

The Antioxidant Power and Level of Lipid Peroxidation Products in the Sera of Apparently Healthy Adult Males

Višnja Katalinić¹, Ilza Salamunić², Senijo Pažanin³, Rosanda Mulić⁴, Marija Milišić⁴ and Darko Ropac⁴

¹ Department of Food Technology and Biotechnology, Faculty of Chemical Technology, University of Split, Split, Croatia

² Department of Medical Laboratory Diagnosis, University Hospital Split, Split, Croatia

³ Department of Occupational Health, Naval Medical Institute, Split, Croatia

⁴ Department of Public Health, School of Medicine, University of Split, Split, Croatia

ABSTRACT

The aim of this study was to determine the antioxidant potential of the serum and the level of lipid oxidation products in the sera of apparently healthy adult males. The »antioxidant power« of the serum, defined as the ability to reduce ferric ions by antioxidants from the serum (FRAP), was taken as the indicator of total antioxidation potential. The formation of lipid oxidation products was evaluated as thiobarbituric reactive species serum test (TBARS). The ferrous oxidation in xylenol orange version 2 (FOX2) assay coupled with triphenylphosphine was used for measurement of total sera hydroperoxides (ROOHs). The following biochemical variables were determined in the sera: aspartat aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transferase (GGT), bilirubin, glucose, creatinine, cholesterol, triglycerides and hemoglobin. Blood sera from apparently healthy subjects (166 adult males) were analyzed. Median age of study participants was 36 years (range 25–50 years). The $X \pm SD$ sera FRAP level of the 166 apparently healthy adult males was $1047 \pm 131 \mu\text{mol/L}$ (779–1410 range). The $X \pm SD$ level of sera TBARS was $1.2 \pm 0.3 \mu\text{mol/L}$ of the sera (0.5–2.2 range). Compared with the level of TBARS in the sera, the level of FOX2-ROOH was significantly higher. The $X \pm SD$ level of lipid hydroperoxides in the fresh sera, determined as FOX2, was $3.9 \pm 1.5 \mu\text{mol/L}$ of the sera (1.9–6.9 range). Observation of correlation between FRAP and determined biochemical variables in the sera have confirmed a statistically significant linear correlation between sera FRAP and bilirubin, hemoglobine, glucose, ALT and triglycerides ($p < 0.05$). The results of sera FRAP, TBARS and FOX2 levels can help in estimating the antioxidant status of humans. Significant correlation between the antioxidant power of blood serum and particular biochemical parameters indicates the complexity of defence mechanisms and various molecules involved in increasing the reduction power of the serum.

Key words: blood sera, antioxidant power, lipid peroxides, FRAP, FOX2, TBARS

Introduction

Potentially harmful reactive oxygen species (ROS) are constantly produced during normal aerobic metabolism and are safely removed by a variety of biological antioxidants. Cells have formidable defenses against oxidative damage, many of which may not be readily recognized as antioxidants. Antioxidant protection can operate at several different levels within cells by preventing radical formation, intercepting radicals when formed, repairing oxidative damage caused by radicals, increasing the elimination of damaged molecules and not repairing exces-

sively damaged molecules. When prooxidants increase or antioxidant fail, a situation of oxidative stress ensues that leads to excessive molecular damage and tissue injury^{1,2}.

Mammals have evolved complex antioxidant strategies to utilize oxygen and to minimize the noxious effects of its partially reduced species. The cooperation among the different antioxidants provides greater protection against attack by ROS, than any compound alone. Thus, the overall antioxidant capacity may give more biologi-

cally relevant information than that obtained from measuring concentrations of individual antioxidants^{3,4}. The extra cellular fluids of the human body, such as blood plasma, tissue fluid, cerebrospinal fluid, synovial fluid and seminal plasma contain little or no catalase activity, and only low activities of superoxide dismutase (SOD) and selenium-containing glutathione peroxidase can be measured. There is also very little reduced glutathione (GSH) in most extra cellular fluids; about 2 μM in human plasma⁵. Plasma contains a variety of redox activity low-molecular mass molecules and many of these have been ascribed primary antioxidant role. Very often, low-mass molecular antioxidants are multifunctional: can act as reducing agents, hydrogen donating antioxidants, singlet oxygen quenchers, or can bind reactive transition metal cations by complexation. Thus chemically different methods for measuring antioxidant activity will produce different »hierarchies« of antioxidants^{2,6–10}.

Many compounds with antioxidant activity are readily oxidizable compounds. In principle it should be possible to correlate the antioxidative effectiveness of a compound toward an oxidizing species such as an electrophilic free radical with its ease of oxidation by an electron transfer process *in vitro*¹⁰. Nonenzymic antioxidants such as ascorbic acid can be described as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reactive species is reduced at the expense of the oxidation of another. In this context, antioxidant power may be referred as reducing ability¹¹.

Disturbance of the balance between the production of ROS such as superoxide; hydrogen peroxide, hypochlorous acid, hydroxyl, alkoxyl, and peroxy radicals; and antioxidant defenses against them produces oxidative stress, which amplifies tissue damage by eroding away protective sacrificial antioxidants¹². Previous investigations mostly deal only with one side of balance: positive aspect (determination of antioxidative efficiency of extra cellular fluids using different assays) or negative aspect (determination of the different products of lipid oxidation).

A related point of interest in this study was parallel investigation of the ferric reducing ability/antioxidant power of serum (FRAP) and amount of lipid oxidation products in the fresh serum of apparently healthy adult males, and correlation of sera FRAP or lipid oxidation products with several biochemical variables.

Materials and Methods

All chemicals and reagents were of analytical grade and were obtained from Sigma – Aldrich Chemical Co. (Steineheim, Germany), Merck (Darmstadt, Germany) and Kemika (Zagreb, Croatia).

Samples

Blood sera from apparently healthy subjects (166 Croatian adult males) were analyzed. The samples were collected during the years 2002 and 2003. Men selected for the study were those who were in good health, as deter-

mined by a history questionnaire during the periodical health examination of the male population. At least one month before participation in the research the subjects had not been under any medication. Median age of study participants was 36 years (range 25–50 years). Fasting blood samples were collected in the morning and immediately chilled. Blood sera were obtained by centrifugation at 2000xg for 15 minutes. Sera were separated and used immediately.

Spectrophotometric measurements

Spectrophotometric measurements were performed by UV-VIS Specord 200 double-beam spectrophotometer (Analytik Jena GmbH, Jena, Germany).

Determination of ferric-reducing/antioxidant power of blood sera (FRAP)

The total antioxidant potential of blood sera was determined using a ferric reducing ability of plasma (FRAP) assay as a measure of »antioxidant power«¹¹. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} form by the action of electron donating antioxidants. The working FRAP reagent was prepared by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 1 volume of 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L hydrochloric acid and with 1 volume of 20 mmol/L ferric chloride. Freshly prepared FRAP reagent (1.5 mL) was warmed to 37 °C and a reagent blank reading was taken at 593 nm/2nm slit width (M1). Subsequently, 50 μL of sample and 150 μL of deionized water was added to the FRAP reagent. Final dilution of sample in reaction mixture was 1:34. The sample was incubated at 37 °C throughout the monitoring period. The change in absorbance between the final reading selected (4-min reading) and the M1 reading were selected for calculation of FRAP values. Aqueous solutions of known Fe^{II} concentration, in range of 100–1000 μmol/L (FeSO₄·7H₂O) were used for calibration. All solutions were used on the day of preparation. In the FRAP assay the antioxidant efficiency of the sera under the test was calculated with reference to the reaction signal given by a Fe^{II} solution of known concentration, this representing a one-electron exchange reaction. All reactions were performed in triplicate.

Determination of lipid peroxidation products with thiobarbituric acid reactive substances (TBARS) assay

The method known as thiobarbituric acid reactive species (TBARS) assay, concerns the spectrophotometric measurement of the pink pigment produced through reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) and other secondary lipid peroxidation products. The evaluation of absorbance at 532 nm gives a measure of the extent of lipid degradation^{12,13}. TBARS in sera were determined by previously described assay¹⁴. Aliquot (0.5 mL) of sera was added to a reaction mixture (1.0 mL) formed by equal parts of 15% trichloroacetic acid,

0.25 N HCl, and 0.375% thiobarbituric acid, followed by 30 min heating at 95 °C. After cooling either incubation, chromogen was extracted with n-butanol and read spectrophotometrically at 532 nm against a reaction mixture »blank« lacking sera but subjected to the entire procedure and extracted with n-butanol. To correct for background absorption, absorbance values at 572 nm were subtracted from those at 532 nm, the latter representing the absorption maximum of the 2:1 TBA:MDA adduct¹⁵. A molar extinction coefficient of 156 000 was used. The level is expressed in terms of malondialdehyde. All determinations were performed in duplicate.

Determination of lipid hydro peroxide concentrations in sera by FOX2 assay

Lipid hydroperoxides (ROOH) were determined by ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine (TPP)¹⁵. The FOX2 reagent comprises two stock solutions, A and B. Stock solution A was prepared by dissolving ammonium ferrous sulphate (98 mg) in 100 mL of 250 mM H₂SO₄. Subsequently Xylenol Orange (76 mg) was added to the ammonium ferrous sulphate solution and the mixture stirred for 10 min at room temperature. Solution B was prepared by dissolving 970 mg BHT in 900 mL methanol (HPLC-grade). A working FOX2 reagent was prepared by mixing one volume of solution A with nine volumes of solution B. Final FOX2-reagent comprised Xylenol Orange (100 µmol/L), BHT (4.4 mmol/L), sulphuric acid (25 mmol/L) in 90% (vol/vol) methanol¹⁶. The extinction coefficient of the FOX2 reagent at 560 nm was routinely checked in solutions of known concentrations of hydrogen peroxide (H₂O₂). Aliquots (180 µL) of sera were transferred into eight centrifuge vials (8 mL). Then 20 µL of 10 mmol TPP in methanol was added to four of the vials to reduce ROOHs, thereby generating a quadruplicate of blanks. Methanol (20 µL) was added to the remaining four vials to produce a quadruplicate of test samples. All vials were then vortexed and incubated at room temperature for 30 min prior to the addition of 1800 µL of FOX2 reagent. After mixing, the samples were incubated at room temperature for another 30 min. The vials were centrifuged at 8000x g for 10 min. Absorbance of supernatant was measured at 560 nm. ROOH concentration in sera samples was calculated using the mean absorbance difference between quadruplicates of test samples and blank samples.

Determination of different sera biochemical variables

Sera biochemical variables were determined (aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transferase (GGT), bilirubin, glucose, creatinine, cholesterol, triglyceride) with commercially available reagents (Olympus Diagnostica, Co Clare, Ireland). All analyses were performed on an Olympus AU 600, automated clinical chemistry analyzer (Olympus Optical Co. Tokyo, Japan). Hemoglobin was determined on an Advia 120 Hematology system (Bayer C., Tarrytown, NY, USA).

Statistical analysis

Statistical analysis was done using GraphPad InStat. Data are presented as means with standard deviations (SDs). The unpaired t-test was used to compare whether the mean of the two variables differs. A two-tailed »p« value less than 0.05 was considered statistically significant. Variation among column means of three age groups for FRAP, TBARS or FOX2 were compared using one-way analysis of variance (ANOVA). Correlation between two variables was calculated using χ^2 test and quantified by the correlation coefficient »r«. The limit for significance was set to p<0.05.

Results

The 166 fresh sera samples of apparently healthy adult males were analyzed. All assays were performed immediately after sera preparation. The results of the determination of different biochemical variables in tested sera samples are given in Table 1, in comparison with reference values. Different methods may yield different values of sera biochemical variables, depending on calibration and other technical considerations¹⁷. The reference values given in this study were extracted from *Harmonisation of general medical biochemistry tests* document of Croatian Chamber of Medical Biochemists¹⁸. According to the obtained results it is evident that determined biochemical variables were not always within referent values. The most number of variations from sera referent values was noticed for triglycerides and cholesterol levels. Almost 50% of sera samples had elevated triglycerides, and cholesterol was not within referent values for 36% of sera samples, mostly from participants' age group 31–40. To calculate the number of sera samples with all controlled biochemical variables within reference values (»normal« sera samples) all tested samples with AST, ALT, GGT, bilirubin, glucose, creatinine, cholesterol, triglyceride and hemoglobin higher or lower of referent values were excluded. According to the obtained results 25.3% of 166 sera samples had »normal« values of controlled biochemical variables (all variables within referent values, n=42).

Ferric-reducing/antioxidant power of blood sera (FRAP, analyzed fresh)

The FRAP assay gives fast and reproducible results with sera and plasma (EDTA-plasma, Li-plasma). The difference between the reducing ability of sera and reducing ability of EDTA-plasma was not statistically significant. Because of the practical reasons in this study sera samples were tested. All tested sera samples (N=166) showed significant reducing ability/antioxidant power (Table 2).

FRAP – ferric reducing/ antioxidant power of sera, TBARS – thiobarbituric acid reactive species, FOX2 ROOH – total sera hydroperoxides determined using FOX2 assay.

TABLE 1
BLOOD SERA BIOCHEMICAL VARIABLES OF APPARENTLY HEALTHY ADULT MALES IN COMPARISON WITH THE REFERENCE VALUES

Biochemical variables in blood sera	Referent values for males	Controlled sera samples		Number of sera within referent values	% of sera within referent values
	(min–max)	(min–max)	mean		
Hemoglobin g/L	138–175	119–172	152	151	90.96
Glucose mmol/L	4.4–6.4	4.3–8.5	5.6	149	89.76
Creatinine $\mu\text{mol/L}$	79–125	63–112	87	160	96.39
Triglyceride mmol/L	0.9–2.0	0.4–13.6	1.3	86	51.81
Cholesterol mmol/L	3.9–5.7	2.7–9.8	5.6	106	63.86
Bilirubin total $\mu\text{mol/L}$	3.0–20	4.7–48.9	14.1	148	89.16
GGT U/L	11–55	8–277	34.9	134	80.72
AST U/L	11–38	13–140	26.2	148	89.16
ALT U/L	12–48	9–126	32.2	121	72.89

GGT – γ -glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase, N=166; median age 36 years; range 25–50 years

The means of sera FRAP in $\mu\text{mol/L}$ were: 1013 (ages 21–30), 1055 (ages 31–40) and 1066 (ages 41–50). The $X \pm \text{SD}$ sera FRAP value of all 166 apparently healthy Croatian males was 1047 ± 131 $\mu\text{mol/L}$ (range: 779–1409). The differences between means of sera FRAP levels of different age groups, determined with one-way ANOVA, were not statistically significant ($p > 0.05$), although slight constant increase of the mean values of sera FRAP can be noticed in older age groups. There was significant correlation (χ^2 test, the limit for significance set to $p < 0.05$, Table 3) between sera FRAP ($n=166$) and bilirubin ($p=0.0213$; bilirubin range 4.7–48.9 $\mu\text{mol/L}$), between sera FRAP and hemoglobin ($p=0.0318$; hemoglobin range 119–172 g/L), between sera FRAP and glucose ($p=0.0241$; glucose range 4.3–8.5 mmol/L), and sera FRAP and ALT ($p=0.0157$, ALT range 9–126 U/L). Also, there was very significant linear correlation between sera FRAP and triglycerides ($p=0.0091$; triglycerides range 0.4–13.6 mmol/L). About 25% (42/166) of sera samples had all determined biochemical variables within referent values («normal» sera samples). The $X \pm \text{SD}$ sera FRAP value of 42 «normal» sera samples was 1007 ± 123 $\mu\text{mol/L}$ (range 779–1313). According the unpaired t-test, there was no significant difference between the means of FRAP of this «normal» group ($n=42$) and FRAP of all tested samples ($n=166$).

Thiobarbituric acid reactive substances (TBARS) in sera, analyzed fresh

Thiobarbituric acid (TBA) reacts with standard lipoperoxidation aldehydes, such as malondialdehyde (MDA); 2,4-nonenal and 2,4-decadienal. TBARS test is often used clinically to investigate radical-mediated lipid peroxidation and oxidative stress^{17,18}. The sera TBARS levels of apparently healthy adult males are summarized in Table 2. As can be seen, the $X \pm \text{SD}$ sera TBARS of apparently healthy adult Croatian males was 1.2 ± 0.3 $\mu\text{mol/L}$ of sera ($n=166$) and same as the result obtained for «normal» sera samples ($n=42$). The results are in agreement with

Lapenna et al. 2001 (1.2 ± 0.2). Inspection of values listed in Table 2 indicates that the differences of TBARS levels between three age groups were not significant. The positive linear correlation between sera TBARS ($n=166$) and biochemical variables: hemoglobin and creatinine was confirmed (Table 3).

Lipid hydroperoxide concentrations in sera analyzed by FOX2 assay

Authentic sera hydroperoxydes were determined by strategy in which the hydroperoxide reductant, triphenylphosphine, is used to discriminate between the background signals generated by hydroperoxide in sera. The assay is based upon oxidation of ferrous ions in ferric ions by hydroperoxides under acidic conditions. Ferric ions are complexed by the ferric ion indicator, xylenol orange, generating a blue-purple complex with an absorbance maximum at 550–560 nm¹⁵. According to the obtained results the $X \pm \text{SD}$ level of lipid hydroperoxydes (FOX2) in fresh analyzed sera of Croatian adult males ($n=166$) was 3.9 ± 1.6 $\mu\text{mol/L}$ (range 1.9–6.9 $\mu\text{mol/L}$) and was significantly higher compared with mean level of FOX2 in the «normal» group of sera samples (Table 2). The difference between means of sera FOX2 levels of different age groups was determined, and FOX2 tended to increase with age. Compared with mean level of TBARS in sera (1.2 $\mu\text{mol/L}$), mean level of FOX2-ROOH in sera was significantly higher. The correlation between sera FOX2 lipid hydroperoxydes and TBARSs was not significant. Positive correlation between FOX-2 and creatinine was confirmed (Table 3).

Discussion

Individual members of antioxidant defense team in biological fluids are deployed to prevent generation of ROS, destroy potential oxidant, and to scavenge ROS. Blood has a central role within the strategy to maintain redox balance because it transports and redistributes an-

TABLE 2
FERRIC REDUCING ABILITY/ANTIOXIDANT POWER OF SERA (FRAP) AND LEVEL OF LIPID PEROXIDATION PRODUCTS (TBARS AND FOX2-ROOH) IN THE FRESH SERA OF APPARENTLY HEALTHY ADULT MALES

Indicators of antioxidative status		Sera samples of apparently healthy adult males				»Normal« sera samples* (N=42)
		Age group years 21–30 (N=46)	Age group years 31–40 (N=84)	Age group years 41–50 (N=36)	Total (N=166)	
FRAP ($\mu\text{mol/L}$)	X \pm SD	1013 \pm 137	1055 \pm 117	1066 \pm 151	1047 \pm 131	1007 \pm 124
	Range	779–1337	829–1302	824–1409	779–1409	779–1313
TBARS ($\mu\text{mol/L}$)	X \pm SD	1.2 \pm 0.3	1.2 \pm 0.3	1.3 \pm 0.3	1.2 \pm 0.3	1.2 \pm 0.3
	Range	0.6–1.1	0.5–2.2	0.8–1.9	0.5–2.2	0.7–2.2
FOX2 ($\mu\text{mol/L}$)	X \pm SD	3.1 \pm 0.8	3.9 \pm 1.3	4.9 \pm 1.6	3.9 \pm 1.5	3.0 \pm 0.6
	Range	2.3–4.4	1.9–6.5	2.1–6.9	1.9–6.9	1.9–3.8

* »Normal« sera samples – Sera samples with all controlled biochemical variables within referent values according *Harmonisation of general medical biochemistry tests* document of Croatian Chamber of Medical Biochemists,

TABLE 3
CORRELATION BETWEEN SERA ANTIOXIDANT POWER (FRAP), LIPID PEROXIDATION PRODUCTS (TBARS AND FOX2) IN FRESH SERA, AND CONTROLLED SERA BIOCHEMICAL VARIABLES

Variable	FRAP		TBARS		FOX-2	
	r	p	r	p	r	p
Hemoglobin g/L	0.1682	0.0318*	0.1798	0.0217*	0.1916	0.4184 ^{ns}
Glucose mmol/L	0.1761	0.0241*	0.0670	0.3938 ^{ns}	0.1923	0.4167 ^{ns}
Creatinine $\mu\text{mol/L}$	0.1184	0.1310 ^{ns}	0.1595	0.0414*	0.4700	0.0365*
Triglyceride mmol/L	0.2036	0.0091*	0.0224	0.7762 ^{ns}	0.1983	0.4020 ^{ns}
Cholesterol mmol/L	0.1157	0.1402 ^{ns}	0.0918	0.2422 ^{ns}	0.4298	0.0586 ^{ns}
Bilirubin $\mu\text{mol/L}$	0.1802	0.0213*	0.1259	0.1093 ^{ns}	0.1039	0.6629 ^{ns}
GGT U/L	0.1468	0.0606 ^{ns}	0.0057	0.9425 ^{ns}	0.1449	0.5422 ^{ns}
AST U/L	0.0646	0.4112 ^{ns}	0.0120	0.8789 ^{ns}	0.0259	0.9136 ^{ns}
ALT U/L	0.1885	0.0157*	0.0978	0.2127 ^{ns}	0.0566	0.8126 ^{ns}

r – correlation coefficient (quantified using χ^2), *p – a limit for significance was set to $p < 0.05$, N=166, GGT – γ -glutamyl transferase, AST – aspartate aminotransferase, ALT – alanine aminotransferase

tioxidants to every part of the body. To evaluate the level of oxidative stress in biological fluids it is very important to have basic information about: a) antioxidant capacity of biological fluid, and b) the »reference« level of lipid peroxidation products in biological fluid^{19–22}. Tests, which measure the combined antioxidant effect of the nonenzymic antioxidants in human sera or plasma, may be useful in providing an index of ability to resist oxidative damage. The speed and easy of the FRAP assay permits several analysis of antioxidant capacity to be performed on fresh samples. According to the obtained results the mean sera FRAP of healthy adult males (all biochemical variables within referent values), fresh analyzed, was 1007 $\mu\text{mol/liter}$, what is in agreement with the previously reported level of mean plasma FRAP of 1017 $\mu\text{mol/L}$ ¹⁰. In our research work the minimum sera FRAP value was 779 $\mu\text{mol/L}$ and was significantly higher (cca 30%) compared with minimum FRAP (614 $\mu\text{mol/L}$) as reported by Benzie and Strain (1996). It is difficult to say what the reason of these differences is. In our study only

adult males were tested, and the females were not included. The next studies have to give information about average sera FRAP of healthy females, and sera FRAP change with getting old (>50). The level of sera FRAP could be influenced by some other factors, too. The contribution of ascorbic acid, α -tocopherol, uric acid, bilirubin, and protein to total level of plasma FRAP is known¹⁰, but it is quite possible that FRAP level can also be significantly influenced by numerous different food constituents²³. Results of this current study indicate that antioxidant capacity of sera determined as ferric reducing ability, can be influenced by different biochemical variables. The strong positive correlation between sera FRAP and bilirubin was confirmed. The results of this research work are in agreement with the results of other authors^{10,24}. Bilirubin is generally regarded as toxic compound when accumulated at abnormally high concentrations in tissue. However it has also been suggested that the bile pigments bilirubin and biliverdin may protect vitamin A and linoleic acid from oxidative destruction in the intes-

tinal tract. Bilirubin contains an extended system of conjugated double bonds and a reactive hydrogen atom and thus could possess antioxidant properties²⁵. The results showed very strong positive correlation between sera FRAP and increased concentrations of triglycerides, too. It is difficult to explain correlation between FRAP and sera triglycerides. Could it be that significant increase of sera antioxidant capacity was induced with abnormally high concentrations of sera triglycerides, as a response of a body to protect them from oxidation?

Despite the biochemical importance of lipid peroxidation it has been difficult to measure. A number of routine laboratory techniques have been employed for the assessment of various lipid peroxidation products in body fluids⁷. Between different methods for hydroperoxide measurement, which are currently available, the TBA methods are those which are most widely used and require the least sophisticated instrumentation, but are most criticized on grounds of their inadequate specificity. The TBARS assay measures many variables in addition to lipid peroxidation and is affected by the lipid content of sample¹⁶. In spite of its inadequate specificity and because of its simplicity and sensitivity, the TBA test is still the most common method of assessing lipid peroxidation in biological samples^{25–27}. According to the results of this study, the physiological level of TBARS of healthy adult males was less than 2.5 (range 0.5–2.2) $\mu\text{mol/L}$ of sera, and there was no significant difference between sera TBARS for tested age groups.

The ferrous oxidation in xylenol orange version (FOX2) assay has recently been employed to determine whole plasma lipid hydroperoxides^{16,25–27}. Sodergren *et al.* have noticed that about 27% of the lipid hydroperoxides is lost during storage and that were large inter-individual differences in the loss after storage¹⁶. For those reasons only fresh sera samples were tested. The obtained result of the mean level of lipid hydroperoxides in fresh sera of healthy adult males (3.0 \pm 0.6 $\mu\text{mol/L}$) is in agreement with the results of the three separate studies conducted in healthy volunteers using FOX2 assay where the de-

tected plasma level of hydroperoxides was 3.02 \pm 1.85; 3.76 \pm 2.48 and 4.1 \pm 2.2 $\mu\text{mol/L}$ ²². The lack of correlation between FOX2 and TBARS assay is not surprising because they use different technologies. The average level of lipid hydroperoxides in sera as detected with FOX2 assay was about 3-folds higher compared with lipid oxidation products determined as TBARS. It is obvious that determination of lipid oxidation products in biological fluids as TBARS can lead to underestimation of extent of lipid peroxidation.

Previous investigations mostly deal only with one side of the balance: positive aspect (determination of antioxidative efficiency of extra cellular fluids using different assays like TEAC, ORAC or FRAP) or negative aspect (determination of lipid peroxidation products). In this study we made a parallel investigation of the basic ability of sera to protect itself from ROS (FRAP) and the level of damage (TBARS and FOX2). The knowledge of the values of sera FRAP, TBARS and FOX-ROOH can be useful tool in evaluating and controlling the level of oxidative stress in the body fluids.

Conclusion

The delicate balance between oxidants and antioxidants in blood sera is essential in health and disease. Knowledge of the antioxidant power (FRAP) and the level of lipid peroxidation products (TBARS, FOX2-ROOH) in the sera of healthy adult males can be of help in evaluating and controlling the antioxidant status in humans.

The level of lipid oxidation products in sera determined as TBARS is underestimated compared with FOX2-ROOH. The significant correlation between sera FRAP and some biochemical variables like bilirubin and triglycerides, indicates that, because of potential confounding effects, determination of sera FRAP has to be accompanied by determination of different biochemical variables.

REFERENCES

1. FREI B, STOCKER R, AMES BN, Proc Natl Acad Sci USA, 85 (1988) 9748. — 2. HALLIVEL B, GUTTERIDGE JMC, Free Radic Biol Med, 18 (1995) 125. — 3. GHISELLI A, SERAFINI M, MAINAI G, ASSINI E, FERRO-LUZZI A, Free Radic Biol Med, 18 (1994) 29. — 4. GHISELLI A, SERAFINI M, NATELLA F, SCACCINI C, Free Radic Biol Med, 29 (2000) 1106. — 5. HALLIVEL B, GUTTERIDGE JMC, Arch biochem biophys, 280 (1990) 1. — 6. GUTTERIDGE JMC, RICHMOND R, HALLIWEL B, FEBS Lett, 112 (1980) 269. — 7. RICE-EVANS C, MILLER NJ, Methods Enzymol, 234 (1994) 279. — 8. PRIOR RL, CAO G, Free Radic Biol Med, 27 (1999) 1173. — 9. LARSON RA, Antioxidant mechanisms of secondary natural products. In: AHMAD S (Ed), Oxidative stress and antioxidant defenses in biology (Chapman & Hall, New York 1995). — 10. BENZIE IFF, STRAIN JJ, Anal Biochem, 239 (1996) 70. — 11. GUTTERIDGE JMC, Clin Chem, 41 (1995) 1819. — 12. KULIŠIĆ T, RADONIĆ V, KATALINIĆ V, MILOŠ M, Food Chem, 85 (2004) 633. — 13. KATALINIĆ V, MODUN D, MUSIĆ B, BOBAN M, Comp Biochem Physiol C Toxicol Pharmacol, 140 (2005) 47. — 14. BUEGE JA, AUST SD, Methods Enzymol, 52 (1978) 302. — 15. LAPENNA D, GIOFANI G, PERDOMENICO SD, GIAMBERARDINO MA, CUCCURULLO F, Free Radic Biol Med, 31 (2001) 331. — 16. SODERGREN E, NOUROOZ-ZADEH J, BERGLUND L, VESSBY B, J Biochem Biophys Methods, 37 (1998) 137. — 17. ČVO-RIŠEĆ D, STAVLJENIĆ-RUKAVINA A, Handbook on Evaluation of Medical Biochemistry Laboratory Tests, In Croatian, (Medicinska naklada Zagreb, 1993). — 18. Croatian Chamber of Medical Biochemists, Novosti: Harmonizacija laboratorijskih nalaza, Available from: URL, accessed August 08, 2006. — 19. NOUROOZ-ZADEH J, TAJJADINI-SARMADI J, WOLF SP, Anal Biochem, 220 (1994) 403. — 20. GHISELLI A, SERAFINI M, NATELLA F, SCACCINI C, Free Radic Biol Med, 29 (2000) 1106. — 21. SANCHEZ-MORENO C, CANO MP, DE ANCOS B, PLAZA L, OLMEDILLA B, GRANADO F, MARTIN A, Am J Clin Nutr, 78 (2003) 454. — 22. TIWARI AK, Curr Sci, 8 (2001) 1179. — 23. MODUN D, MUSIĆ I, KATALINIĆ V, DUJIĆ Ž, BOBAN M, Clin Chem, 52 (2006) 785. — 24. OU B, HUANG D, HAMPSCH-WOODILI M, FLANAGAN JA, FASEB J, 17 (2003) 127. — 25. CAO G, PRIOR RL, Clin Chem, 44 (1998) 1309. — 26. YAGI K. Free Radicals in Diagnostic Medicine (Plenum Press, New York, 1994) — 27. NOUROOZ-ZADEH J, SMITH CC, BETTERIDGE DJ, Atherosclerosis, 156 (2001) 435.

R. Mulić

School of Medicine, University of Split, Šoltanska 2, 21 000 Split, Croatia
e-mail: rosanda.mulic1@st.htnet.hr

ANTIOKSIDACIJSKA SNAGA I PRODUKTI LIPIDNE PEROKSIDACIJE U SERUMU NAIZGLED ZDRAVIH ODRASLIH MUŠKARACA

S A Ž E T A K

Cilj ovog rada bio je odrediti antioksidacijski potencijal seruma i razinu produkata oksidacije lipida u serumu naizgled zdravih odraslih muškaraca te istražiti korelaciju između ovih pokazatelja antioksidacijskog statusa seruma i pojedinih biokemijskih parametra. Antioksidacijska snaga seruma određena je FRAP metodom (Ferric-Reducing/Antioxidant Power). Količina produkata oksidacije lipida kvantificirana je određivanjem kemijskih vrsta u serumu koje reagiraju s tiobarbiturnom kiselinom (TBARS test). Udio ukupnih hidroperoksida (ROOH) u serumu kvantificiran je metodom FOX2 (Ferrous oxidation in xylenol orange version 2). U serumu su određivani sljedeći biokemijski parametri: aspartat aminotransferaza (AST), alanin aminotransferaza (ALT), γ -glutamil transferaza (GGT), bilirubin, glukoza, kreatinin, kolesterol, trigliceridi i hemoglobin. Analizirani su serumi odraslih naizgled zdravih muškaraca ($n=166$) starosne dobi od 20 do 50 godina (median 36). Prosječna vrijednost FRAP-a u kontroliranim serumima ($n=166$) bila je 1047 ± 131 $\mu\text{mol/L}$ (raspon 779–1410). Prosječni udio TBARS u serumu iznosio je $1,2\pm 0,3$ $\mu\text{mol/L}$ seruma (raspon 0,5–2,2). U usporedbi s udjelom TBARS u serumu, udio FOX2-ROOH bio je znatno veći. Prosječni $\pm\text{SD}$ udio lipid hidroperoksida u serumu, određen FOX2 metodom iznosio je $3,9\pm 1,5$ $\mu\text{mol/L}$ seruma (raspon 1,9–6,9). Potvrđena je statistički značajna linearna korelacija između FRAP-a seruma i bilirubina, hemoglobina, glukoze, ALT i triglicerida ($p<0,05$). Rezultati određivanja serumskog FRAP-a, TBARS-a i FOX2-a mogu pomoći u procjeni antioksidacijskog statusa čovjeka. Značajna korelacija između antioksidacijske snage seruma i pojedinih biokemijskih parametra ukazuje na složenost mehanizama obrane i sudjelovanje različitih molekula u jačanju redukcijske snage seruma.