

## AGE-RELATED BLOOD ANTIOXIDANT CAPACITY IN MEN AND WOMEN STARENJE I ANTIOKSIDANTNI KAPACITET U KRVI KOD MUŠKARACA I ŽENA

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**Summary:** The aim of the study was to assess the blood antioxidant capacity in men and women in relation to age. The subjects were 19 men (YM) and 19 women (YW) aged 25–32 years, and 11 men (OM) and 11 women (OW) aged 63–71 years, all sedentary. The following factors were determined: the activity of erythrocyte superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX), catalase (CAT), total antioxidant status (TAS), as well as plasma retinol,  $\alpha$ -tocopherol, uric acid and total protein concentrations. The sum of standardized activities of antioxidant enzymes was calculated to compare age-related changes in the total capacity of the erythrocyte antioxidant defense. No significant age-related changes in SOD activity were observed; mean CAT activity was higher in older women and men than in younger subjects. Mean activity of GPX was higher and that of GR lower in older subjects compared to the younger ones. The calculated total erythrocyte antioxidant enzyme capacity in younger and older subjects rendered similar values. No significant differences in plasma retinol and  $\alpha$ -tocopherol concentrations in relation to sex or age were noted. The plasma total protein level was significantly lower in younger women and men compared to their older mates. It was concluded that the total erythrocyte enzymatic antioxidant capacity did not change with age. The results obtained clearly show that multiple factors may contribute to the ageing process.

**Keywords:** antioxidant enzymes, non-enzymatic antioxidants, men, women, ageing

**Kratak sadržaj:** Cilj studije bio je da se odredi antioksidantni kapacitet u krvi kod muškaraca i žena u odnosu na njihovu starost. Ispitano je 19 muškaraca (YM) i 19 žena (YW) starosti 25–32 godine, kao i 11 muškaraca (OM) i 11 žena (OW) starosti 63–71 godine, koji su svi bili neaktivni. Određeni su sledeći faktori: aktivnost superoksid dismutaze (SOD), glutation-reduktaze (GR), glutation-peroksidaze (GPX), katalaze (CAT), ukupni antioksidantni status (TAS) u eritrocitima, kao i retinol, alfa-tokoferol, mokraćna kiselina i ukupne koncentracije proteina u plazmi. Izračunat je zbir standardizovanih aktivnosti antioksidantnih enzima kako bi se uporedile promene u ukupnom kapacitetu antioksidantne odbrane u eritrocitima vezane za starosno doba. Nisu uočene značajne promene vezane za starosno doba u aktivnosti SOD; srednja aktivnost CAT bila je viša kod starijih žena i muškaraca nego kod mlađih ispitanika. Srednja aktivnost GPX bila je viša a aktivnost GR niža kod starijih ispitanika u poređenju s mlađima. Izračunati ukupni antioksidantni enzimski kapacitet u eritrocitima dao je slične vrednosti kod mlađih i kod starijih ispitanika. Nisu uočene značajne razlike u koncentracijama retinola i alfa-tokoferola u plazmi u odnosu na pol ili starosno doba. Ukupni nivo proteina u plazmi bio je značajno niži kod mlađih žena i muškaraca u poređenju sa starijim ispitanicima. Zaključeno je da se ukupni enzimski antioksidantni kapacitet u eritrocitima ne menja s godinama. Dobijeni rezultati jasno pokazuju da u procesu starenja učestvuje više faktora.

**Ključne reči:** antioksidantni enzimi, neenzimski antioksidanti, muškarci, žene, starenje

### Introduction

The reactive oxygen species (ROS), both exogenous and endogenous, affect all organisms including human beings. The mitochondrial electron transport chain is the main source of endogenous ROS, especially superoxide radicals (1, 2). The rate of ROS production in cells is physiologically balanced with the activities of antioxidant systems. When that equilibrium becomes affected and an overproduction

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of ROS takes place, the symptoms of oxidative stress are observed (3).

Antioxidative defense of the organism includes enzymatic and non-enzymatic systems. The enzymatic system consists of antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC1.11.1.6), glutathione peroxidase (GPX; EC1.11.1.9) and glutathione reductase (GR; EC1.6.4.2). The low-molecular, non-enzymatic system contains reduced glutathione, uric acid, and exogenous antioxidants, mainly  $\beta$ -carotene, retinol, vitamin C and  $\alpha$ -tocopherol (4). Reactive oxygen species can affect macromolecules of amino acids, proteins, lipids, carbohydrates and DNA, progressively leading to physiological cell dysfunction (3). Erythrocytes are particularly susceptible to the damaging action of ROS, due to the autooxidation of haemoglobin and high content of polyunsaturated fatty acids in cell membranes (5).

It is accepted that reactive oxygen species are responsible for age-related damages at the cellular and tissue levels (6). Free radical theory of ageing comprises several hypotheses which take into account the role of cellular organelles in that process and the type of cell damage (7, 8). One of such hypotheses involves the impact of reactive oxygen species on the mutations of mitochondrial DNA which brings about changes in the activities of enzymes of the electron transport chain (9). It is also generally accepted that the efficiency of the electron transport chain decreases with age. This is related to an increased generation of superoxide radicals which initiates the chain of free radical reactions and becomes the source of highly reactive hydroxyl radicals. Another hypothesis points to the accumulation of oxidized forms of proteins in cells (10) since the capacity to degrade them declines with age (11). The main ageing factor seems to be an increased oxidation of cell membrane lipids which brings about an increased permeability and cell damage and, eventually, cell death (12). It was demonstrated that ROS affect the rate of ageing by reacting with the cell genome (13) and Beckman and Ames (7) found that the frequency of DNA damage is

increased in elderly people. It was noted that genetic mutations of *Drosophila melanogaster*, shown to greatly increase the lifespan over the wild type strain, result in increased SOD and CAT activities (14). The effects of ROS on ageing of organisms were confirmed in studies on mice with a mutated gene responsible for SOD synthesis; these mice lived shorter than those without this mutation (13). Other studies showed a high association between high superoxide dismutase activity and the onset of the ageing process (15).

The existing data on age and sex-related changes in antioxidant enzyme activities and non-enzymatic antioxidant concentrations in blood are contradictory. The present study is an attempt to contribute to the understanding of this issue by comparing blood antioxidant capacity in women and men in relation to their age.

## Material and Methods

Four groups of subjects were studied: young men and women aged 25–32 years (YM and YW respectively,  $n = 19$  each) and old men and women aged 63–71 years (OM and OW respectively,  $n = 11$  each), all of them sedentary. All subjects gave their written consents to participate in the study, which was approved by the local Ethics Committee according to the Declaration of Helsinki. Body mass and height were measured and the body mass index (BMI) was calculated. Body fat content was determined from 4 skinfolds according to Durnin and Womersley (16). Physical characteristics of all groups are shown in Table 1.

Blood samples were collected in the preprandial state from the antecubital vein into heparinized tubes. Part of the collected blood was centrifuged at 3000 g for 15 min at 4 °C, and the erythrocytes were washed 3 times with cold saline. Whole blood, erythrocytes and plasma were frozen at –70 °C until assayed.

The activities of superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) were

**Table 1** Physical characteristics of studied groups of women and men (mean  $\pm$ SD).

Group* Variable	YW (n=19)	YM (n=19)	OW (n=11)	OM (n=11)
Age (years)	28.8 $\pm$ 2.3	28.8 $\pm$ 2.4	67.1 $\pm$ 4,5	66.7 $\pm$ 4.9
Body height (cm)	160.5 $\pm$ 9.1 <sup>A</sup>	179.4 $\pm$ 7.5	160.0 $\pm$ 6.8 <sup>A</sup>	172.0 $\pm$ 5.8
Body mass (kg)	60.5 $\pm$ 9.1 <sup>A</sup>	83.7 $\pm$ 14.4	72.6 $\pm$ 9.5	78.0 $\pm$ 9.1
BMI	22.3 $\pm$ 3.6 <sup>A,C</sup>	25.9 $\pm$ 3.0	28.1 $\pm$ 6.1	26.4 $\pm$ 5.4
Fat content (%)	27.6 $\pm$ 4.1 <sup>A,C</sup>	19.6 $\pm$ 5.6 <sup>A</sup>	43.8 $\pm$ 4.2 <sup>B</sup>	26.9 $\pm$ 5.2

Legend:

\* YW – Young women; YM – Young men; OW – Old women; OM – Old men

<sup>A</sup> Significantly ( $p < 0.01$ ) different compared to the respective male group

<sup>B</sup> Significantly higher ( $p < 0.001$ ) compared to older men

<sup>C</sup> Significantly lower ( $p < 0.01$ ) compared to older women

determined in erythrocyte hemolysates using commercial kits (SOD and GR: Randox, Great Britain; CAT: Oxis, USA). Glutathione peroxidase (GPX) activity was determined in whole blood hemolysates using commercial kits (Ransel, Randox, Great Britain). Haemoglobin concentrations in whole blood and in erythrocyte hemolysates were determined using Drabkin's method. Enzyme activities were expressed in U/mg Hb or U/g Hb. Commercial kits were used to determine plasma concentrations of total protein and of uric acid (Alpha Diagnostics, Poland), and of total antioxidant status (TAS; Randox, Great Britain). Retinol and  $\alpha$ -tocopherol were determined using the HPLC (Varion, USA) method. Samples, blanks and reference substances (ChromaDex, USA) were applied onto C18 DB 150x4.6 mm column with 5  $\mu$ m particle size (Restek, USA). The mobile phase was acetonitrile-methanol (70:30, v/v) and the flow rate was 1.4 mL/min. Fluorescence was read at 280 nm. The retention times for retinol and  $\alpha$ -tocopherol were 2.3 and 7.8 min, respectively.

The sum of standardized values of antioxidant enzyme activities was computed to compare the age-related changes in the total capacity of the erythrocyte antioxidant defense (17). The distributions were assessed graphically by correlating the group SD's with the respective means and by correlating combined sorted values with the »Normal SD's« (NORMSINV function in MS Excel™). Except GPX the values of SOD, CAT and GR required logarithmic transformation. Since the mean values for YM and YW groups were fairly alike, the means of individual variables computed for both groups combined served as a reference, reference standard deviations being computed from combined variances for both groups. Data from all groups were standardized using reference means and standard deviations. Since the contributions of individual enzymes to the sum of four standardized variables (18) were alike, no weighting factors were applied to the mean standardized values of enzymatic variables. The data were then subjected to two-way ANOVA (gender x age) with the *post-hoc* t-test, the level of significance being set at  $p < 0.05$ .

## Results

Body height and mass as well as BMI were significantly lower in young women compared to young men but percent of body fat was significantly higher in women, reflecting typical sex differences (Table I). Old women had lower body height and significantly higher percent of body fat than old men. Young women had lower BMI and body fat content than older ones while the BMI in young and old men was similar but percent of fat was higher in the latter group.

The activity of erythrocyte superoxide dismutase (SOD) was significantly lower in old women compared to old men (Table II). There was no difference in SOD activity between the young groups. Catalase activity (CAT) was significantly lower in young men compared to the older ones, but between young and old women only a tendency to lower values was seen in Group YW. Glutathione peroxidase (GPX) activity was significantly lower and that of glutathione reductase (GR) significantly higher in both young groups than in the respective older ones. Additionally, glutathione reductase (GR) activity was significantly higher in young women compared to young men. Plasma total antioxidant status (TAS) and concentrations of selected non-enzymatic antioxidants are presented in Table III. A significantly higher TAS level was noted in the young men group compared to both young women and old men. There were no significant gender- or age-related differences in plasma retinol and  $\alpha$ -tocopherol concentrations. Plasma concentrations of uric acid were significantly lower in young women compared to both young men and old women. The total protein level in plasma was significantly lower in young women and men compared to respective groups of older subjects but no significant differences between men and women were noted within the given age category.

Mean standardized values of enzymatic variables are shown in Figure 1. ANOVA revealed no significant differences in SOD activities; in case of CAT and

**Table II** Erythrocyte antioxidant enzyme activities in studied groups of women and men (mean  $\pm$ SD).

Group* Variable*	YW (n=19)	YM (n=19)	OW (n=11)	OM (n=11)
SOD (U/g Hb)	1338.5 $\pm$ 301.7 <sup>A</sup>	1374.3 $\pm$ 509.8	1143.5 $\pm$ 148.6 <sup>B</sup>	1331.6 $\pm$ 242.8
CAT (U/mg Hb)	222.2 $\pm$ 38.9	213.1 $\pm$ 49.4 <sup>B</sup>	267.8 $\pm$ 79.3	266.5 $\pm$ 59.2
GPX (U/g Hb)	49.3 $\pm$ 16.5 <sup>C</sup>	49.7 $\pm$ 16.7 <sup>B</sup>	71.0 $\pm$ 12.9	69.2 $\pm$ 14.4
GR (U/g Hb)	11.8 $\pm$ 2.3 <sup>A,D</sup>	10.2 $\pm$ 1.6 <sup>E</sup>	7.9 $\pm$ 1.2	6.5 $\pm$ 1.2

\* SOD – superoxide dismutase, CAT – catalase, GPx – glutathione peroxidase, GR – glutathione reductase

\* See Table I

<sup>A</sup> Significantly higher ( $p < 0.05$ ) compared to older women

<sup>B</sup> Significantly lower ( $p < 0.001$ ) compared to older men

<sup>C</sup> Significantly lower ( $p < 0.05$ ) compared to older women

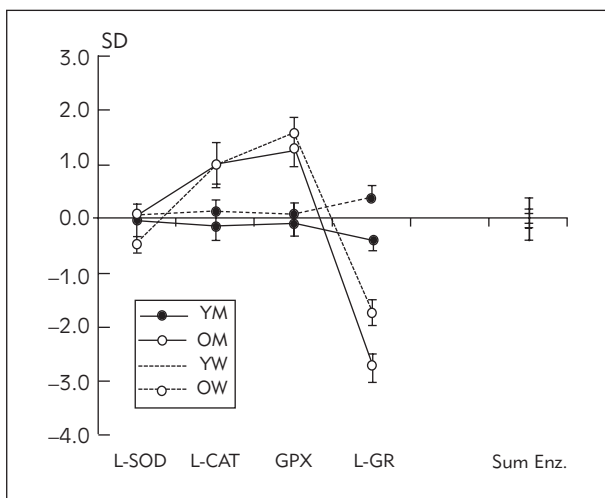
<sup>D</sup> Significantly higher ( $p < 0.001$ ) compared to younger men

<sup>E</sup> Significantly higher ( $p < 0.001$ ) compared to older men

**Table III** Plasma antioxidant capacity in studied groups of women and men (mean  $\pm$ SD).

Group* Variable <sup>x</sup>	YW (n=19)	YM (n=19)	OW (n=11)	OM (n=11)
TAS (mmol/L) <sup>x</sup>	1.31 $\pm$ 0.2 <sup>A</sup>	1.50 $\pm$ 0.2 <sup>B</sup>	1.22 $\pm$ 0.3	1.26 $\pm$ 0.2
Retinol ( $\mu$ g/mL)	0.79 $\pm$ 0.2	0.90 $\pm$ 0.3	0.90 $\pm$ 0.2	0.92 $\pm$ 0.1
$\alpha$ -Tocopherol ( $\mu$ g/mL)	7.43 $\pm$ 1.6	7.32 $\pm$ 1.8	8.44 $\pm$ 2.3	7.23 $\pm$ 2.7
Uric acid (mmol/L)	0.23 $\pm$ 0.05 <sup>A,C</sup>	0.38 $\pm$ 0.1	0.31 $\pm$ 0.1	0.34 $\pm$ 0.1
Total protein (g/L)	70.9 $\pm$ 5.6 <sup>C</sup>	75.4 $\pm$ 8.5 <sup>D</sup>	85.1 $\pm$ 5.5	87.5 $\pm$ 8.6

\* See Table I

<sup>x</sup> TAS – plasma total antioxidant status<sup>A</sup> Significantly lower ( $p < 0.01$ ) compared to younger men<sup>B</sup> Significantly higher ( $p < 0.01$ ) compared to older men<sup>C</sup> Significantly lower ( $p < 0.001$ ) compared to older women<sup>D</sup> Significantly lower ( $p < 0.001$ ) compared to older men**Figure 1** Mean ( $\pm$ SE) standardized values of enzymatic variables obtained for 4 groups of subjects.

Legend: YM – Young men (n = 19); OM – Old men (n = 11); YW – Young women (n = 19); OW – Old women (n = 11); L – Logarithmic transformation; Sum Enz. – Mean value of all enzymatic variables

GPX, old subjects attained significantly ( $p < 0.001$ ) higher values than the young ones; in case of GR women had higher values than men and old subjects had higher values than the young ones ( $p < 0.001$  each); in case of the combined enzymatic variables (Sum Enz.) all means were practically identical.

## Discussion

The ageing process is accompanied by progressive impairment of a variety of physiological systems. One of the theories of ageing, although controversial, claims that reactive oxygen species are responsible for cellular oxidative damage and increasing homeostatic imbalance. The data concerning the antioxidant capacity of blood including erythrocyte activities of antioxidant enzymes, as well as concentrations of non-enzymatic plasma antioxidants in relation to age

are inconsistent. In a study on men and women aged 20–70 years, Kasapoglu and Ozben (19) demonstrated that erythrocyte SOD and CAT activities were higher and GPX activity lower in old subjects than in the younger ones. On the other hand, Jozwiak and Jasnowska (20) noted lower SOD activity in old blood donors than in the younger ones, and Ho et al. (21) demonstrated that old healthy Chinese adults had higher erythrocyte SOD activity than younger subjects. Contrary results were reported by Jozwiak and Jasnowska (20) who indicated that CAT and GPX were increased in old blood donors compared to younger ones. Age-related increase in glutathione reductase (GR) activity and decreased GPX activity in healthy individuals were demonstrated by Erden-Inal et al. (22), but Ho et al. (21) noted in healthy Chinese adults an age-related increase in CAT activity while GPX activity was similar in all studied subjects independent of age. However, Mendoza-Nunez et al. (23) found no age-associated changes in SOD and GPX activities in healthy subjects. This result was confirmed by Bogdanska et al. (24) who stressed no significant effect of age on the erythrocyte antioxidant enzyme activities in men. In this study no significant age-related changes in SOD activity in men were noted; however, SOD activity was lower in older women compared with the younger ones. On the other hand, CAT activity was higher in old women and men than in younger subjects. Activity of GPX was higher and GR activity lower in old women and men than in the younger ones. These data show that there are no one-way changes of antioxidant enzymes activity associated with ageing. A lower SOD activity observed in older women compared with the younger ones is difficult to explain in the light of the report of Lutosławska et al. (25) who found that decreased estradiol concentrations were associated with increased SOD activity in erythrocytes. On the other hand, Massafra et al. (26) found no differences in SOD activities in both phases of the menstrual cycle. The decreased GR activity noted in older subjects may be indicative of a diminished capacity to produce reduced glutathione (GSH), an important endogenous



antioxidant. Gil et al. (27) suggested that GSH concentrations in blood were lower in older women than in the younger ones or in men. Also, Rizvi et al. (28) showed an age-dependent decrease in intracellular reduced glutathione and in membrane sulphhydryl groups. On the other hand, higher GPX activity in the elderly subjects compared with the younger ones may represent an adaptive response to intensified oxidative stress like in the case of CAT. The total antioxidant enzyme capacity of erythrocytes was in the young and old subjects alike, indicating the existence of compensatory changes in antioxidant enzyme activities (29, 30).

It follows from our results that the total antioxidant status was higher in the young than in the old men, but no such difference was found for women. Mendoza-Nunez et al. (23) demonstrated age-related TAS levels in healthy women and men, the values in old individuals being lower than in the young ones. This is contradicted by Kostka et al. (31) who noted that in young (21.1 years) and old (71.0 years) men TAS levels were alike. The total antioxidant status is reflected by plasma concentrations of all non-enzymatic antioxidants which depend on diet and, probably, on metabolic disturbances in old subjects. Habdous et al. (32) emphasized that plasma albumin and uric acid levels were the main TAS determinants. It is well known that a high uric acid level increases the plasma antioxidant capacity (33). In this study, the relationship between TAS and uric acid concentra-

tions was seen only in young men. However, the possibility that the high level of total protein in old subjects, found in this study, reflects a compensating mechanism to maintain appropriate plasma antioxidant capacity, cannot be ruled out. The concentrations of retinol and  $\alpha$ -tocopherol depend mainly on their contents in the diet (34). The lack of age-related differences in the concentrations of those compounds suggested their intakes being in all groups alike.

In conclusion, the total erythrocyte enzymatic antioxidant capacity was not related to age, although young and old subjects differed in CAT, GPX and GR activities. Age- and gender-related differences between subjects in the non-enzymatic antioxidant levels can be interpreted in various ways. The data obtained in this study clearly show that many factors may contribute to the ageing process, and further research is needed to confirm the oxygen stress hypothesis of ageing.

*Acknowledgements.* The study was supported by grant DS-119 from University of Physical Education, Warsaw, Poland. The authors wish to thank Elzbieta Baczkowska-Posnik for her excellent technical assistance in the data collection.

### Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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*Received: November 9, 2010*

*Accepted: December 14, 2010*