

Katedra Nauk Fizjologiczno-Medycznych, Zakład Biochemii<sup>1</sup>,  
Akademia Wychowania Fizycznego  
Department of Physiological and Medical Sciences, Biochemistry Unit,  
Academy of Physical Education, Katowice,  
Szpital Geriatryczny, Katowice<sup>2</sup>  
Geriatric Hospital, Katowice

BARBARA KŁAPCIŃSKA<sup>1</sup>, KATARZYNA KEMPA<sup>1</sup>,  
JAROSŁAW DEREJCZYK<sup>2</sup>, MAŁGORZATA MICHALCZYK<sup>1</sup>,

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***Blood antioxidant defense during aging***

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**Obrona antyoksydacyjna krwi w procesie starzenia**

According to the free radical theory of aging (Harman 1991), different types of reactive oxygen species (ROS) generated during normal aerobic metabolism are proposed to be among the most important contributors to the increased risk of age-related diseases. Blood antioxidant enzymes and vitamins are important components of the complex system of defense against oxidative damage in the organism. This study was aimed at the evaluation of the capacity of the antioxidant defense in centenarians and the elderly subjects.

**SUBJECTS AND METHODS**

A total of 165 centenarians (27 male and 138 female) and 82 elderly subjects (28 male and 54 female), whose characteristics is presented in Table 1, were enrolled for the study. Venous blood samples were drawn into heparinized test tubes. After their thorough mixing by vortexing the aliquots of 200  $\mu$ l were transferred into heparinized Eppendorf tubes for subsequent fluorometric assay for Se content according to Danch and Dróżdź (1996). The remaining blood was centrifuged (1000xg, 15 min) to collect plasma and erythrocytes, that were washed three-times with cold (4°C) saline and kept frozen at -70°C until being analyzed for activities of antioxidant enzymes, i.e. SOD using a commercially available RANSOD kit (Randox Laboratories, UK), CAT by the method of Aebi (1984), GPX with the use of RANSEL (Randox) diagnostic kit and GR according to Glatzle et al. (1970). Plasma samples were assayed for uric acid with the use of a reagent kit (ANALCO),  $\alpha$ -tocopherol by using a reverse-phase-HPLC method according to Sobczak et al. (1999) and MDA by the thiobarbituric acid (TBA) test (Buege and Aust 1978) with extraction of the chromogene to n-butanol. Cognitive status of subjects was assessed based on the results of the Mini Mental State Examination (Folstein et al. 1975) performed by a geriatrician and a qualified consultant reviewer. The study was performed in compliance with the guidelines of the Helsinki Declaration as revised in 1996 regarding the use of human subjects.

**Statistics**

Mean and standard deviation (SD) were calculated for all the variables. Between-group differences, with respect to gender and Mini Mental State Examination score as independent variables, were identified with the non-parametric Mann-Whitney U-test. Differences with P values <0.05 were considered significant. In addition, Spearman rank order correlation coefficients were computed to reveal

associations between variables. All statistical analyses were performed using STATISTICA 5.0 (StatSoft, Inc. 1995) software.

## RESULTS AND DISCUSSION

The cognitive status of all subjects included in the study was assessed with the MMSE test validated for educational level and physical disabilities. Each subject's cognition was defined as normal or impaired according to the cut-off scores, and further classified as normal (score  $\geq 24$ ) or impaired (mild impairment: score 18-23 and severe: score 0-17). Among centenarians there was a nearly five-fold prevalence of women over men. Twenty eight of 165 centenarians (11 male and 17 female) were able to complete all MMSE items which gave them the highest score ( $\geq 24$ ) indicating normal cognition, whereas 25 persons (4 men and 21 women) were scored 0. As compared to the control group, the mean MMSE score in centenarians was significantly lower (Table 1).

**Table 1. Characteristics of subjects (M-men, F-women)**

Subjects		N	Age. yr	MMSE score
Centenarians	M/F	165	101.1 $\pm$ 1.3	15.6 $\pm$ 9.1*
	M	27	100.8 $\pm$ 0.9	19.3 $\pm$ 9.7*
	F	138	101.2 $\pm$ 1.4	14.8 $\pm$ 8.8* <sup>&amp;</sup>
Control group	M/F	82	66.1 $\pm$ 1.4	27.7 $\pm$ 1.7
	M	28	66.1 $\pm$ 0.6	27.2 $\pm$ 1.6
	F	54	66.1 $\pm$ 1.7	28.0 $\pm$ 1.8 <sup>&amp;</sup>

Note: \* significantly ( $p < 0.05$ ) different from the appropriate values in the control group; <sup>&</sup>-significantly ( $p < 0.05$ ) different from the appropriate values in men

Interestingly, from all centenarians examined for cognitive state by the MMSE 17 women (~12 % of female) and 11 men (44 % of male subjects) were classified as having normal cognition (MMSE score  $\geq 24$ ) whereas 21 women (~15 % of female) and 4 men (~15 % of male subjects) were scored 0, which was indicative of the most severe cognitive impairment. The high prevalence of men with the highest MMSE score among centenarian subjects may suggest that men are less vulnerable to a decline in cognitive function during the extreme edge of their life span. The contrary trend was observed among the elderly controls as women with the highest MMSE score were in prevalence.

The free radical theory of aging (Harman 1991) postulates that free radical reactions are the major cause of aging, therefore a reduction in their levels by the body antioxidant defense system should, in principle, retard aging and increase the maximum life span (Ashok and Ali, 1999). One may hypothesize that in centenarians, who represent a highly selected group of successfully aged people, the antioxidant status would be better than that of the normally aged elderly humans. In this study we evaluated the capacity of the blood antioxidant defense system in centenarians and compared it to that of the elderly group of subjects aged 66.1 $\pm$ 1.4 yr. The activities of RBC antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR), whole blood selenium content as well as plasma levels of  $\alpha$ -tocopherol, uric acid and malondialdehyde (MDA), as recorded in both groups, are listed in Table 3.

The major finding of this study is that centenarians were characterized by significantly lower GPX activity and selenium content in whole blood, which is conform to the observation of other authors (in Ashok et al. 1999, Kłapcińska et al. in press). As expected, the activity of GPX appeared to be Se-dependent (R Spearman = 0.0196,  $p < 0.05$ ). Interestingly, a positive association was found between selenium level in blood and MMSE score R Spearman = 0.335,  $p < 0.0001$ ), which may suggest that the efficiency of antioxidant defense system may, at least to some extent, play a protective role in prevention of age-related neurodegeneration. It is noteworthy that selenium supplementation has been reported to increase longevity (Simonoff et al. 1992).

The activity of SOD was comparable in both groups, although it tended to be lower in male centenarians, especially in those with the highest MMSE score. On the other hand, the highest SOD activity was recorded in centenarians with the lowest cognitive function (MMSE = 0). It seems that high SOD activity might be induced by a parallel increase in free radicals and might be indicative of strongly enhanced oxidative stress in those individuals. The contrary trend was observed in CAT activity that

adopted the lowest values in male centenarians with the most severe cognitive impairment. Although such a trend was not observed in female centenarians, it may be conceivable that under condition of an enhanced oxidative stress, GPX and CAT acting in concert to scavenge hydrogen peroxide generated by SOD, are not sufficiently effective players in the antioxidant defense. The main inter-group difference in the enzymatic antioxidant defence was that GPX was lower whereas CAT activities higher in centenarians, which supports the hypothesis of a synergistic action of these two enzymes in scavenging hydrogen peroxide. However, only in female centenarians the activity of CAT appeared to be significantly higher than in the appropriate group of control subjects.

As to non enzymatic antioxidants, no difference in plasma urate were found between the groups, although in both groups women had lower uric acid levels. Significant inter-group differences in plasma  $\alpha$ -tocopherol concentration was observed with the elderly controls having higher levels. We cannot exclude that some individuals from the control group received the vitamin supplements. It should be stressed, however, that plasma vitamin E concentration in centenarians was high ( $> 17$  mg/l) as compared to young adults ( $\sim 12$  mg/l) (Kłapcińska et al. 2002). The lowest plasma  $\alpha$ -tocopherol level was recorded in centenarians (both men and women) with the lowest MMSE score (MMSE=0), which may imply that this antioxidant vitamin is of particular importance for prevention of neurological dysfunction.

The level of MDA, considered as a marker of oxidative stress, did not differ much between groups, the highest level was recorded in male centenarians with the lowest MMSE score.

In conclusion, centenarians examined within the frame of our study presented a peculiar antioxidant profile, in which important role in the defence against the oxidative stress may be attributed to  $\alpha$ -tocopherol. The efficiency of antioxidant defence depended also on the activity of antioxidant enzymes. The lower activity of GPX, resulting from an age-related decline in selenium status, was compensated by higher activities of CAT, whereas activity of SOD was elevated but comparable with that recorded in the elderly controls.

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## SUMMARY

This study was aimed at the evaluation of the capacity of the blood antioxidant defense in centenarians and the elderly subjects. We have measured the activities of red blood cell superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and glutathione reductase (GR) as well as whole blood selenium and plasma levels of uric acid,  $\alpha$ -tocopherol and malondialdehyde as a marker of oxidative stress in 160 centenarians and 82 healthy elderly subjects aged 66.1 $\pm$ 1.5 yr. The centenarians were characterized as having lower activity of GPX and lower whole blood selenium and plasma  $\alpha$ -tocopherol, whereas activities of the remaining antioxidant enzymes as well as uric acid and MDA levels were comparable. The lowest level of  $\alpha$ -tocopherol was recorded in centenarians with the most severe cognitive impairment.

## STRESZCZENIE

Celem niniejszej pracy była ocena skuteczności obrony antyoksydacyjnej krwi u stulatków oraz osób w podeszłym wieku. Oznaczano aktywność dysmutazy nadadtlenkowej (SOD), peroksydazy glutationowej (GPX), katalazy (CAT) i reduktazy glutationowej (GR) w hemolizatach erytrocytów, jak również stężenia selenu w pełnej krwi oraz stężeń  $\alpha$ -tokoferolu, kwasu moczowego i dialdehydu malonowego (MDA), jako markera stresu oksydacyjnego, u 160 stulatków oraz 82 zdrowych osób starszych w wieku 66.1 $\pm$ 1.5 lat. Osoby stuletnie charakteryzowały się obniżoną aktywnością GPX oraz obniżonym stężeniem selenu i  $\alpha$ -tokoferolu, natomiast aktywności pozostałych enzymów antyoksydacyjnych, podobnie jak poziom kwasu moczowego i MDA były zbliżone do wartości zarejestrowanych u osób z grupy kontrolnej. Najniższy poziom  $\alpha$ -tokoferolu zanotowano u stulatków z ciężkimi zaburzeniami zdolności kognitywnych.

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**Table 2. Activities of the blood antioxidant enzymes (SOD, GPX, CAT, GR), whole blood selenium, plasma concentrations of uric acid,  $\alpha$ -tocopherol and malondialdehyde (MDA) in centenarian and control subjects**

Variable	Centenarians						Control			
	N	Total	N	MMSE $\geq$ 24	N	MMSE=0	N	Total	N	MMSE $\geq$ 24
SOD U/g Hb	M/F 157	1046.8 $\pm$ 329.9	M/F 26	998.7 $\pm$ 216.8	M/F 23	1125.4 $\pm$ 409.9	M/F 82	1059.4 $\pm$ 246.5	M/F 69	1072.1 $\pm$ 254.1
	M 26	950.5 $\pm$ 329.9*	M 11	873.9 $\pm$ 159.2*	M 4	1127.4 $\pm$ 687.9	M 28	1109.9 $\pm$ 260.5	M 24	1111.6 $\pm$ 261.7
	F 131	1065.9 $\pm$ 327.9 <sup>&amp;</sup>	F 15	1090.3 $\pm$ 211.1	F 10	1125.0 $\pm$ 355.6	F 54	1033.1 $\pm$ 237.1	F 45	1050.9 $\pm$ 250.3
GPX U/g Hb	M/F 157	33.0 $\pm$ 11.2*	M/F 26	29.4 $\pm$ 11.6*	M/F 23	32.2 $\pm$ 10.5	M/F 82	40.3 $\pm$ 15.2	M/F 69	39.2 $\pm$ 14.6
	M 26	31.5 $\pm$ 13.2*	M 11	31.8 $\pm$ 13.8*	M 23	32.2 $\pm$ 10.5	M 28	41.5 $\pm$ 16.8	M 24	42.3 $\pm$ 17.3
	F 131	33.3 $\pm$ 10.8*	F 15	27.7 $\pm$ 9.9*	F 4	37.6 $\pm$ 17.3	F 54	39.7 $\pm$ 14.5	F 45	37.5 $\pm$ 12.9
CAT k/g Hb	M/F 157	155.8 $\pm$ 10.8*	M/F 26	154.2 $\pm$ 50.4	M/F 23	161.2 $\pm$ 61.6	M/F 82	137.1 $\pm$ 49.5	M/F 69	137.8 $\pm$ 49.9
	M 26	139.9 $\pm$ 47.0	M 11	145.1 $\pm$ 52.1	M 4	121.4 $\pm$ 60.7	M 28	132.8 $\pm$ 38.3	M 24	137.2 $\pm$ 39.2
	F 131	158.9 $\pm$ 57.2*	F 15	160.9 $\pm$ 49.8	F 19	169.6 $\pm$ 59.9	F 54	139.4 $\pm$ 54.7	F 45	138.2 $\pm$ 55.3
GR U/g Hb	M/F 156	22.3 $\pm$ 6.2	M/F 26	21.2 $\pm$ 6.7	M/F 23	21.9 $\pm$ 6.5	M/F 82	22.4 $\pm$ 6.9	M/F 69	22.5 $\pm$ 6.9
	M 25	22.8 $\pm$ 7.3	M 11	22.2 $\pm$ 5.9	M 4	24.5 $\pm$ 8.6	M 28	21.7 $\pm$ 6.4	M 24	21.6 $\pm$ 6.4
	F 131	22.2 $\pm$ 5.9	F 15	20.4 $\pm$ 7.4	F 19	21.4 $\pm$ 6.2	F 54	22.7 $\pm$ 7.3	F 45	22.6 $\pm$ 7.3
Selenium ng/ml	M/F 117	46.2 $\pm$ 19.5*	M/F 19	47.6 $\pm$ 21.4*	M/F 20	43.0 $\pm$ 21.9	M/F 78	63.9 $\pm$ 20.3	M/F 65	62.8 $\pm$ 19.8
	M 18	53.8 $\pm$ 22.9	M 7	52.4 $\pm$ 26.7	M 2	44.8 $\pm$ 23.5	M 24	64.1 $\pm$ 15.9	M 20	62.6 $\pm$ 15.3
	F 99	44.9 $\pm$ 18.6*	F 12	44.7 $\pm$ 18.4*	F 18	42.8 $\pm$ 22.4	F 54	63.9 $\pm$ 22.1	F 45	62.9 $\pm$ 21.6
Uric acid mg/dl	M/F 165	5.08 $\pm$ 1.59	M/F 28	5.63 $\pm$ 1.70*	M/F 23	5.32 $\pm$ 1.97	M/F 77	4.85 $\pm$ 1.39	M/F 69	4.85 $\pm$ 1.39
	M 27	5.62 $\pm$ 1.49	M 11	5.17 $\pm$ 1.52	M 4	5.62 $\pm$ 1.88	M 27	5.54 $\pm$ 1.67	M 24	5.60 $\pm$ 1.76
	F 138	4.97 $\pm$ 1.59 <sup>&amp;</sup>	F 17	5.94 $\pm$ 1.78	F 19	5.25 $\pm$ 2.03	F 50	4.47 $\pm$ 1.05 <sup>&amp;</sup>	F 45	4.55 $\pm$ 1.00
$\alpha$ - tocopherol mg/l	M/F 155	17.7 $\pm$ 5.7*	M/F 25	17.7 $\pm$ 5.7*	M/F 25	15.4 $\pm$ 4.3	M/F 81	22.0 $\pm$ 6.3	M/F 68	22.0 $\pm$ 6.7
	M 25	16.6 $\pm$ 5.2*	M 10	16.1 $\pm$ 5.9*	M 4	15.9 $\pm$ 1.3	M 28	20.7 $\pm$ 5.2	M 24	20.6 $\pm$ 5.6
	F 130	17.9 $\pm$ 5.8*	F 15	18.8 $\pm$ 5.5*	F 21	15.3 $\pm$ 4.7	F 53	22.7 $\pm$ 6.8	F 44	22.8 $\pm$ 7.2
MDA nmol/l	M/F 160	4.2 $\pm$ 5.7	M/F 27	3.9 $\pm$ 1.9	M/F 25	3.7 $\pm$ 2.2	M/F 81	4.4 $\pm$ 1.05	M/F 68	4.3 $\pm$ 2.9
	M 26	4.9 $\pm$ 2.2	M 11	4.1 $\pm$ 1.5	M 4	5.3 $\pm$ 4.1	M 28	4.3 $\pm$ 2.8	M 24	4.2 $\pm$ 3.0
	F 134	4.1 $\pm$ 1.9	F 16	3.8 $\pm$ 2.1	F 21	3.4 $\pm$ 1.6	F 53	4.4 $\pm$ 2.8	F 44	4.3 $\pm$ 2.9

Note: \* significantly ( $p < 0.05$ ) different from the appropriate values in the control group;

&-significantly ( $p < 0.05$ ) different from the appropriate values in men<sup>77</sup>