reaktivní sloučeniny kyslíku (ROS)

K nejvýznamnějším ROS se počítají superoxidový anion $^{\cdot}O_2^-$, hydroxylový radikál $^{\cdot}OH$ a látka neradikálové povahy peroxid vodíku H_2O_2. K hlavním zdrojům $^{\cdot}O_2^-$ patří v lidském organismu reakce provázející oxidativní fosforylaci v mitochondriích, reakce katalyzované peroxizomálními oxidázami, NAD(P)H oxidázami, xantinoxidázou nebo jednoelektronová redukce kyslíku syntázami NO v případě deficitu argininu nebo tetrahydrobiopterinu (obr. 1). Při oxidativní fosforylaci probíhá v dýchacím řetězci v mitochondriích redukce molekuly atmosférického kyslíku na dvě molekuly vody, spojená s tvorbou ATP (4). Redukce molekulárního kyslíku na vodu vyžaduje celkem čtyři elektrony, pokud se uskuteční transfer pouze jednoho elektronu, vzniká superoxidový anion (4).

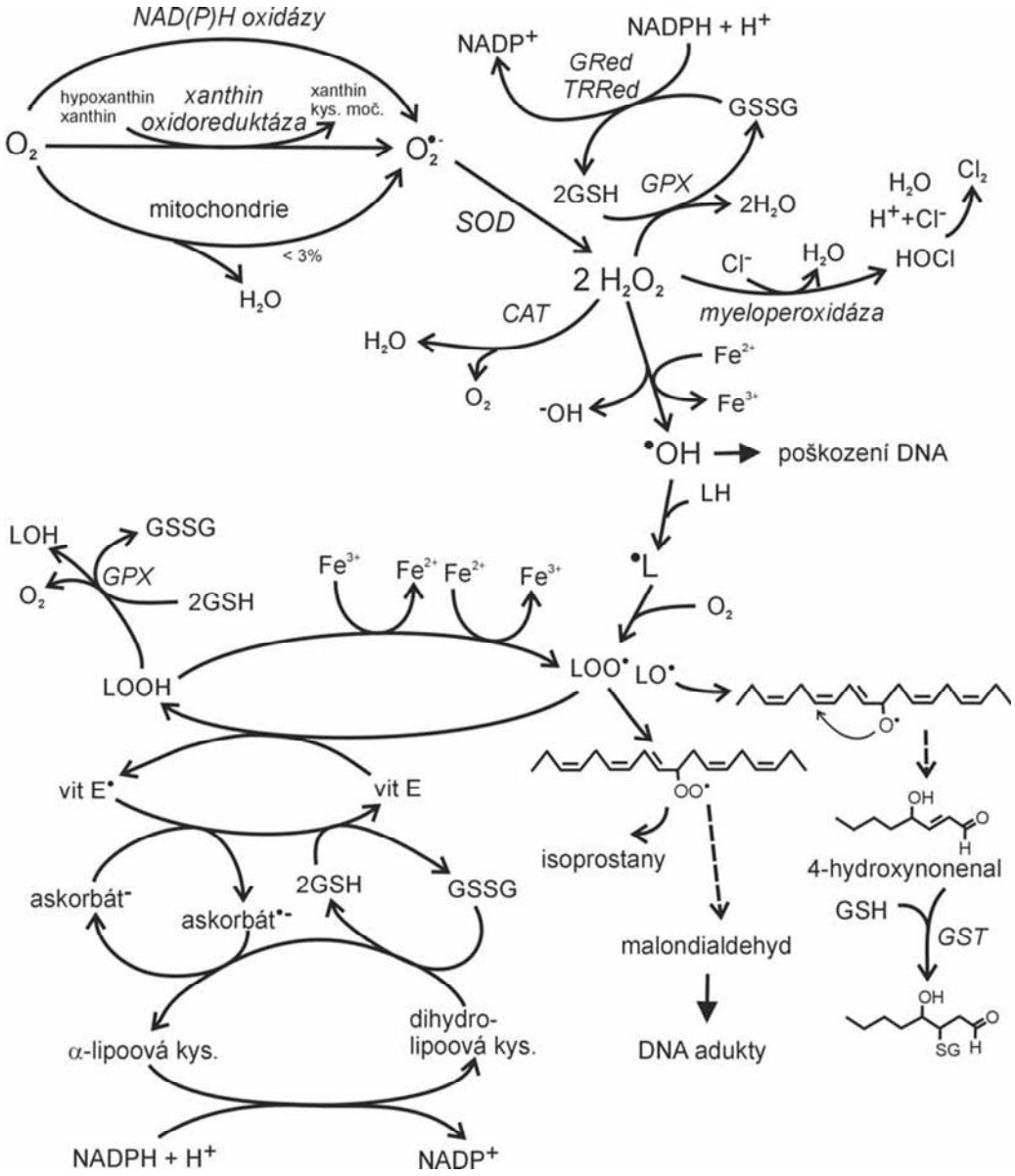
$$O_2 + e^- \rightarrow O_2^{\cdot-} \qquad [1]$$

Na superoxidový anion je převedeno až 3 % molekul mitochondriálního kyslíku (5). NAD(P)H oxidáza je enzym vázaný na buněčnou membránu, který používá elektrony pocházející z NADPH k redukci molekulárního kyslíku na $^{\cdot}O_2^-$:

$$NAD(P)H + 2\ O_2 \rightarrow NAD(P)^+ + H^+ + 2\ ^{\cdot}O_2^- \qquad [2]$$

Enzym se nachází v neutrofilních leukocytech, monocytech či makrofázích, kde je zdrojem velkého množství $^{\cdot}O_2^-$, který má baktericidní účinky. Strukturálně poněkud odlišná je NAD(P)H oxidáza obsažena v endotelu cév. Její produkce $^{\cdot}O_2^-$ je ve srovnání s formou obsaženou ve fagocytech o několik řádů nižší. Aktivita cévní NAD(P)H oxidázy a následná tvorba $^{\cdot}O_2^-$ je zvyšována působením řady faktorů účastnících se v patogenezi aterosklerózy, jako jsou angiotenzin II působící vazokonstrikci, PDGF (platelet derived growth factor) působící hyperplazii hladkých svalových buněk nebo trombin (6). Superoxid vzniká také působením enzymu xantinoxidoreduktázy. Tento flavoproteinový enzym obsahující molybden existuje ve dvou formách: xanthin oxidáza (XO) a xantin dehydrogenáza (XD). Enzym katalyzuje postupnou oxidativní hydroxylaci hypoxanthinu na xanthin a dále na kyselinu močovou (ve formě XD), ale může také redukovat kyslík na $^{\cdot}O_2^-$ (forma XO). Je zajímavé, že je lokalizován hlavně v endoteliálních a epiteliálních buňkách, což dobře nekoresponduje s jeho funkcí v metabolismu purinů, ale spíše naznačuje význam v systému antimikrobiální ochrany (7). Xantinoxidáza hraje významnou úlohu v patofyziologii reperfuzního syndromu. Při hypoxii způsobené nedostatečným přívodem kyslíku krví (nízká perfuze tkání např. při infarktu myokardu) dochází k vzestupu hladiny ADP, který je za fyziologických okolností přeměňován působením XO na hypoxantin, xantin a močovou kyselinu. Při hypoxii je enzym inhibován; poté, co dojde k reperfuzi a opětovnému obnovení dodávky kyslíku, zvýší XO svoji aktivitu s cílem odstranit nahromaděné ADP a jako vedlejší produkt jsou ve zvýšeném množství produkovány enzymem XO i ROS s následným paradoxním prohloubením poškození po obnově dodávky kyslíku.

Vznikající $^{\cdot}O_2^-$ je působením enzymu superoxid dismutázy (SOD) přeměňován na H_2O_2, ze kterého pak účinkem lyzozomální katalázy nebo mitochondriální glutathion peroxidázy (GPx) vzniká voda a kyslík (obr. 2). Glutathion, používaný GPx jako donor vodíku během eliminace H_2O_2, je regenerován glutathion reduktázou (GR). Součástí obranných mechanismů lidského organismu proti infekčním agens je také enzym myeloperoxidáza (MPO), nacházející se v azurofilních granulech



Obr. 1. Vznik reaktivních forem kyslíku v lidském organismu a jejich další osud (dle 6, 19)
vit – vitamin, GSH – glutathion, GSSG – glutathion disulfid, GPx – glutahion peroxidáza, GRed – glutathion reduktáza, GST – glutathion S-transferáza, TRRed – thioredoxin reduktáza, CAT – kataláza, LH – mastná kyselina, SOD – superoxid dismutáza

neutrofilů a lyzozomech monocytů. Enzym, který hraje roli ve fagocytóze, vytváří kyselinu chlornou (HClO) z peroxidu vodíku (H_2O_2) a chloridů. Reakce HClO se superoxidem může vést ke vzniku mimořádně reaktivního hydroxylového radikálu (8).

$$HOCl + {}^{\cdot}O_2^- \rightarrow {}^{\cdot}OH + Cl^- + O_2 \quad [3]$$
$$HOCl + Fe^{2+} \rightarrow {}^{\cdot}OH + Cl^- + Fe^{3+} \quad [4]$$

Chlornanové anionty reagují také s nízkomolekulárními aminy za vzniku chloraminů, které stejně jako chlornany mají silný baktericidní účinek (4). Hemo-peroxidázy, jako je MPO i eozinofilní peroxidázy, katalyzují také v přítomnosti H_2O_2 a nitritu NO_2^- nitraci tyrozinu v proteinech, a mohou tak nepříznivě modifikovat jejich funkci, jako např. v případě apolipoproteinu A-1 a apolipoproteinu B.

Reaktivní sloučeniny dusíku

Mezi nejvýznamnější reaktivní formy dusíku patří radikál oxidu dusnatého ·NO a peroxynitrit $ONOO^-$. Radikál oxidu dusnatého ·NO je tvořen oxidací L-argininu působením syntázy oxidu dusnatého (NOS) za vzniku citrulinu a NO. U člověka lze rozlišit tři hlavní izoformy NOS: endoteliální NOS (eNOS), inducibilní (iNOS) a neuronální (nNOS). nNOS a eNOS jsou konstitutivně exprimované enzymy, aktivované vzestupem hladiny intracelulárního kalcia (Ca^{2+}). Ca^{2+} se váže na kalmodulin a komplex Ca^{2+}/kalmodulin aktivuje nNOS nebo eNOS. iNOS obsahuje pevně vázaný kalmodulin s kalciem a její syntéza je indukována zánětlivými cytokiny, jako je interleukin-1 (IL-1), tumor nekrotizující faktor alfa (TNF-α), interferon gamma (IFN-γ), ale i antigeny bakterií a nádorových buněk.

Za fyziologických okolností se ·NO významně podílí na tzv. endotel-dependentní vazodilataci a regulaci cévního tonu, má protizánětlivé účinky, inhibuje agregaci krevních destiček a adhezi leukocytů i destiček na endotel a reguluje proliferaci a diferenciaci buněk cévní stěny. V buňce hladkého svalu cévy aktivuje ·NO enzym guanylát cyklázu. Aktivace guanylát cyklázy vede k syntéze cyklického GMP a k vazorelaxaci.

Ke správné funkci vyžadují NOS pět kofaktorů: flavinadenindinukleotid (FAD), flavinmononukleotid (FMN), hem, tetrahydrobiopterin (BH_4) a Ca^{2+}-kalmodulin. Jestliže chybí L-arginin – substrát pro NOS nebo jeden z jeho kofaktorů, může NOS produkovat $\cdot O_2^-$ místo ·NO, což je označováno jako roz-

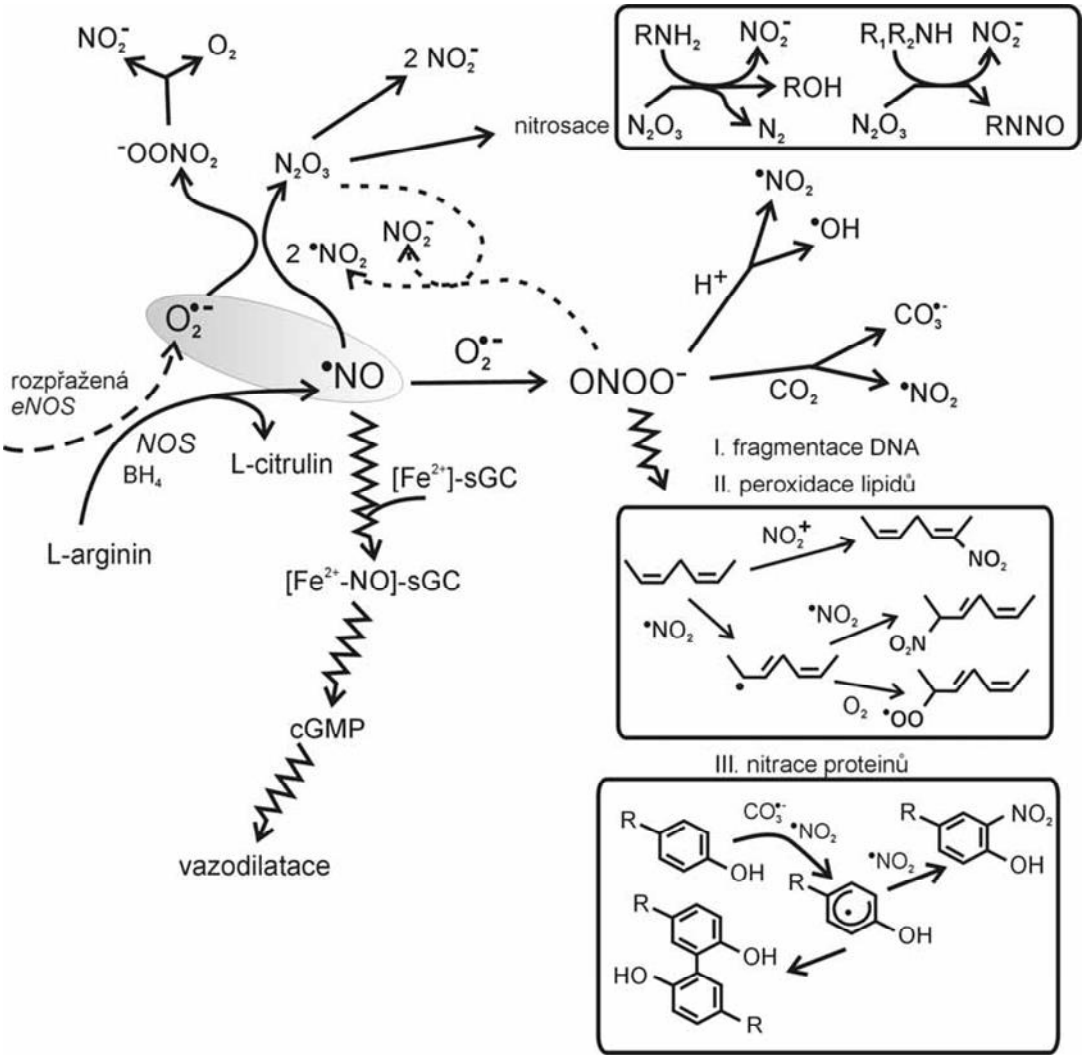
přažený (uncoupled) stav NOS (9). K rozpřažení reakce vede též zvýšení hladiny inhibitoru NOS, asymetrického dimetylargininu (ADMA), jehož zvýšené koncentrace jsou spojeny s endoteliální dysfunkcí u hypercholesterolémie, inzulínové rezistence či hyperhomocysteinémie. Rozpřažení eNOS v cévní stěně působí oxidační stres jednak poklesem tvorby ·NO, jednak zvýšenou tvorbou $\cdot O_2^-$. Pokud jsou tvořeny současně ·NO a $\cdot O_2^-$, vzniká toxický peroxynitrit $ONOO^-$. Peroxynitrit reaguje s CO_2, který je v tělesných tekutinách obsažen ve vysokých koncentracích, a vytváří jednoelektronové oxidanty $\cdot NO_2$ a $CO_3^{\cdot -}$, které oxidací aminokyseliny tyrozinu vedou ke vzniku tyrozinového radikálu $Tyr^{\cdot}$ a následně 3-nitrotyrozinu, 3-NO_2-Tyr . Z $ONOO^-$ může také vzniknout ·OH, působící peroxidaci lipidů, mutace DNA, jejich fragmentaci nebo modifikace proteinů (obr. 2). Peroxynitrit vedle přímého toxického působení oxiduje BH_4, což rovněž přispívá k rozpřažení eNOS.

Lipoxygenáza

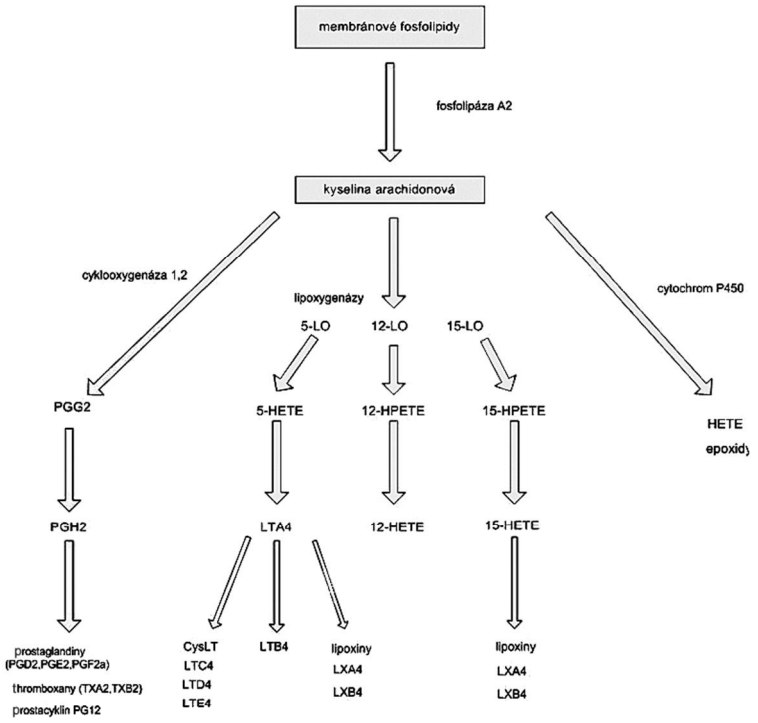
Lipoxygenázy jsou dioxygenázy obsahující železo, které katalyzují stereospecifickou inzerci molekulárního kyslíku do molekuly vícenenasycené mastné kyseliny. Aktivní forma enzymu obsahuje v katalytickém centru trojmocné železo, forma lipoxygenázy s dvojmocným železem není aktivní. Působení lipoxygenáz na kyselinu arachidonovou vede k tvorbě 5-, 11- a 15- hydroperoxyeikosatetraenových mastných kyselin (HPETE), které jsou v tkáních rychle redukovány na odpovídající hydroxyeikosatetraenové kyseliny (HETE), jako jsou 5*S*-hydroxy-6t,8c,11c,14c-, 12*S*-hydroxy-5c,8c,10t,14c- a 15*S*-hydroxy-5c,8c,11c,13t-eikosatetraenové mastné kyseliny (10). Z těchto derivátů jsou 5-hydroxy izomery předchůdci biologicky aktivních leukotrienů a lipoxinů (obr. 3), které hrají významnou roli v patofyziologii zánětu. 5-HETE má chemotaktický účinek na neutrofily, leukotrien B_4 (LTB_4) působí chemotakticky na fagocyty, LTC_4, LTD_4 a LTA_4 působí vazokonstrikci, bronchokonstrikci a zvyšují permeabilitu cév. Lipoxiny mají naopak účinky protizánětlivé.

Myeloperoxidáza

Myeloperoxidáza (MPO) je enzym, obsahující hem, který se nachází v azurofilních granulech neutrofilů a lyzozomech monocytů. Enzym vytváří kyselinu chlornou (HClO) z peroxidu vodíku (H_2O_2) a chloridů. MPO hraje roli při fagocytóze a produkty jejího působení se podílejí na destrukci bakterií, intracelulárních parazitů i nádorových buněk. Reakce kyseliny chlorné se superoxidem může vést ke vzniku mimořádně reaktivního hydroxylového radikálu (8). Chlornanové anionty reagují také s nízkomolekulárními aminy za vzniku chloraminů, které stejně jako chlornany mají silný baktericidní účinek (4). Hemo-peroxidázy, jako je MPO i eozinofilní peroxidázy, katalyzují také v přítomnosti H_2O_2 a nitritu NO_2^- nitraci tyrozinu v proteinech, a mohou tak nepříznivě modifikovat jejich funkci, jako např. v případě apolipoproteinu A-1 a apolipoproteinu B. Oxidované produkty působení MPO jsou ve vysokých



Obr. 2. Vznik a působení reaktivních forem dusíku (dle 6, 11, 16, 17)
BH_4 – tetrahydrobiopterin, eNOS – endoteliální syntháza NO, sGC – solubilní guanylát cykláza, cGMP – cyklický guanylmonofosfát



Obr. 3. Metabolizmus eikosanoidů
HETE – hydroxyeikosatetraenová kyselina, HPETE – hydroperoxyeikosatetraenová kyselina, LO-x – lipoxygenáza, COX – cyklooxygenáza, LX – lipoxiny, LT – leukotrieny, cysLT – cysteinylleukotrieny

koncentracích prokazovány v částicích LDL lokalizovaných v aterosklerotických plátech. Předpokládá se, že aktivita MPO souvisí s vulnerabilitou ateromových plátů (11). MPO může také modifikovat lipoprotein HDL, což vede k poruše reverzního transportu cholesterolu. Nejvyšší kvartily MPO v krvi a leukocytech významně korelovaly s přítomností koronární aterosklerózy. Ve studii u nemocných s AKS předpovídaly hladiny MPO rozvoj IM nezávisle na jiných rizikových faktorech, jako např. CRP (12). Zdá se, že MPO je významným činitelem, podílejícím se na destabilizaci ateromového plátu a stanovení MPO by mohlo sloužit jako nezávislý prognostický ukazatel u nemocných přijatých k observaci pro bolest na hrudi.

MECHANISMY PŮSOBENÍ REAKTIVNÍCH ČÁSTIC

Nukleové kyseliny, lipidy a proteiny mohou být poškozeny působením RONS, což může vést až k buněčné smrti (5). Jejich působení není však jen nepříznivé, aktivují také různé buněčné signální kaskády, které regulují proliferaci, detoxifikaci, reparaci DNA nebo apoptózu. V případě snížené tvorby RONS může dojít k poruše imunitní odpovědi na cizorodou noxu nebo k poruše proliferace. V závislosti na koncentraci a typu RONS mohou být aktivovány buď signální cesty protektivní (např. reparace DNA) anebo buněčná apoptóza.

Poškození lipidů

Působením radikálové látky, nejčastěji $^{\cdot}OH$, na lipidy, zejména na vícenenasycené mastné kyseliny (polyunsaturated fatty acids, PUFA), vede k lipoperoxidaci (viz obr. 2). Oxidující látka vytrhne elektron z metylenové skupiny uhlovodíkového řetězce PUFA ($-CH_2^{\cdot}-$) za vzniku lipidového radikálu ($L^{\cdot}$). Po vytržení vodíku dojde ke změně elektronového uspořádání v uhlovodíkovém řetězci PUFA tak, že mezi dvěma dvojnými vazbami je jedna vazba jednoduchá (konjugovaný dien) (4). Konjugované dieny se snadno spojují s molekulárním kyslíkem za vzniku peroxylového radikálu $LOO^{\cdot}$. Peroxylový radikál může vytrhnout elektron ze sousední mastné kyseliny, která se stává radikálem, zatímco peroxyl se mění na hydroperoxid LOOH. Radikálová reakce se pak řetězovitě šíří dál (propagace), dokud není ukončena (terminace) setkáním radikálu mastné kyseliny s jiným radikálem nebo vitaminem E (4). Lipoperoxidačními pochody vznikají hydroperoxidy a cyklické peroxidy mastných kyselin, které v přítomnosti přechodných kovů (jako Ca^{2+}, Fe^{2+}) podléhají tzv. Fentonově reakci za vzniku alkoxylového radikálu $LO^{\cdot}$ a hydroxidového aniontu OH^-.

$$LOOH + Fe^{2+}\{Cu^{+}\} \rightarrow LO^{\cdot} + Fe^{3+}\{Cu^{2+}\} + OH^- \quad [5]$$

Lipoperoxidace nakonec vede ke vzniku stabilních látek, které lze laboratorně stanovit, jako je např. MDA (malondialdehyd) a nebo 4-hydroxynonenal (4-HNE). Malondialdehyd velmi ochotně reaguje s nukleofilními skupinami (aminoskupiny), a způsobuje tak modifikaci struktury a následně funkce proteinů (zesíťování kolagenu). Další produkt oxidace vícenenasycených mastných kyselin 4-hydroxynonenal, elektrofilní α,ß-nenasycený aldehyd, způsobuje kovalentní modifikaci DNA, což způsobuje vznik mutací, a proteinů signálních drah, a ovlivňuje tak genovou expresi zodpovědnou za produkci složek antioxidačního systému, heat shock proteinů a proteinů účastnících se reparace poškozené DNA. 4-hydroxynonenal je též užíván jako biomarker oxidativního poškození buněk. Jinými významnými produkty působení oxidačního stresu na lipidy jsou F_2-izoprostany (13). Izoprostany jsou látky podobné F_2-prostaglandinu vznikající neenzymatickou peroxidací kyseliny arachidonové působením radikálů. F_2-izoprostany však *in vivo* prodělávají další přeměnu v E_2-, D2-, A2-, J2-izoprostany, izotromboxany a vysoce reaktivní ketoaldehydy zvané izoketaly. Podobné sloučeniny vznikají též z kyseliny dokosahexaenové, která je hojná v neuronech, a proto se sloučeniny vzniklé její radikálovou neenzymatickou peroxidací nazývají neuroprostany či neuroketaly. F_2-izoprostany jsou nejenom markery lipoperoxidace, ale jako ligandy specifických receptorů způsobují i vazokonstrikci. U různých onemocnění (např. diabetu) dochází ke vzniku oxidativně modifikovaných LDL částic tzv. oxLDL, jejichž hlavními kompo-

nentami jsou 9-hydroxy-10,12-oktadekadienová (9-HODE) a 13-hydroxy-9,11-oktadekadienová (13-HODE) kyselina. Vznikají působením ROS na linolovou kyselinu. Bylo zjištěno, že 9-HODE i 13-HODE jsou endogenními aktivátory PPAR-γ (peroxisome proliferator-activated receptor gamma) a hrají významnou úlohu například při rozvoji diabetické nefropatie tím, že stimulují mezangiální proliferaci.

Poškození DNA

Podobně jako lipidy jsou nukleové kyseliny poškozovány především ·OH. Hydroxylový radikál reaguje se všemi složkami DNA a poškozuje jak purinové, tak pyrimidinové báze i strukturu deoxyribózy. Dochází k vyjmutí vodíkového atomu z deoxyribózy s následnou destrukcí sacharidu a přerušení řetězce. Hydroxylový radikál vytváří addukty s purinovými i pyrimidinovými bázemi a modifikované báze pak slouží jako marker poškození DNA, např. 8-hydroxydeoxyguanozin (8-OH-dG), 8-hydroxy-guanin a 8-hydroxy-guanozin (4). Modifikace nukleových kyselin pak vede k chybným párováním bázi při replikaci DNA a k následným změnám genetické informace.

Poškození bílkovin

Oxidativní modifikace poškozuje strukturu bílkovin. Oxidace aminokyselin v proteinech vede k nevratným změnám. Dochází k fragmentaci a agregaci bílkovin. V důsledku konformačních změn se zvyšuje citlivost k proteolytickému štěpení. Citlivost proteinů vůči oxidaci je ovlivňována také přítomností iontů kovů schopných katalyzovat reakci Fentonova typu (14). Modifikovány jsou zejména aminokyseliny postranních řetězců, zejména cystein a methionin, přičemž oxidací cysteinových zbytků vznikají smíšené disulfidy mezi thiolovými skupinami bílkovin (-SH) a nízkomolekulárními thioly, zejména GSH (15). Oxidace proteinů vede také k fragmentaci polypeptidových řetězců a k intra- i intermolekulárnímu síťování (cross-linking). Takto modifikované proteiny snáze podléhají degradaci. Je známo, že glykovaný kolagen zvyšuje tvorbu malondialdehydu a 4-hydroxynonenalu, produktů oxidace vícenenasycených mastných kyselin. Malondialdehyd je velice reaktivní sloučenina vytvářející vazby především s amino-skupinami aminokyselin, což zapříčiňuje zesíťování proteinů a ztrátu jejich funkce. U diabetiků se zvyšuje glykace kolagenu, jenž se působením malondialdehydu zesíťuje a tento děj sekundárně urychluje rozvoj aterosklerózy.

ANTIOXIDAČNÍ SYSTÉM V LIDSKÉM ORGANISMU

Složité biochemické děje neustále probíhající v živých organismech vytvářejí RONS, které mohou mít jak nepříznivé, tak i příznivé účinky. Pro správné fungování metabolických procesů je tak nutné stále ustavovat rovnovážný stav mezi vznikem a odbouráváním RONS. K udržení homeostázy v situaci, kdy jsou neustále vytvářeny RONS, slouží systém antioxidantů (tab. 2). Dříve se pomýšlelo, že RONS mají pouze negativní účinky, ukázalo se však, že mají i příznivé účinky. V leukocytech slouží k likvidaci infekčních částic cestou respiračního vzplanutí za účasti NADPH oxidázy, hrají důležitou úlohu v signálních dráhách (ovlivňují nukleární faktor κB, mitogen- activated proteinkinázu atd.), proliferaci, přežívání, migraci a adhezi buněk.

Enzymatické antioxidanty

Mezi enzymatické antioxidanty patří např. superoxid dismutáza (SOD), kataláza (CAT), glutathion peroxidáza, glutathion reduktáza. Enzymy glutathion peroxidáza a glutathion reduktáza se nacházejí v cytoplazmě, mitochondriích i v jádře. Glutathion peroxidáza mění H_2O_2 na vodu za spoluúčasti glutathionu (GSH) jako dárce vodíku. Vznikající glutathion disulfid (GSSG) je přeměňován zpět na GSH působením GR, jejímž kofaktorem je NADPH. Působením SOD dochází k přeměně $^{\Sigma}O_2^-$ na H_2O_2, který je detoxikován buď katalázou, která v lyzozomech rozkládá H_2O_2 na vodu a kyslík, nebo účinkem GPx v mitochondriích (viz obr. 1). Glutathion reduktáza regeneruje GSH, který je používán jako donor vodíku glutathion peroxidázou během eliminace H_2O_2.

Tab. 2. Důležité komponenty antioxidačního systému (dle 30, 31, 36)

Lokalizace		
Intracelulární Antioxidanty	**buněčná membrána**	**extracelulární antioxidanty**
enzymové složky		
superoxiddismutáza kataláza glutathionperoxidáza peroxidáza DT-diaforáza proteolytické enzymy hem oxygenáza I	fosfolipázy	SOD-3 EC-CAT GPx-3 paraoxonáza selenoprotein P? peroxiredoxiny
neenzymové složky		
Intracelulární Antioxidanty	**buněčná membrána**	**extracelulární antioxidanty**
Glutathion kyselina askorbová kyselina lipoová vazebné proteiny kovů – feritin (Fe^{2+}ġFe^{3+}), metallothioneiny (Cu^{+}) opravné systémy DNA – excize basí glykosylázami, homologní rekombinace, spojování nehomologních konců	vitamín E ß-karoten	glutathion kyselina askorbová vitamin E * proteiny vázající přechodné kovy – transferin (Fe^{3+}), laktoferin (Fe^{3+}), ceruloplasmin (Cu^{2+}), haptoglobiny (hemoglobin) a hemopexin (hem) albumin, bilirubin, kyselina močová, thioredoxin

* Vitamin E je nejsilnějším antioxidantem v membránách, mimo ně vykazuje pouze slabé antioxidační schopnosti.

Neenzymatické antioxidanty

Mezi neenzymatické antioxidanty patří vitaminy A, C a E, glutathion, kyselina alfa-lipoová, dále karotenoidy, stopové prvky jako měď, zinek a selen, koenzym Q_{10} (Co Q_{10}) a kofaktory jako kyselina listová, vitaminy B_1, B_2, B_6 a B_{12}, dále též močovina, albumin či bilirubin. Hlavním intracelulárním antioxidantem je GSH, který působí jako přímý scavenger a současně jako kosubstrát pro GPx. Vitamin E je označení pro skupinu osmi příbuzných tokoferolů a tokotrienolů, které zabraňují peroxidaci lipidů. U lidí je nejaktivnější forma α-tokoferolu. Hydroxylový radikál reaguje s tokoferolem za vzniku stabilního fenolického radikálu, který je redukován zpět na fenol askorbátem a NAD(P)H dependentními reduktázami (16). Koenzym Q_{10} působí jako elektronový nosič v komplexu II mitochondriálního elektronového transportního řetězce. Je to v tucích rozpustný antioxidant, který ve vyšších koncentracích působí jako scavenger $^{\Sigma}O_2^-$ (17). Vitamin C (kyselina askorbová) stabilizuje kofaktor NOS, tetrahydrobiopterin (BH_4), což podporuje tvorbu NO (18). Kyselina α-lipoová je hydrofilní antioxidant, působící jak ve vodném, tak v lipidovém prostředí. Její redukovaná forma, dihydrolipoát, je schopna regenerovat jiné antioxidanty, jako jsou vitamin C nebo vitamin E (18). Bilirubin je v poslední době intenzivně studován jako neenzymatický antioxidant. Kromě toho působí antiaterogenně tím, že inhibuje oxidaci LDL částic a lipidů obecně a pohlcuje kyslíkové radikály. Mnohé studie prokázaly inverzní vztah hladin bilirubinu k výskytu kardiovaskulárních chorob. Lidé s Gilbertovým syndromem (nekonjugovaná hyperbilirubinémie) mají nižší incidenci koronární choroby (19).

UPLATNĚNÍ RONS V KLINICKÉ MEDICÍNĚ

Volné radikály i ostatní RONS plní v organismu významné funkce. Jsou součástmi **obranného systému** organismu proti bakteriální infekci, intracelulárním parazitům, cizorodým látkám i nádorovým buňkám. V případě bakteriální infekce se v neutrofilních leukocytech a makrofázích aktivuje enzym NAD(P)H oxidáza, vzniká superoxidový anion. Takto aktivované buňky zvýší spotřebu O_2 (tzv. oxidační nebo respirační vzplanutí, respiratory burst). Vznikající $^{\Sigma}O_2^-$ se přeměňuje na H_2O_2. Enzym myeloperoxidáza zase v polymorfonukleárních leukocytech katalyzuje tvorbu kyseliny chlorné z H_2O_2 a chloridového iontu. Významnou součástí obrany organismu proti různým mikrobům, intracelulárním parazitům i nádorovým buňkám, je aktivita iNOS. Exprese enzymu je indukována působením mikrobů a různých cytokinů a vede k produkci NO mnohem vyšší, než ke které dochází v důsledku aktivity eNOS. Současně vytvářený $^{\Sigma}O_2^-$ však působí zvýšení koncentrací peroxynitritu $ONOO^-$, který má baktericidní účinky (4). V nízkých koncentracích se RONS podílejí na nitrobuněčných signálních pochodech. V tzv. **signální transdukci** je zprostředkován přenos informace přicházející zvenčí prostřednictvím hormonů, cytokinů, růstových faktorů či neurotransmiterů až do buněčného jádra. Transkripční faktory po vazbě na specifické sekvence DNA regulují aktivitu RNA polymerázy II. Některé signální cesty v buňce jsou zprostředkovány RONS, které v tomto případě hrají roli „druhotných poslů" (second messengers). Pravděpodobně nejvýrazněji se RONS uplatňují při ovlivnění systému MAP kináz (mitogen associated protein kinase), který představuje kaskádu fosforylačních reakcí, ve kterých se postupně aktivují enzymy a další proteiny s výsledným ovlivněním jaderných transkripčních faktorů, regulujících buněčný růst, diferenciaci i apoptózu. Patří sem faktory NF-κB (nuclear factor kappa B), významný u zánětlivých procesů, AP-1 (activated protein-1), ovlivňující růst a diferenciaci buněk a p53, což je protein, který pomáhá udržovat stabilitu genomu (zásahem do reparačních mechanismů DNA a také do regulace proliferace a diferenciace buňky) (20).

VYBRANÁ ONEMOCNĚNÍ, V JEJICHŽ ETIOPATOGENEZI JSOU VÝZNAMNÉ VOLNÉ RADIKÁLY

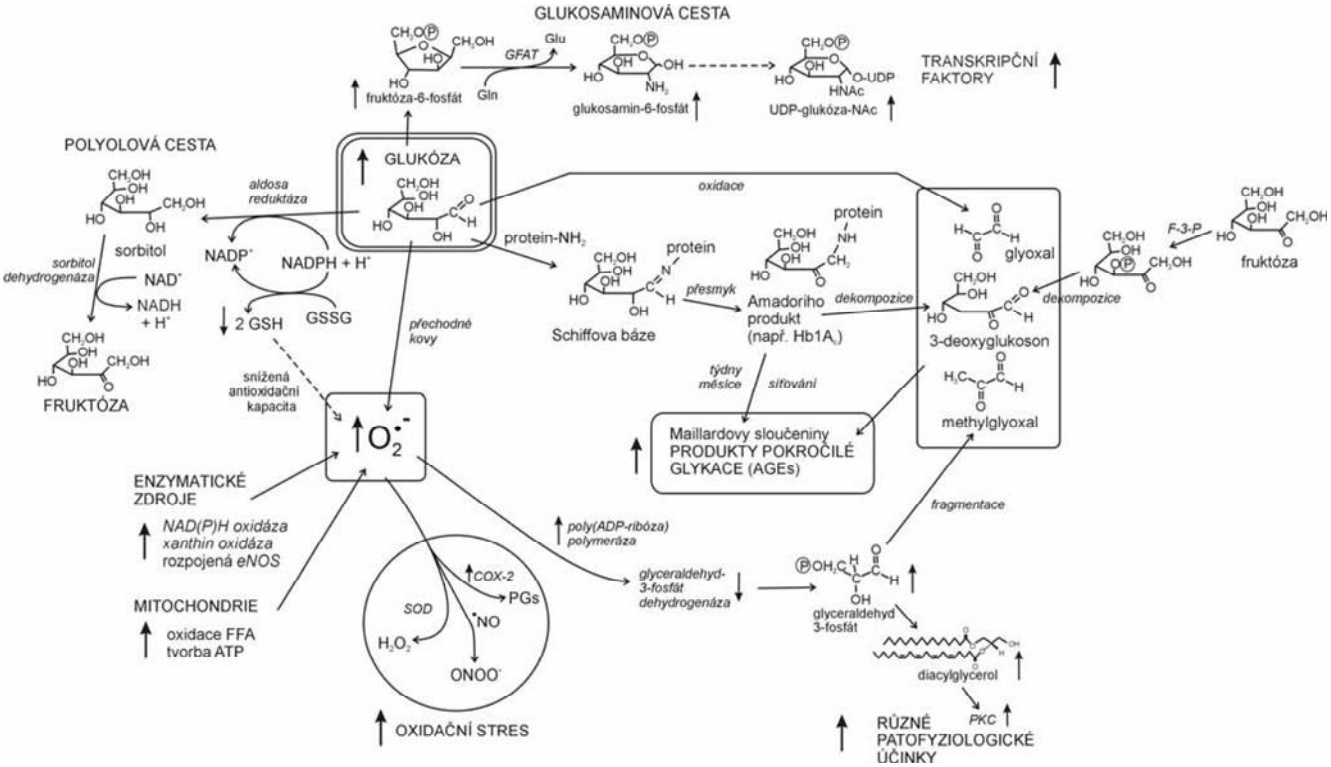
Ateroskleróza a její komplikace

Aterosklerózu je možno charakterizovat jako chronické zánětlivé fibroproliferativní onemocnění, ve kterém hraje podstatnou roli proliferace hladkých svalových buněk (SMC) a makrofágů, tvorba pojivové tkáně buňkami hladké svaloviny a hromadění lipidů, zejména volného (FC) a esterifikovaného cholesterolu (CE), v buňkách a mezibuněčné hmotě. V aterogenezi hraje oxidační stres významnou roli. Uplatňuje se zejména $^{\cdot}O_2^-$, H_2O_2 a $^{\cdot}NO$. Nadměrná tvorba RONS vede k aterogenním a trombogenním změnám ve smyslu zvýšené adheze monocytů, agregace krevních destiček a porušené vazodilatace. Působením RONS jsou ve stěně cévy modifikovány LDL částice a vznikají tzv. minimálně modifikované a oxidované LDL (mmLDL – minimally modified and oxLDL-oxidized), mmLDL mají zoxidovanou pouze lipidovou složku a u oxLDL dochází k oxidační modifikaci i proteinové složky. Tyto částice následně inhibují vazodilataci a působí proaterogenně tím, že aktivují zánětlivou odpověď, proliferaci buněk, ale i jejich apoptózu. Syntéza proaterogenních adhezních molekul je zvyšována cytokiny (interleukiny, tumor necrosis factor alfa, angiotenzin II, endoteliální růstový faktor VEGF) mechanismy zahrnujícími RONS. Inaktivace $^{\cdot}NO$ působením $^{\cdot}O_2^-$ a zvýšená tvorba H_2O_2 inhibuje vazodilataci. Oxidační stres působí také zvýšenou apoptózu endoteliálních buněk cestou aktivace signální cesty proteinkinázy C (21). H_2O_2 i $^{\cdot}O_2^-$ významně ovlivňují migraci hladkých svalových buněk do cévní stěny indukcí MCP-1 (monocyte chemotactic protein-1) i proliferaci hladkých svalových buněk. Zvýšením sekrece i aktivity metaloproteinázy 9 se RONS podílejí i na zvýšeném odbourávání extracelulární matrix (22), což má význam při vývoji nestabilního ateromového plátu a následné trombózy.

Diabetes mellitus

U diabetes mellitus zvýšený oxidační stres pochází z několika zdrojů: 1. neenzymatické zdroje, tj. zejména hyperglykémie, 2. zdroje enzymatické, kde vznikají RONS v důsledku aktivity enzymů, hlavně NAD(P)H oxidázy, xanthinoxidázy, cyklooxygenázy a 3. mitochondriální elektronový řetězec v průběhu oxidativní fosforylace (23). Hyperglykémie zvyšuje tvorbu volných radikálů několika způsoby (24). Během tzv. autooxidace glukózy, katalyzované přechodnými kovy, dochází ke vzniku redukovaných kyslíkových derivátů, jako jsou $^{\Sigma}O_2^-$, $^{\Sigma}OH$ a H_2O_2 ale i reaktivních ketoaldehydů. Glukóza je dále schopna vázat se neenzymaticky adicí k aminoskupině proteinu (glykace), tímto vznikají přes meziprodukt (Schiffovy báze) tzv. Amadoriho produkty. Důsledkem intramolekulárních přesmyků v Amadoriho produktech je vznik vysoce reaktivních dikarbonylových látek, glyoxalu, methylglyoxalu a 3-deoxyglukosonu. Řádově během týdnů jsou Amadoriho produkty po intra- a intermolekulárních přestavbách přeměňovány na novou třídu molekul, tzv. Maillardovy sloučeniny, neboli AGE (advanced glycation end products). U hyperglykémie se metabolismus glukózy ubírá i polyolovou cestou, která také vede ke zvýšené tvorbě $^{\Sigma}O_2^-$. Glukóza je nejprve redukována aldozoreduktázou za účasti NADPH na sorbitol, ten je oxidován NAD^+ s následným zvýšením poměru NADH/NAD^+ v cytosolu (hyperglykemická pseudohypoxie).

Zdrojem zvýšené tvorby superoxidu $^{\Sigma}O_2^-$ jsou u diabetu vedle hyperglykémie také enzymatické aktivity NAD(P)H oxidázy, xanthinoxidázy i cyklooxygenázy, jejichž působením vzniká $^{\Sigma}O_2^-$ jednoelektronovou redukcí kyslíku. Se vzestupem hladiny $^{\Sigma}O_2^-$, možná v důsledku poklesu BH_4 jsou spojeny stavy spojené s inzulinovou rezistencí jako obezita, arteriální hypertenze a diabetes mellitus (25). Superoxid, tvořený v mitochondriálním systému za hyperglykemických podmínek,



Obr. 4. Oxidační stres a hyperglykémie v patofyziologii diabetu (dle 26)
COX-2 – izoforma 2 cyklooxygenázy, SOD – superoxid dismutáza, PKC – proteinkináza C, F-3-P – fruktóa 3-fosfatáza, GFAT – glutamin: fruktóza 6-fosfát aminotransferáza, Gln – glutamin, Glu – glutamát, PGs – prostaglandiny

aktivuje enzym poly (ADP-ribóza) polymerázu (PARP). To působí inhibici glyceraldehydfosfát dehydrogenázy (GAPDH) s následným zvýšením koncentrace glyceraldehyd-3-fosfátu a aktivací 4 patologických mechanismů: 1. polyolové cesty, 2. glukozaminové cesty, 3. zvýšené tvorby methylglyoxalu a AGE, 4. vzniku diacylglycerolu (DAG), který aktivuje PKC (proteinkinázu C) (26). Aktivací PKC je možné vysvětlit některé cévní abnormality pozorované u diabetu (změny funkcí buněk endoteliálních, mezangiálních, buněk hladkého svalstva cév s výslednými změnami permeability, kontraktility a syntézy bazální membrány). PKC může také modulovat působení hormonů, růstových faktorů a iontových kanálů. Nástin působení oxidačního stresu v patofyziologii komplikací diabetu je podán na obrázku 4.

Hyperlipidémie

Hypercholesterolémie i hypertriglyceridémie (HTG) jsou zdrojem zvýšeného oxidačního stresu. Je u nich zjišťována zvýšená tvorba $^{\Sigma}O_2^-$, zřejmě v důsledku zvýšené aktivity xantin oxidázy (27) a NAD(P)H oxidázy (28). Léčiva užívaná k léčbě těchto dvou stavů, statiny a fibráty, nesnižují pouze hladinu lipidů, ale mají tzv. pleiotropní účinky, mezi které patří i příznivé účinky na oxidační stres. U nemocných s HTG léčených fibráty byl popsán pokles hladiny konjugovaných dienů, prodloužení lag fáze lipoproteinových částic VLDL a LDL i vzestup aktivity SOD a GPx (29).

Neurodegenerativní onemocnění

Mozek je vůči oxidačnímu stresu vysoce citlivý, protože využívá 20 % kyslíku spotřebovávaného organismem (30). Mozek také obsahuje velké množství vícenenasycených mastných kyselin a železa a nízkou koncentraci antioxidačních enzymů.

Parkinsonova nemoc. U Parkinsonovy nemoci dochází k degeneraci neuronů v substantia nigra, secernujících dopamin, které se podílí na kontrole a plánování pohybu. Předpokládá se, že v patofyziologii choroby se uplatňuje tvorba RONS a oxidace dopaminu (31). U pacientů s Parkinsonovou nemocí jsou silné důkazy o působení oxidačního stresu. V mozku pacientů s Parkinsonovou nemocí byla zjištěna zvýšená množství oxidovaných forem proteinů, lipidů a nukleových kyselin, jako jsou karbonyly proteinů, 4-hydroxy-2-nonenol a 8-hydroxy-2-deoxyguanozin (2, 3, 5, 9–11).

Alzheimerova nemoc. Podobně se oxidační stres podle současných názorů uplatňuje také v patogenezi Alzheimerovy nemoci. Alzheimerova nemoc (AD) je heterogenní onemocnění, za jejíž hlavní rys je považováno ukládání amyloidu beta (Aß) v mozku. Beta-amyloid je ukládán extracelulárně v tzv. senilních placích a je tvořen z těla vlastního amyloidového prekurzorového proteinu (APP). Dalším patologickým proteinem u AD je degenerovaný protein tau, uložený intracelulárně (32). Tvorba RONS, jako např. H_2O_2 provázející redukci kovových iontů, vedla k oxidačnímu poškození neuronů a vzniku Aß. Aß sám je zdrojem oxidačního stresu. Během progrese AD byla prokázána lipoperoxidace, oxidační poškození proteinů i DNA.

V mozkové tkáni jsou u pacientů s AD prokazovány markery oxidačního stresu, jako je zvýšená aktivita hem-oxygenázy 1 (HO-1) a koncentrace 8-hydroxyguaninu (8-OHG). Senilní plaky nesou známky oxidativního poškození jako modifikace Maillardovými sloučeninami (AGE), karbonylace, „síťování“ (cross-linking) proteinů. V mozkové tkáni nemocných s AD jsou také prokazovány zvýšené koncentrace železa a mědi. Přesné mechanismy spojení mezi oxidačním stresem a smrtí neuronů, vedoucí k poruchám poznávacích procesů, však zatím nebyly objasněny (33).

Psychiatrická onemocnění

V poslední době je úloha oxidačního stresu sledována i u některých psychiatrických onemocnění, zejména schizofrenie, ale i depresivních poruch, obsedantní kompulzivní poruchy (OKP) a autismu.

Schizofrenie. U pacientů se schizofrenií je většinou prokazována dysfunkce antioxidačního systému spojená s vystupňovanou lipoperoxidací. Mechanismy vzniku a působení oxidačního stresu u schizofrenie nejsou jasné, někteří autoři ukazují na význam zvýšeného obratu katecholaminů u nemocných se schizofrenií (34).

Depresivní poruchy. V etiopatogenezi deprese se předpokládá účast oxidačního stresu, a protože mozek obsahuje velké množství vícenenasycených mastných kyselin, železa a nízkou koncentraci antioxidačních enzymů je k jeho působení náchylný. Deprese je často spojena i se subklinickým zánětem provázeným zvýšenými hladinami zánětlivých cytokinů, zvyšujících tvorbu reaktivních částic (35). Byla popsána korelace závažnosti symptomů deprese s hladinou lipoperoxidů v séru.

ZÁVĚR

RONS i antioxidační systémy hrají v organismu důležitou úlohu. Jejich vývoj šel ruku v ruce s vývojem aerobního metabolismu a ochrany před toxicitou kyslíku. Role RONS není pouze negativní účastí v patofyziologických mechanismech různých chorob, ale RONS mají též řadu příznivých účinků a jsou součástí přirozených buněčných signálních drah. Jejich patologické působení závisí hlavně na nerovnováze prooxidačních a antioxidačních systémů. V současné době je jejich studiu věnována zvýšená pozornost a lze doufat v brzké uplatnění poznatků pro léčbu chorob, v jejichž rozvoji se RONS uplatňují.

Zkratky

13-HODE – a 13-hydroxy-9,11-oktadekadienová kyselina
4-HNE – 4-hydroxynonenal
8-OH-dG – 8-hydroxydeoxyguanozin
8-OHG – 8-hydroxyguanin
9-HODE – 9-hydroxy-10,12-oktadekadienová kyselina
AD – Alzheimerova nemoc
ADMA – asymetrický dimetylarginin
AGE – advanced glycation end products
AP-1 – activated protein-1
APP – amyloidový prekurzorový protein
Aß – amyloid beta
BH_4 – tetrahydrobiopterin
Ca^{2+} – intracelulární kalcium
CAT – kataláza
CE – esterifikovaný cholesterol
cGMP – cyklický guanylmonofosfát
Co Q_{10} – koenzym Q_{10}
COX – cyklooxygenáza
COX-2 – izoforma 2 cyklooxygenázy
CRP – C-reaktivní protein
cysLT – cysteinylleukotrieny
DAG – diacylglycerol
eNOS – endotheliální syntáza NO
F-3-P – fruktóza 3-fosfatáza
FAD – flavinadenindinukleotid
FC – volný cholesterol
FMN – flavinmononukleotid
GAPDH – glyceraldehydfosfát dehydrogenázy
GFAT – glutamin: fruktóza 6-fosfát aminotransferáza
Gln – glutamin
Glu – glutamát
GPx – glutahion peroxidáza
GR – glutathion reduktáza
GSH – glutathion
GSSG – glutathion disulfid
GST – glutathion S-transferáza
H_2O_2 – peroxid vodíku
HclO – kyselina chlorná
HETE – hydroxyeikosatetraenová kyselina
HO-1 – hem-oxygenáza
HPETE – hydroperoxyeikosatetraenová mastná kyselina
HTG – hypertriglyceridémie
IFN-γ – interferon gamma
ICHS – ischemická choroba srdeční
IL – interleukin
iNOS – inducibilní NOS
LDL – lipoproteiny o nízké hustotě
LH – mastná kyselina
LO-x – lipoxygenáza
LT – leukotrieny
LX – lipoxiny
MCP-1 – monocyte chemotactic protein-1
MDA – malondialdehyd
mmLDL – minimálně modifikované a oxidované LDL (minimally modified and oxLDL-oxidized)
MPO – myeloperoxidáza
NF-κB – nuclear factor kappa B
nNOS – neuronální NOS
NO – oxid dusnatý
NOS – syntáza oxidu dusnatého
OKP – obsedantní kompulzivní porucha
$ONOO^-$ – peroxynitrit
OS – oxidační stres
PDGF – platelet derived growth factor
PGs – prostaglandin
PKC – proteinkináza C
PPAR-γ – peroxisome proliferator-activated receptor gamma
PUFA – polyunsaturated fatty acids
RONS – reactive oxygen and nitrogen species
ROS – reaktivní sloučeniny kyslíku
sGC – solubilní guanylát cykláza
SMC – proliferace hladkých svalových buněk
SOD – superoxid dismutáza
TNF-α – tumor nekrotizující faktor alfa
TRRed – thioredoxin reduktáza
XD – xanthin dehydrogenáza
XO – xanthaxanthin oxidáza

LITERATURA

1. **Ignarro LJ, Cirino G, Casini A, Napoli C.** Nitric oxide as a signalling molecule in the vascular system: an overview. J Cardiovasc Pharmacol 1999; 34: 879–886.
2. **Sies H.** Role of reactive oxygen species in biological processes. Klin Wochenschr 1991; 69(21–23): 965–968.
3. **Halliwell B, Whiteman M.** Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 2004; 142: 231–255.
4. **Štípek S. (ed.)** Antioxidanty a volné radikály ve zdraví a nemoci. Praha: Grada Publishing 2000.
5. **Valko M, Leibfritz D, Moncola J, Cronin MTD, Mazura M, Telser J.** Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem & Cell Biol 2007; 39: 44–84.
6. **Madamanchi NR, Vendrov A, Runge MS.** Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol 2005; 25: 39–38.
7. **Harrison R.** Physiological Roles of Xanthine Oxidoreductase Drug Metab Rev 2004; 36(2): 363–375.
8. **Racek J, Holeček V.** Enzymy a volné radikály. Chem Listy 1999; 93: 11A–780.
9. **Maritim AC, Sanders RA, Watkins JB, 3rd.** Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003; 17: 24–38.
10. **Smith WL.** The eicosanoids and their biochemical mechanisms of action. Biochem. J 1989; 259: 315–324.
11. **Hazen SL, Heinecke JW.** 3-chlorotyrosine, a specific marker of myeloperoxidase- catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. J Clin Invest 1997; 99(9): 2075–2081.
12. **Brennan ML, Hazen SL.** Emerging role of myeloperoxidase and oxidant stress markers in cardiovascular risk assessment. Curr Opin Lipidol 2003; 14: 353–359.
13. **Lawson JA, Rokach J, Fitzgerald GA.** Isoprostanes: formation, analysis and use as indices of lipid peroxidation in vivo. J Biol Chem 1999; 274 (35): 24441–24444.
14. **Stadtman ER.** Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. Free Radical Biol Med 1990; 9: 315–325.

15. **Grune T, Reinheckel T, Davies Kja.** Degradation of oxidized proteins in mammalian cells. FASEB J 1997; 11: 526–534.
16. **Hensley K, Robinson KA, Gabbita SP, et al.** Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med 2000; 28(10): 1456–1462.
17. **Hodgson JM, Wats GF.** Can koenzyme Q10 improve vascular fiction and blood pressure? Potential for effective therapeutic reduction in vascular oxidative stress. Biofactors 2003; 18 (1–4): 129–136.
18. **Heller R, Unbehaun A, Schellenberg B, et al.** L-ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin. J Biol Chem 2001; 276(1): 40–47.
19. **Vítek L, et al.** Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis 2002; 160(2): 449–456.
20. **Piette J, Piret B, Bonini G, Schoonbroodt S, Merville MP, Legrand-Poels S, Bours V.** Multiple redox regulativ in NF-kappa B transcription factor activation. Biol Chem 1997; 378 (11): 1237–1245.
21. **Brunt KR, Fenrich KK, Kiani G, et al.** Protection of human vascular smooth cells from H_2O_2-induced apoptosis through functional codependence between HO-1 and Akt. Arterioscler. Thromb. Vasc Biol 2006; 26: 2027–2034.
22. **Libby P, Ridker PM, Maseri A.** Inflammation and Atherosclerosis. Circulation 2002; 105: 1135–1143.
23. **Griendling KK., FitzGerald GA.** Oxidative stress and cardiovascular injury: Part I: Basic mechanisms and in vivo monitoring of ROS. Circulation 2003; 108: 1912–1916.
24. **Giugliano D, Ceriello A, Paolisso G.** Oxidative stress and diabetic vascular complications. Diabetes Care 1996; 19: 257–267.
25. **Das UN.** Folic acid says NO to vascular diseases. Nutrition 2003; 19: 686–692.
26. **Brownlee M.** The Pathobiology of diabetic complications. A unifying mechanism. Banteng Lemure 2004. Diabetes 2005; 54: 1615–1625.
27. **Ohara Y, Peterson TE, Harrison DG.** Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993; 6: 2546–2551.
28. **Guzik TJ, Mussa S, Gastaldi D, et al.** Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxid synthese. Circulation 2002; 105: 1656–1662.
29. **Zeman M, Žák A, Vecka M, Tvrzická E, Romaniv S, Konárková M.** Treatment of hypertriglyceridemia with fenofibrate, fatty acid composition of plasma and LDL, and their relations to parameters of lipoperoxidation of LDL. Ann NY Acad Sci 2002; 967: 336–341.
30. **Juurlink BH, Patison PG.** Review of oxidative stress in brain and spinal cord Indry: suggestions for pharmacological and nutritional management strategies. J Spinal Cord Med 21 1998; 309–334.
31. **Zhang J, Perry G, Smith MA, et al.** Parkinsonęs dinase is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol 1999; 154: 1423–1429.
32. **Jirák R, Koukolík F.** Demence. Praha: Galén 2004.
33. **Perry G, Cash AD, Smith MA.** Alzheimer disease and oxidative stress. J Biomed Biotechnol 2002; 23: 120–123.
34. **Mahadik SP, Mukherjee S.** Free radical pathology and antioxidant defense in schizophrenia: A review. Schizophrenia Research 1996; 19(1): 1–17.
35. **Maziere C, Auclair M, Maziere JC.** Tumor necrosis factor enhances low density lipoprotein oxidative modification by monocytes and endothelial cells. FEBS Lett 1994; 338: 43–46.
36. **Tkáč I, Molčányiová A, Javorský M, Kozárová M.** Fenofibrate treatment reduces circulating conjugated diene level and increases glutathione peroxidase activity. Pharmacol Res 2006; 53: 261–264.

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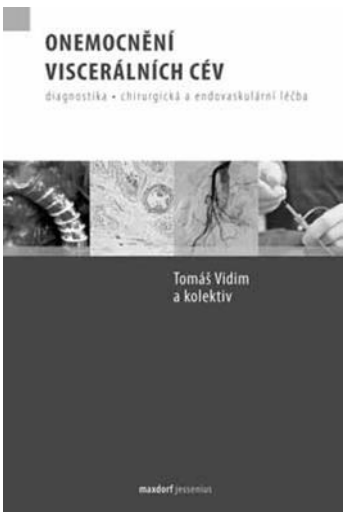

ONEMOCNĚNÍ VISCERÁLNÍCH CÉV
Diagnostika, chirurgická a endovaskulární léčba

MUDr. Tomáš Vidim a kol.

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Postižení viscerálních cév je provázeno vysokou morbiditou a ve svých akutních projevech také vysokou mortalitou. Chronická ischemie splanchnických tepen je však dobře léčitelná a při včasné diagnostice lze snížit fatální riziko nepoznaného onemocnění.
Viscerální ischemie vyžaduje mezioborovou spolupráci. Týká se to jak diagnostiky, na níž se podílejí gastroenterologové, angiologové i další interní a chirurgické obory, tak zejména léčby. Endovaskulární léčba je již samostatným oborem a kapitola byla zpracována předním specialistou v oboru. Cévně chirurgická léčba je v kompetenci cévního chirurga. Kniha uvádí popis operačních přístupů spolu s obrazovou dokumentací, která by měla být instruktivní pro všechny, kteří v daných oblastech neoperují příliš často.

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