Activity of erythrocyte antioxidant enzymes in healthy women depends on age, BMI, physical activity and diet

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Abstract

Introduction

Antioxidant enzymes protect the human body against the harmful effects of oxidative stress. The activity of antioxidant enzymes changes with age, and depends on dietary nutrients such as fats and vitamins, which can have a significant impact on minimizing or exacerbating oxidative stress.

Aim

Examine the effect of age, BMI, diet, physical activity and smoking status on the activity of erythrocyte antioxidant enzymes catalase, glutathione reductase, glutathione peroxidase glutathione S-transferase, superoxide dismutase and glutathione concentrations in healthy women.

Material and methods

This study included 98 healthy women aged between 20 and 65 years. All women underwent anthropometric tests: body weight, height, hip and waist circumference. Antioxidant activity in erythrocytes was measured by spectrophotometric methods.

Results

Catalase activity increased significantly with age ($p<0.001$), while superoxide dismutase activities and glutathione decreased with age ($p =0.008$, $p =0.023$, respectively). Women with a lower BMI (emaciation) had higher superoxide dismutase activity than those in the first degree of obesity ($p = 0.009$

Conclusions

1. Increased catalase activity with age may be a sign of a large amount of hydrogen peroxide, resulting from poorly functioning antioxidant systems in older age. 2. Decreased superoxide dismutase activity with age may indicate inactivation of this enzyme by excessive hydrogen peroxide, as well as glycation of superoxide dismutase molecules or reactions with lipid peroxidation products, the intensity of which increases with age. 3. The negative correlation between superoxide dismutase activity and BMI index indicates reduced enzymatic activity in obese subjects, despite increased ROS production by adipose tissue.

Introduction

The oxygen that we breathe is reduced in the body, resulting in a water molecule. The products of incomplete reduction of the oxygen molecule are called reactive oxygen species (ROS). These molecules or free radicals quickly form chain reactions, reacting with proteins, sugars, lipids, and nucleic acids in cells, leading to the formation of free radical products [1].

Physiologically, ROS are formed in the respiratory chain, during purine nucleotide metabolism, and in the microsomal hydroxylation cycle, in a reaction taking place with oxidoreductases. Their task is to induce cell differentiation and apoptosis, influence the synthesis, release, or inactivation of the endothelial vasodilator endothelial-derived relaxing factor (EDRF), extend or contract the wall of blood vessels, and stimulate glucose transport into cells, or serotonin into platelets. ROS also take part in immunological processes [1]. The most important enzymes involved in the neutralization of ROS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH). Among the non-enzymatic antioxidants, we studied glutathione (GSH), which directly and indirectly binds the majority of enzymatic antioxidants [2, 3].

The amount of ROS synthesis correlates with the activity of intracellular antioxidant systems [38]. The resulting generation of ROS can lead to undesirable effects on the function of the body, including metabolic disorders and changes in nucleic acids. Oxidative stress occurs when there is a lack of balance between the production and removal of ROS, and the antioxidant system aims to rebalance ROS levels.

The activity of antioxidant enzymes may change with age. In recent years, processes connected with the aging of the body with the action of reactive oxygen species have been increasingly investigated. In order to prevent the accumulation of ROS, the body has developed antioxidant mechanisms. Changes in their activity and concentration depend on the race, sex, organ and location of the subcellular enzyme. The reduction in their activity observed with age is caused by the direct or indirect modification of enzyme molecules by ROS. In turn, increasing their activity should be treated as a compensation response to overproduction of free oxygen radicals. With age, GSH synthesis decreases as a result of the reduced availability of cysteine and methionine and reduced activity of γ-glutamyl cysteine synthetase and cystathionase on the one hand, as well as increased GSH consumption in reactions with free radicals, which are generated in excessive amounts [4].

In the literature you can also find reports on the nutrients contained in the diet (fats, vitamins) that can have a significant impact on the minimization or intensity of oxidative stress. For years, there has also been a discussion on whether physical activity or smoking affects the activity of antioxidant systems in the body and, if so, how these factors affect antioxidant activities. Current research also indicates that the antioxidant systems undergo significant changes in response to acute and chronic exercise. This is also related to age, likely because physical activity usually decreases in older people. Acute exercise may increase the activity of some antioxidative enzymes in various tissues, but the mechanism of this activation is unclear. Exercise training has little effect on liver enzymes or cardiac muscle systems, but it can cause adaptive reactions in antioxidant enzymes within skeletal muscle, particularly in GPx. These findings suggest that aging, physical exercise, and diet may impose oxidative stress on the body [2]. The purpose of this work is to answer the above questions.

Materials And Methods

1. Ethical approval and consent

The Bioethical Commission at the Pomeranian Medical University in Szczecin approved the research carried out (no KB-0012/36/11). All participants, were informed about the purpose and scope of the study and gave their consent to donate samples and for the resulting data to be published.

2. Study group

The study covered 98 healthy women between the ages of 20 and 65. Their health status was confirmed on the basis of previous morphological and biochemical tests (total cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), total protein, albumin, glucose, uric acid) in the Department of Laboratory Diagnostics at the Independent Public Clinical Hospital No. 2 in Szczecin. No results were found that departed from the standards adopted at the Central Laboratory and in methodologies from producers (Biomaxima, Lublin, Poland). All women underwent anthropometric tests: body weight, height, hip and waist circumference. A survey was also carried out to assess the diet and physical activity of the women surveyed, and whether they smoked cigarettes. The questionnaire regarding the occurrence of chronic diseases was also asked in the survey, and all subjects studied denied the presence of chronic disease. Healthy volunteers did not have to be on a special diet or show increased physical strength, both before and during the study. Detailed data on age, body mass index (BMI), waist-to-hip ratio (WHR), physical activity, diet, and smoking are presented in Tables 1-4. The BMI index criteria were: 16-16.99 - emaciation, 17-18.99 - underweight, 19-24.99 - standard, 25-29.99 - overweight, 30-34.99 – I degree of obesity. All women agreed to participate in the study. The study was approved by the Bioethical Commission at the Pomeranian Medical University in Szczecin.

3. Samples

Venous blood was collected in 5ml tubes and allowed to clot before centrifugation (10 minutes, 3000 rpm, 20 °C), and the serum was transferred to subsequent tubes and frozen at -80 °C until the analyses were performed. Additional samples of venous blood were collected into 5ml tubes containing an anticoagulant (K_3EDTA), and morphological parameters of the blood were assessed. The blood was centrifuged (10 minutes, 3000 rpm, 20 °C) and the plasma was transferred to another tube and frozen at -80 °C until analyses were performed. The remaining red blood cells were rinsed 3 times with 0.9% NaCl. After the last rinsing and removal of NaCl, the erythrocytes were transferred to appropriately labeled tubes and frozen at -80 °C until analyses were performed.

4. Activity of antioxidant enzymes

The activity of the antioxidant enzymes sodium dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione (GSH) was measured by spectrophotometric methods.

4.1 Determination of superoxide dismutase activity in erythrocytes

Reagents: adrenaline, carbonate buffer, chloroform, ethanol. Reagents were purchased from Sigma Aldrich (Poznań, Poland).

An extract was prepared by centrifuging 300 µl water, 200 µl chloroform: ethanol and 250 µl hemolysate II. The tested and blank samples were determined. 1425 µl carbonate buffer, 25 µl of obtained extract and 50 µl of adrenaline were added to the test sample. On the other hand, 1475 µL of carbonate buffer and 25 µL of obtained extract were added to the blank. Then both samples were incubated in a water bath for 3 minutes and the extinction measured at 320 nm for 3 minutes [3,8].

4.2 Determination of catalase activity in erythrocytes

Reagents: phosphate buffer, hydrogen peroxide. Reagents were purchased from Sigma Aldrich (Poznań, Poland).

Hemolysate IV was made by adding 10 mL of Hemolysate II buffer to 5000 mL. At the same time both the tested and blank samples were determined. 1000 µl of hemolysate IV and 500 µl of hydrogen peroxide were added to the test sample, while an additional 500 µl of phosphate buffer was added to the blank. Extinction was measured at 240 nm over 30 seconds [3,8].

4.3 Determination of glutathione peroxidase activity in erythrocytes

Reagents: phosphate buffer, glutathione reductase in phosphate buffer, reduced glutathione, NADPH + H +, tert-butyl hydroxide (T-BOOH). Reagents were purchased from Sigma Aldrich (Poznań, Poland).

250 µl transforming reagent was added to 500 µl hemolysate III and incubated for 5 minutes at room temperature. Then 250 µl of hemolysate with transforming reagent, 50 µl of reduced glutathione, 50 µl of NADPH + H + and 50 µl of glutathione reductase were added to 550 µl of phosphate buffer, followed by incubation for 10 minutes in a water bath. After incubation, 50 µL of T-BOOH was added and the extinction decline at 340 nm was measured against distilled water as a blank [3,8].

4.4 Determination of glutathione concentration in erythrocytes

Reagents: precipitation solution (glacial metaphosphoric acid, disodium / dipotassium EDTA, bidestilled water), DTNB, phosphate buffer pH 7.9. Reagents were purchased from Sigma Aldrich (Poznań, Poland).

50 µl hemolysate II was added to 450 µl of distilled water. 750 ml of the precipitation solution was mixed with the hemolysate and incubated for 5 minutes at 4 ° C, followed by centrifugation (550 g, 10 minutes). 250 ml of supernatant was added to 1 ml of phosphate buffer followed by 125 ml of DTNB solution and incubated 15 minutes at 4 ° C. Extinction was determined at λ 412 nm at 25 ° C [3,8].

4.5 Determination of glutathione transferase activity in erythrocytes

Reagents: phosphate buffer, GSH solution, CDNB solution (1-chloro-2,4-dinitrobenzene). Reagents were purchased from Sigma Aldrich (Poznań, Poland).

Hemolysate II with a hemoglobin concentration of 5 g / dl diluted 10-fold with distilled water (9: 1) was prepared. Each sample was then assayed separately by adding 850 µL phosphate buffer, 50 µL GSH solution and 50 µL CDNB solution. The extinction increase at 340 nm was measured against distilled water as a blank [3,8].

4.6 Determination of glutathione reductase activity in erythrocytes

Reagents: diluted erythrocyte hemolysate (1250 µl water and 62.5 µl erythrocytes), triethanolamine buffer (EDTA, pH 7.5), diluted RI working reagent (900 µl EDTA and 100 µl RI), working reagent RII. Reagents were purchased from Sigma Aldrich (Poznań, Poland).

25 ml of hemolysate was added to 1 ml of RI solution and incubated 5 minutes at 30 ° C. Then 0.1 ml RII reagent was added and extinction measured at λ 340 nm over 5 minutes at 30 ° C [3,8].

5. Statistical analysis

Descriptive statistics (arithmetic mean, standard deviation (SD), minimum and maximum values) of body mass index (BMI), waist-to-hip ratio (WHR), antioxidant enzymes, and GSH are reported. The Shapiro-Wilk test was used to check the assumption of normality for the data on GSH concentration and enzyme activity, which in the case of most variables showed a non-normal distribution of parameters. Differences in enzyme activity and GSH levels in the age groups and BMI categories were analyzed. The non-parametric Kruskal-Wallis ANOVA test was used for intergroup comparisons. The analysis also included differences in enzyme activity and GSH concentration depending on physical activity and cigarette smoking. The non-parametric Mann-Whitney U test was used for intergroup comparisons. The correlation strength between the parameters was measured using Spearman's rank correlation.

In order to determine a multifactorial evaluation of relationships between the parameters studied, a linear multiple regression model was used. The antioxidative enzymes and GSH concentrations were analyzed as dependent variables. The age and BMI of healthy volunteers were introduced as independent variables.

Statistical analysis of the results was carried out using Statistica PL 12 statistical program (StatSoft). P values ≤ 0.05 was assumed to be statistically significant.

Results

This study analyzed the relationship between the age of healthy volunteers and the activity of antioxidative enzymes and the concentration of GSH. There was a statistically significant relationship between age and CAT activity ($p < 0.001$), with the highest CAT activity in the 46-55 age group, and the lowest in the youngest group (Fig. 1). In the case of other enzymes, there was no significant effect of age on their activity and on the concentration of GSH. The results are shown in Table 6.

This study also analyzed the relationship between individual age groups of healthy volunteers and the activity of antioxidative enzymes and GSH concentrations. A statistically significant relationship was

found between CAT activity and the age range of 20-35 years and 36-45 years ($p = 0.002$). Statistical significance was also demonstrated in the case of GST activity ($p = 0.047$) in the same age ranges (Table 7). Comparison of the age ranges of 20-35 years and 46-55 years showed a statistically significant result in the activity of CAT ($p < 0.001$ (Table 8).

Statistical significance was also demonstrated by comparing the age groups of 20-35 years and 56-65 years for SOD activity ($p = 0.008$), CAT ($p = 0.027$) and GSH concentration ($p = 0.023$) (Table 9).

The differences in enzyme activity in individual BMI categories were analyzed. A statistically significant relationship was found between SOD activity and BMI ($p = 0.009$). The highest activity of SOD was observed in subjects who were classified by BMI as underweight, and the lowest activity was observed in people with I degree of obesity (Table 10).

The study also analyzed the relationship between individual BMIs of healthy volunteers and the activity of antioxidant enzymes and GSH concentration. A statistically significant relationship was demonstrated in the case of SOD activity in people who were underweight and with normal body mass ($p = 0.006$). Statistical significance was also demonstrated in the case of this enzyme by comparing people with the 1st degree of obesity and those who were underweight ($p = 0.036$). Detailed results are presented in Tables 11 and 12.

There were no significant differences in enzyme activity and GSH concentration depending on physical activity levels (Table 13) and cigarette smoking (Table 14).

The correlation between GSH concentration and enzyme activity, age, waist-to-hip ratio, body mass index, number of hours of physical activity, and cigarette smoking was analyzed. There was a statistically significant negative correlation between age and SOD activity ($p = 0.007$) and GST ($p = 0.025$). The activity of these enzymes decreases with age. In contrast, a statistically significant positive correlation was demonstrated between age and CAT activity. The activity of CAT increases with age. Detailed results are presented in Table 15.

There was no correlation between WHR and the activity of antioxidant enzymes and GSH concentration (Table 16). There was a statistically significant negative correlation between BMI and SOD activity ($p = 0.014$) (Table 17). There was no statistically significant correlation between physical activity levels (taking into account the number of hours of effort per week) and the activity of antioxidant enzymes and GSH concentration (Tables 18 and 19). There was no statistically significant correlation between the activity of antioxidant enzymes and GSH concentration, and smoking. The results are shown in Table 20. Multivariate regression analysis was performed. The influence of age, WHR and BMI (independent variables) on the activity of individual enzymes (dependent variable) was examined.

In the case of SOD, it has been shown that age affects its activity in an average of 5%. With increasing age, the activity of SOD decreases by an average of 0.0033 U/mgHb per year. In the case of CAT it was

shown that age affects its activity in an average of 17%. With increasing age, CAT activity increases by an average of 0.0057 U/mgHb during the year (Table 21).

Discussion

Changes in the activity of antioxidant enzymes and GSH level, in relation to age are commonly described in many articles and scientific publications. However, the specificity of the change in activity of these enzymes and GSH in relation to physical activity, diet or smoking is less known.

The literature describes the importance of oxidative stress and the reduced efficiency of repair processes in the process of aging. The most visible effects of these pathological conditions are revealed in DNA. DNA damage caused by the action of reactive oxygen species can lead to the formation of mutations, which in turn may be a cause of cancer development. Therefore, with increasing age, humans experience a higher incidence of various diseases, mainly including cancers but also neurodegenerative diseases and atherosclerosis, among other disease. Well-functioning repair systems remove damage and prevent harmful changes in cells. Unfortunately, with age, they are weakened, which contributes to an increase in the number of damaged cells [5].

Changes related to the aging of the body and increasingly becoming associated with the operation of ROS. In order to avoid the accumulation of ROS, the body has developed mechanisms of antioxidant defense, which include, among others, the action of enzymes such as CAT, SOD, GST, GPx, or non-enzymatic antioxidants such as GSH. Changes in their activity and concentration depend on the organ or subcellular location of the enzyme, as well as race and sex, and other variables. The reduction of their activity and concentration with age is caused by the modification of the enzyme molecule, which is caused directly or indirectly by ROS. Increasing their activity, on the other hand, should be treated as a compensatory response to overproduction of reactive oxygen species. With age, there may also be a decrease in GSH synthesis due to the much lower availability of methionine and cysteine, and the activity of γ-glutamyl cysteine synthetase and cystathionase, as well as increased GSH consumption in reactions with ROS, produced in too large quantities [4].

An important enzyme involved in the defense of the body against oxidative stress is CAT. CAT reacts with hydrogen peroxide (H_2O_2) to form water and molecular oxygen, as well as compounds such as methanol, ethanol, formic acid, phenol, etc. CAT thus protects the body against the effects of hydrogen peroxide produced in cells and is one of the most efficient enzymes in the fight against oxidative stress [6]. This study analyzed differences in the levels of antioxidant enzymes in particular age groups. A statistically significant increase in CAT activity with age was demonstrated. The highest activity was observed in the 46–55 age group. These results also seem to be confirmed by multivariate regression analysis, which showed an increase in CAT activity with increasing age in healthy volunteers (an average increase of 0.0057 U/mgHb per year). These data support the generally accepted hypothesis regarding the increase in the activity of CAT in an aging organism [7]. The increase in the activity of this enzyme may also be related to the activity of GST. There was a statistically significant ($p = 0.047$) decline in its activity among

the elderly. These enzymes, due to their function to reduce peroxides, can complement one another. Therefore, when one of these enzymes increases, the growth of the other is inhibited [8].

Another important enzyme in antioxidant defense is GPx. This enzyme reduces both inorganic peroxides, e.g. H_2O_2, as well as organic peroxides (ROOH) to form selenic acid as an intermediate [9, 10]. Peroxidase has a greater affinity for hydrogen peroxide than CAT, therefore it performs a more important function in most physiological situations, when the amount of hydrogen peroxide formed is not too high. Insufficient CAT activity is therefore compensated by an increase in GPx activity and, conversely, reduced peroxidase activity is compensated by an increase in CAT activity [3, 11]. In the current studies reported here, no increased GPx activity was observed, in any age range, as well as in dependence from BMI. This may be related to the greater activity of CAT, which, despite its lower affinity for H_2O_2, performs this reaction with greater efficiency. This is evidenced by the increase in the activity of CAT with age. However, this does not confirm that peroxidase is more active in physiological situations.

Olędzki et al. compared the activity of SOD and CAT in erythrocytes taken from young healthy people (aged 20–29) and older individuals (> 60 years of age). They reported reduced SOD activity in the older patients. The study also showed statistically significant differences in the activity of SOD between the group of the youngest and the oldest women. According to the study reported here, the SOD activity decreases by an average of 0.0033 U/mgHb per year. In the case of CAT, decreased activity in the elderly was observed in relation to younger people, which is in opposition to the data presented here. Based on these results, it was found that the antioxidant defense level of erythrocytes, during their more than 100-day duration, is not stable in both young and old people and that it decreases in the physiological aging process [12].

The reduction of SOD activity and the increase in CAT activity among aging women can be explained by enzyme inactivation by excess hydrogen peroxide, as well as by glycation of SOD molecules or reactions with lipid peroxidation products, the intensity of which increases with age [11]. This study also analyzed antioxidative enzyme activities and GSH concentration in relation to the BMI of healthy volunteers. A statistically significant increase in the activity of SOD was found in people who were underweight and a significant decrease in people with the first degree of obesity. This enzyme is an antioxidant that catalyzes the superoxide anion radical dismutation reaction for hydrogen peroxide and molecular oxygen, thus contributing to effective defense against oxidative stress [7, 13].

Karolkiewicz and colleagues evaluated the relationship between body mass and insulin resistance parameters, as well as the relationship between body weight and oxidative stress markers in older women. The population studied consisted of 34 women aged 60–90 who were divided into three subgroups based on their BMI: normal weight, overweight and obese. The total antioxidative status (TAS), the concentration of substances reacting with thiobarbituric acid and the level of protein were measured in the plasma C-reactive protein (CRP). However, the concentration of GSH and GPx activity were determined in haemolysate of red blood cells. The results did not reveal any significant differences between the three groups of women surveyed in relation to antioxidant status parameters. There was also no disturbed

balance between oxidants and antioxidants [14]. However, the relationship in the case of SOD has not been studied, therefore it is unknown what the reaction would have been with this enzyme. The current study reported here also did not show statistical significance with respect to GSH concentration and GPx activity.

The impact of diet and increased plant sterol supply on the parameters of oxidative stress in the group of obese women was examined previously by Stelmach-Mardas and co-workers. The study covered 101 women with a BMI > 30 kg / m². They were divided into two groups: the study group (60 women) and the control group (41 women). Anthropometric measurements were made, such as body weight, height, waist and hip circumference. Parameters were calculated, including the value of the body mass index and waist-hip index and percentage of adipose tissue. The lipid profile, oxidative stress parameters (malondialdehyde (MDA), oxidized protein, hydroxydioxides, CAT, SOD) were measured by enzymatic-colorimetric methods. The results showed statistically significant ($p < 0.05$) differences between the examined groups in relation to oxidative stress parameters after supplementing the diet with plant sterols.

In this study group, a decrease in the amount of hydroxydioxides ($p = 0.0011$) and a tendency to lower the activity of malondialdehyde ($p = 0.0018$), oxidized protein, and most importantly SOD ($p = 0.0004$) [15]. Xiao-Liao et al. conducted a study for SOD in a group of 136 young and middle-aged men. The men were divided into three groups based on BMI: group I - obese (43 people), group II - overweight (46 people) and a control group with normal weight (47 people). Statistical analysis was performed, the results of which, in relation to oxidative stress parameters, showed a significant decrease in the activity of MDA and SOD in overweight and obese people [16]. With regard to our current study, it can be assumed that anthropometric features are also strongly related to the activity of individual antioxidant enzymes. It is true that healthy volunteers, before and during the study, did not have to be on a special diet, as was the case in the study by Stelmach-Mardas et al., although SOD activity was similar. The decrease in the activity of SOD in the case of people with I degree of obesity, and its increase in underweight individuals has also been demonstrated.

Obesity is defined as the body mass exceeding the upper limit of physiological needs caused by excessive fat accumulation [16, 17]. The human body has brown and white adipose tissue. White adipose tissue contains fibroblasts, adipocyte, and macrophages, which are characterized by an obvious heterogeneity associated with their location, e.g. subcutaneous or visceral. In addition, white adipose tissue is not only the tissue that stores energy in the body, but also has an endocrine, paracrine, and autocrine function [16, 18]. Bioactive substances secreted by adipose tissues are called adipocytes or adipocytokines (including leptin and adiponectin) [16, 19]. The latter two can increase energy consumption, insulin sensitivity, and fatty acid oxidation, while leptin can suppress appetite and fat aggregation. In addition, adipokines increase the production of reactive oxygen species and cause oxidative stress respectively. Therefore, obesity correlates significantly with the growth of oxidative stress markers [16, 20].

Of course, diet should be taken as a factor having a significant impact on the increase or decrease in the activity of individual antioxidant enzymes and GSH concentration. A diet rich in fat reduces the activity

and concentration of antioxidants. On the contrary, eating a large amount of fruits and vegetables increases the activity and concentration of antioxidants [10]. In this case, attention should be paid to the characteristics of the women surveyed in Table 3. Almost 70% of women limited fat intake, and the majority did not limit consumption of fruit and vegetables in their diet. This applies mainly to older people, because the diet changes with age and health status, which often determines the consumption of proper foods in the elderly.

Epidemiological studies indicate that fruit and vegetables have a protective effect against diseases typical of old age, such as, joint degeneration, cardiovascular disease, stroke, or various types of cancer. The benefits of a diet rich in fruits and vegetables may also result from the avoidance of ingredients of animal origin less desirable for this age, such as saturated fats, oxidized cholesterol, etc., and may result from the intake of various antioxidant compounds, such as vitamin C and major carotenoids and dietary polyphenols [10, 21, 22].

The relationship between the change in the activity of antioxidant enzymes and physical activity is not completely clear. Current research indicates that the systems of antioxidant enzymes undergo significant changes in response to acute and chronic exercise. Acute exercise may increase the activity of some antioxidant enzymes in various tissues. A small effect of physical activity on hepatic enzymes or cardiac muscle has been shown, but changes in the activity of these enzymes in skeletal muscles have been observed, especially in the case of GPx [7]. In our own study, there were no differences in the activity of antioxidant enzymes and GSH concentration in dependence from physical activity, which may be due to the fact that there was minimal physical activity in the characteristics of the study group. More than half of the women surveyed did not show any physical activity, and the part who led an active lifestyle only spent a small number of hours exercising each week.

In the literature, a small number of studies indicate the relationship between antioxidant enzyme activity and cigarette smoking, however these studies are predominantly in pregnant women. In this case, it has been shown that smoking cigarettes during pregnancy and lactation results in disruption of the antioxidant balance in the woman's milk. It is also found that cigarette smoking reduces the total antioxidative capacity in colostrum and this is directly related to the number of cigarettes smoked by pregnant women [9, 23, 24]. As the literature presents, tobacco smoke contains large amounts of reactive oxygen species, which, as we know, intensify oxidative stress and cause of various pathological changes in the human body. In the serum of people who smoke, there is a significant increase in the products of oxidative damage DNA, proteins, and lipids, and at the same time a significant decrease in the activity of antioxidants. It is assumed that in one portion of inhaled tobacco smoke there are as many as 1015 ROS molecules.

These molecules include mainly semiquinone ($QH^{\cdot}$) radicals, but also oxygen radicals such as the hydroxyl radical, superoxide anion radical, or hydroperoxide radical, and molecules that do not belong to free oxygen radicals but are easily transformed

in these forms. These include mainly hydrogen peroxide, which is a precursor of the reactive hydroxyl radical. In the human body, the concentration of hydrogen peroxide is very low, mainly due to the reductive function of CAT and GPx. Oxygen radicals react very easily with the molecules present in cigarette smoke. These molecules include, for example, hydrocarbons, and as a result of their reaction, alkoxy radicals ($RO^{\cdot}$) or alkyl radicals ($R^{\cdot}$) [25]. Based on these data, it should be assumed that in smokers, there should be a significant increase in the activity of antioxidant enzymes, which is adequate to increase the level of reactive oxygen species contained in tobacco smoke. In our study, 57% of women smoke cigarettes. However, there were no significant differences in enzyme activity and GSH concentrations in dependence from smoking cigarettes. It does not confirm, therefore, the reports of other scientists on the increase in oxidative stress as a result of smoking cigarettes. It may be associated with a well-functioning antioxidant system in the group of women surveyed, especially considering most of them were women in the 20–35 age group.

Conclusions

1. Increased CAT activity with age may be a sign of a large amount of hydrogen peroxide resulting from poor-functioning antioxidant systems in older age.
2. Decreased SOD activity with age may indicate inactivation of this enzyme by excessive hydrogen peroxide, as well as glycation of SOD molecules or reactions with lipid peroxidation products, the intensity of which increases with age.
3. The negative correlation between SOD activity and BMI index, indicates a reduced activity of SOD in obese subjects, despite increased ROS production by adipose tissue. This indicates that obese subjects have a reduced capacity to compensate for increased levels of ROS.
4. The concentration of GSH decreases with age, which confirms the results obtained by other researchers.

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Conflict of Interest:

The authors declare that they have no competing interests.

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Ethical approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors' contributions

Elżbieta Cecerska-Heryć - writing an article, project originator

collecting material

Angelika Szczęsniak, Aleksandra Goryniak- Mikołajczyk, Daria Sleboda-Taront, Roksana Jacek – collecting material

Klaudia Krauze- determination of enzymes

Rafał Heryć - statistical analysis

Natalia Serwin - translation, literature analysis

Barbara Dołęgowska - substantive correction of the article

Consent for publication

Not applicable

References

1. Łuszczewski A., Matyska-Piekarska E., Trefler J., Wawer I., Łącki J., Śliwińska-Stańczyk P.: Reactive oxygen species - meaning in physiology and state of pathology of the organism. Rheumatology 2007; 15:284-289.
2. Gałecka E., Jacewicz R., Mrowiska M., Florkowski A., Gałecki P.: Antioxidative enzymes - construction, properties, functions. *Merk. Lek.* 2008; 25:147:266.
3. Dołęgowska B.: Predictors of delayed transplanted kidney function - analysis of prooxidation and antioxidant balance and arachidonic acid metabolism in blood platelets and plasma during ischemia-reperfusion period. Szczecin: Publishing House Pomeranian Medical University; 2009.
4. Augustyniak A.,Skrzydlewska E.: Antioxidant abilities in an aging body. *Postepy Hig Med. Dosw.* 2004; 58:194-201.

5. Michalak A., Krzeszowiak J., Markiewicz-Górka I.: Aging of the body and oxidative stress and reduced efficiency of repair systems, 1483-1491, Postepy Hig Med Dosw, 68, 2014
6. Ścibior D., Czeczot H.: Catalase – construction, properties, function, Postepy Hig Med Dosw, 170-180, 60, 2006
7. Ji L. L.: Antioxidant enzyme response to exercise and aging, Med Sci Sports Exerc, 225-231, 25, 1993
8. Stępniewska J., Dołęgowska B., Cecerska-Heryć E., Gołembiewska E., Malinowska-Jędraszczyk A., et al.: The activity of antioxidant enzymes in blood platelets in different types of renal replacement therapy: a cross-sectional study. Int Urol Nephrol. 2016 ;48:593-9.
9. Bałajewicz-Nowak M., Pityński K., Migdał M.: Influence of cervical canal infections in pregnant women caused by Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum on antioxidant system, Ginekol Pol., 732-737, 82, 2011
10. Gryszczyńska B., Iskra M.: Interaction of exogenous and endogenous antioxidants in the human body, Nowiny Lekarskie, 50-55, 77, 2008.
11. Bartosz G.: Second face of oxygen. Free Radicals in nature. Publishing House of PWN, Warszawa 2003
12. Olędzki R., Harasym J.: Changes in the activity of superoxide dismutase and catalase in age-diverse red blood cells in the elderly, Probl Hig Epidemiol, 89-98, 98(1), 2017
13. Zagierski M.: Wpływ palenia papierosów na równowagę antyoksydacyjno – prooksydacyjną w mleku kobiecym, Rozprawa doktorska, Gdańsk, 2008
14. Karolkiewicz J., Pilaczyńska-Szcześniak Ł., Elegańczyk-Kot H., Nowak A., Kasprzak Z., Lawendowska M.: Body mass index and insulin resistance and parameters of oxidative stress in elderly women, Gerontol. Pol., 64-70, 17, 2, 2009
15. Stelmach-Mardas M.: Wpływ sposobu żywienia oraz zwiększonej podaży steroli roślinnych na profil lipidowy i parametry stresu oksydacyjnego w grupie kobiet otyłych, Rozprawa doktorska, Poznań 2011.The impact of diet and increased supply of plant sterols on the lipid profile and oxidative stress parameters in the group of obese women, PhD dissertation,
16. Xiao-Jiao Jia, Lan-Xiang Liu, Yi-Ming Tian, Rui Wang, Qiang Lu: The correlation between oxidative stress level and intra-abdominal fat in obese males, Medicine, 98:7, 2019.
17. Polyzos S.A, Margioris A.N. Sarcopenic obesity. Hormones (Athens) 2018;17:321–31.
18. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci 2014;15:6184–223
19. Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. Ann N Y Acad Sci 2010;1212:E1–9. [PMC free article]
20. Ueda Y, Hajri T, Peng D, et al. Reduction of 8-iso-prostaglandin F2α in the first week after Roux-en-Y gastric bypass surgery. Obesity (Silver Spring) 2011;19:1663–8.

21. Mates J. M.: Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology, Toxicology, 83-104, 16, 2000
22. Wawrzyniak A., Krotki M., Stoparczyk B.: Antioxidant properties of fruits and vegetables, Medycyna Rodzinna 1, 19-23, 2011
23. Saczko Z., Saczko J., Kulbacka J., Chwiłkowska A., Żórawski K.: Hypertension in pregnant women.Eiopathogenesis, Arterial Hypertension, 199-205, 13, 2009
24. Skólmowska M., Kmieć M.: Antioxidative enzymes - properties and application, Postepy Hig Med Dosw, 640-644, 65, 2011
25. Krzyścin M.: Effect of cigarette smoking and inhalation of tobacco smoke in the aspect of newborn status after birth, PhD dissertation, Poznań, 2013

Tables

Table 1. Characteristics of the study group in terms of age and BMI (body mass index)

Age		
Age range	**n**	**%**
20-35 years	61	62,2
36-45 years	17	17,3
46-55 years	11	11,2
56-65 years	9	9,2
BMI		
Indicator	**n**	**%**
Emaciation	3	3,1
Underweight	11	11,2
Normal	67	68,4
Overweight	13	13,3
I degrees of obesity	4	4,1

n – number of people

Table 2. Characteristics of the examined group in terms of physical activity.

Physical activity	n	%
No	56	57,1
Yes	42	42,9
Number of hours per week	**n**	**%**
0	56	57,1
1	10	10,2
2	15	15,3
3	10	10,2
4	2	2,0
5	5	5,1

n – number of people

Table 3. Characteristics of the study group in terms of diet and smoking cigarettes

Limitation of sugar consumption	**n**	**%**
No	54	55,1
Yes	44	44,9
Limitation of bread consymption	**n**	**%**
No	88	89,8
Yes	10	10,2
Limitation of fishes consumption	**n**	**%**
No	95	96,9
Yes	3	3,1
Limitation of meat consumption	**n**	**%**
No	83	84,7
Yes	15	15,3
Limitation of raw vegetables consumption	**n**	**%**
No	89	90,8
Yes	9	9,2
Limitation of raw fruits consumption	**n**	**%**
No	98	100,0
Limitation of dairy consumption	**n**	**%**
No	89	90,8
Yes	9	9,2
Limitation of fats consumption	**n**	**%**
No	67	68,4
Yes	31	31,6
Smoking cigarettes	**n**	**%**
No	56	57,1
Yes	42	42,9

n – number of people

Table 4. Characteristics of the examined group in terms of BMI and WHR (Avg - arithmetic mean, SD - standard deviation, Min - minimum value, Max - maximum value)

Indicator	Avg	SD	Min	Max
BMI	22,3	3,5	16,0	32,6
WHR	0,8	0,1	0,7	0,9

BMI (body mass index); WHR (waist-hip ratio)

Table 5. Antioxidant enzyme activity and GSH concentration (Avg - arithmetic mean, SD - standard deviation, Min - minimum value, Max - maximum value)

Antioxidant	Avg	SD	Min	Max
SOD [U/mgHb]	0,34	0,20	0,05	0,9
CAT [U/mgHb]	0,31	0,17	0,01	0,9
GPx [U/gHb]	0,05	0,04	0,01	0,2
GSH [µmol/gHb]	9,87	2,29	5,34	19,4
GST [U/gHb]	0,04	0,03	0,00	0,2
GR [U/gHb]	7,37	11,93	0,04	77,1

SOD - superoxide dismutase; CAT - catalase; GPx - glutathione peroxidase; GSH - glutathione; GST - glutathione transferase; GR - glutathione reductase

Table 6. Antioxidant enzymes activity and GSH concentration in particular age groups (Av - arithmetic mean, SD - standard deviation, Min - minimum value, Max - maximum value)

Parameter	20-35 years		36-45 years		46-55 years		56-65 years		p
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	
SOD [U/mgHb]	0,37	0,20	0,35	0,25	0,29	0,17	0,20	0,09	0,062
CAT [U/mgHb]	0,24	0,13	0,39	0,21	0,46	0,16	0,36	0,16	**<0,001**
GPx [U/gHb]	0,05	0,04	0,04	0,02	0,03	0,01	0,03	0,02	0,684
GSH [µmol/gHb]	9,93	1,87	10,90	3,47	9,17	2,00	8,40	1,72	0,107
GST [U/gHb]	0,04	0,03	0,03	0,02	0,06	0,06	0,03	0,02	0,230
GR [U/gHb]	8,67	14,51	5,23	4,09	3,47	2,86	7,35	7,84	0,524

p - statistical significance of the relationship between age of healthy volunteers and activity of antioxidant enzymes and concentration of GSH - Kruskal-Wallis ANOVA

Table 7. Activity of antioxidant enzymes and concentration of GSH in age groups 20-35 and 36-45 years

	Median 20-35 years	Median 36-45 years	p
SOD [U/mgHb]	0,34	0,22	0,438
CAT [U/mgHb]	0,23	0,33	**0,002**
GPx [U/gHb]	0,04	0,04	0,743
GSH [µmol/gHb]	9,84	9,89	0,549
GST [U/gHb]	0,04	0,03	**0,047**
GR [U/gHb]	3,51	4,00	0,990

p - statistical significance of the relationship between individual age groups, antioxidant enzyme activity and GSH concentration - Kruskal-Wallis ANOVA

Tabela 8. Activity of antioxidant enzymes and concentration of GSH in age groups 20-35 and 46-55 years

	Median 20-35 years	Median 46-55 years	p
SOD [U/mgHb]	0,34	0,22	0,193
CAT [U/mgHb]	0,23	0,44	**<0,001**
GPx [U/gHb]	0,04	0,04	0,402
GSH [µmol/gHb]	9,84	8,83	0,406
GST [U/gHb]	0,04	0,03	0,863
GR [U/gHb]	3,51	2,70	0,259

p - statistical significance of the relationship between individual age groups, antioxidant enzyme activity and GSH concentration - Kruskal-Wallis ANOVA

Tabela 9. Activity of antioxidant enzymes and concentration of GSH in age groups 20-35 and 56-65 years

	Median 20-35 years	Median 56-65 years	p
SOD [U/mgHb]	0,34	0,18	**0,008**
CAT [U/mgHb]	0,23	0,37	**0,027**
GPx [U/gHb]	0,04	0,04	0,493
GSH [µmol/gHb]	9,84	8,37	**0,023**
GST [U/gHb]	0,04	0,03	0,250
GR [U/gHb]	3,51	3,50	0,466

p - statistical significance of the relationship between individual age groups, antioxidant enzyme activity and GSH concentration - Kruskal-Wallis ANOVA

Table 10. Antioxidant enzymes activity and GSH concentration in particular BMI groups (Avg - arithmetic

Parametr	Emaciation		Underweight		Normal		Overweight		I degree of obesity		p
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	
SOD [U/mgHb]	0,48	0,15	0,52	0,23	0,31	0,18	0,37	0,23	0,23	0,14	**0,009**
CAT [U/mgHb]	0,33	0,02	0,25	0,09	0,31	0,19	0,30	0,14	0,35	0,24	0,806
GPx [U/gHb]	0,09	0,06	0,06	0,04	0,04	0,03	0,05	0,04	0,05	0,05	0,540
GSH [µmol/gHb]	12,12	2,23	10,37	1,39	9,67	2,47	9,62	1,56	11,13	2,72	0,103
GST [U/gHb]	0,05	0,02	0,05	0,03	0,04	0,03	0,04	0,06	0,04	0,02	0,438
GR [U/gHb]	5,09	4,09	4,93	4,57	8,20	13,94	7,00	6,67	3,06	2,40	0,632

mean, SD - standard deviation, Min - minimal value, Max - maximum value)

p - statistical significance of the relationship between BMI, antioxidant enzyme activity and GSH concentration

Table 11. Antioxidant enzymes activity and GSH concentration in people with underweight and normal body mass

	Median underweight	Mediana normal	p
SOD [U/mgHb]	0,53	0,26	**0,006**
CAT [U/mgHb]	0,26	0,28	0,417
GPx [U/gHb]	0,07	0,04	0,201
GSH [µmol/gHb]	10,18	9,35	0,077
GST [U/gHb]	0,04	0,04	0,332
GR [U/gHb]	3,00	3,24	0,397

p - statistical significance of the relationship between underweight and normal BMI index, and the activity of antioxidant enzymes and GSH concentration - Kruskal-Wallis ANOVA

Table 12. Antioxidant enzymes activity and GSH concentration in people with I degree of obesity and underweight

	Median I degree of obesity	Median underweight	p
SOD [U/mgHb]	0,19	0,53	**0,036**
CAT [U/mgHb]	0,35	0,26	0,556
GPx [U/gHb]	0,04	0,07	0,794
GSH [µmol/gHb]	10,92	10,18	0,647
GST [U/gHb]	0,05	0,04	0,844
GR [U/gHb]	1,99	3,00	0,948

p - statistical significance of the relationship between people with I degree of obesity and underweight, and the activity of antioxidant enzymes and concentration of GSH - Kruskal-Wallis ANOVA

Table 13. Antioxidant enzymes activity and GSH concentration depending on the physical activity undertaken (Avg - arithmetic mean, SD - standard deviation)

Parameter	Physical activity: No		Physical activity: Yes		p
	Avg	SD	Avg	SD	
SOD [U/mgHb]	0,32	0,18	0,38	0,22	0,238
CAT [U/mgHb]	0,32	0,15	0,28	0,19	0,148
GPx [U/gHb]	0,04	0,03	0,06	0,04	0,064
GSH [µmol/gHb]	9,66	1,97	10,16	2,66	0,560
GST [U/gHb]	0,05	0,03	0,04	0,02	0,075
GR [U/gHb]	5,45	5,63	9,93	16,81	0,658

p - statistical significance of the relationship between the physical activity undertaken, the activity of antioxidant enzymes and the concentration of GSH - non-parametric U Mann-Whitney test

Tabela 14. Antioxidant enzymes activity and GSH concentration depending on the smoking (Avg - arithmetic mean, SD - standard deviation)

Parametrer	Smoking:No		Smoking: Yes		p
	Śr	SD	Śr	SD	
SOD [U/mgHb]	0,34	0,20	0,32	0,15	0,869
CAT [U/mgHb]	0,32	0,18	0,22	0,10	0,094
GPx [U/gHb]	0,05	0,04	0,04	0,03	0,272
GSH [µmol/gHb]	9,91	2,36	9,58	1,60	0,920
GST [U/gHb]	0,04	0,03	0,04	0,02	0,878
GR [U/gHb]	7,68	12,51	4,58	3,37	0,782

p - statistical significance of the relationship between the smoking undertaken, the activity of antioxidant enzymes and the concentration of GSH - non-parametric U Mann-Whitney test

Table 15. Correlation coefficients between activity antioxidant enzymes and GSH concentration and age

Antioxidant	Age	p
SOD [U/mgHb]	**-0,27**	**0,007**
CAT [U/mgHb]	**0,39**	**0,000**
GPx [U/gHb]	-0,06	0,570
GSH [µmol/gHb]	-0,13	0,212
GST [U/gHb]	**-0,23**	**0,025**
GR [U/gHb]	-0,02	0,881

p - statistical significance of the relationship between age and activity of antioxidant enzymes and GSH concentration - Spearman's rank correlation analysis

Table 16. Antioxidant enzymes activity and concentration of GSH depending on cigarette smoking (Avg - arithmetic mean, SD - standard deviation)
p - statistical significance of the relationship between cigarette smoking and the activity of antioxidant enzymes and GSH concentration - non-parametric U Mann-Whitney test

Parameter	Smoking: No		Smoking: Yes		p
	Śr	SD	Śr	SD	
SOD [U/mgHb]	0,34	0,20	0,32	0,15	0,869
CAT [U/mgHb]	0,32	0,18	0,22	0,10	0,094
GPx [U/gHb]	0,05	0,04	0,04	0,03	0,272
GSH [µmol/gHb]	9,91	2,36	9,58	1,60	0,920
GST [U/gHb]	0,04	0,03	0,04	0,02	0,878
GR [U/gHb]	7,68	12,51	4,58	3,37	0,782

Table 17. Correlation coefficients between activity antioxidative enzymes and GSH concentration, and body mass index - BMI

Antioxidant	BMI	p
SOD [U/mgHb]	**-0,25**	**0,014**
CAT [U/mgHb]	0,05	0,642
GPx [U/gHb]	-0,12	0,241
GSH [µmol/gHb]	-0,11	0,300
GST [U/gHb]	-0,12	0,246
GR [U/gHb]	0,16	0,120

p - statistical significance of the relationship between BMI, antioxidant enzyme activity and GSH concentration - Spearman's rank correlation analysis

Tabela 18. Results of multivariate regression analysis in relation to SOD and CAT

Dependent variable	Independent variable	β0	β1	p	R^2	F
SOD					**0,050**	2,721
	Age	**-0,217**	**-0,003**	**0,041**		
	WHR	-0,021	-0,069	0,829		
	BMI	-0,120	-0,006	0,254		
CAT					0,170	7,655
	Age	**0,437**	**0,005**	**0,000**		
	WHR	0,163	0,452	0,080		
	BMI	-0,093	-0,004	0,343		

β - standardized coefficient in the regression equation; β1 - unknown (and determined) regression coefficient; β0 - word free; R^2 - coefficient of determination;

p - value of materiality coefficient; SOD - superoxide dismutase; CAT - catalase

Tables 19-21 not provided with this version

Figures

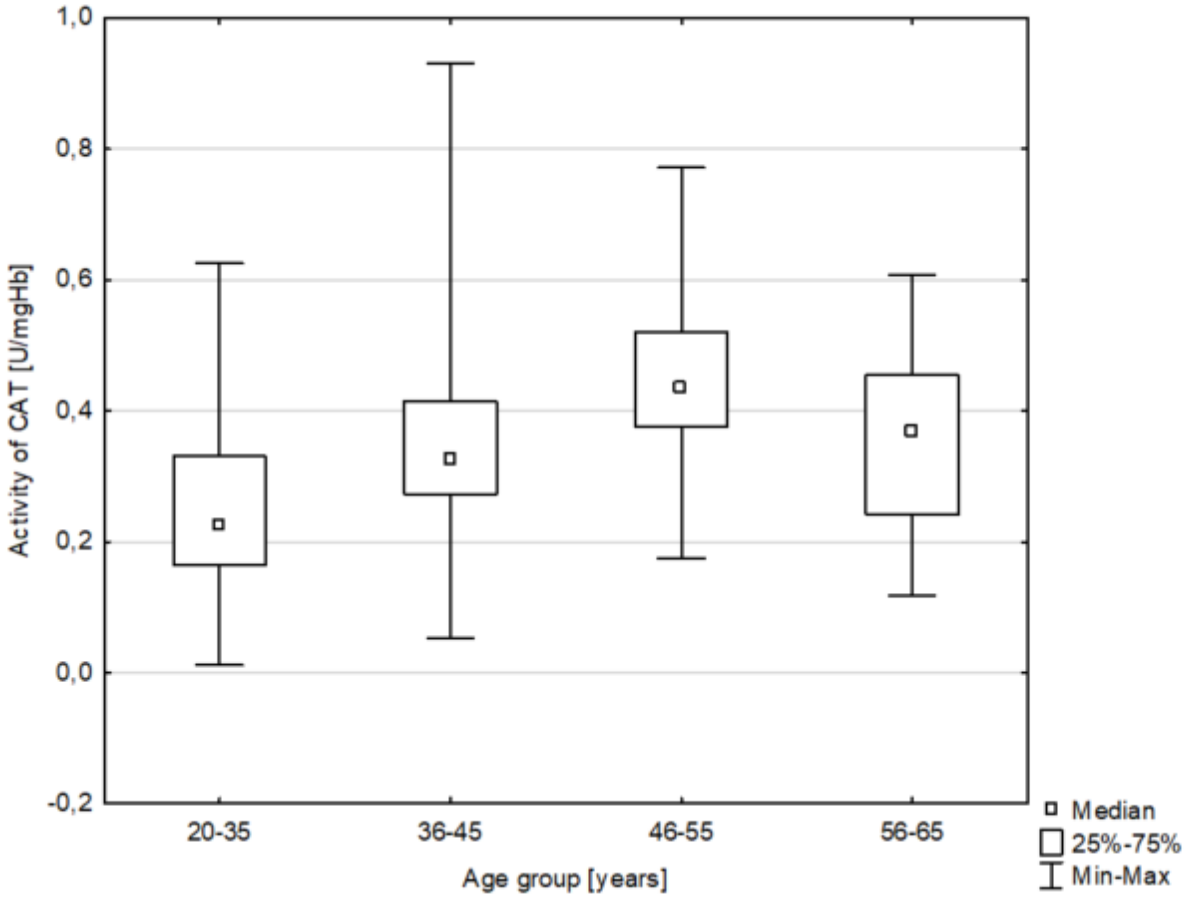

Figure 1

Kruskal-Wallis ANOVA analysis of age influence on CAT activity (p <0.001) CAT – catalase.

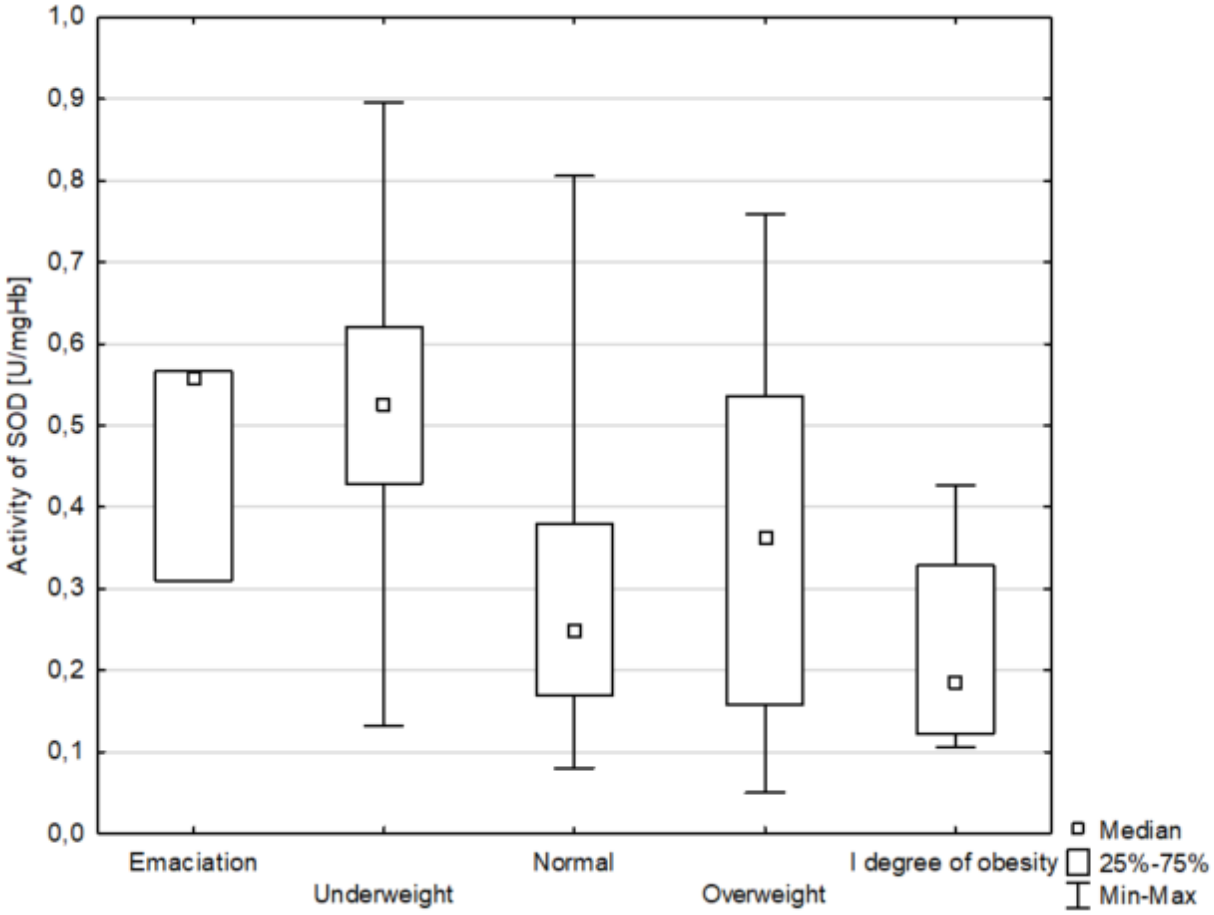

Figure 2

Kruskal-Wallis ANOVA analysis of age influence on SOD activity (p =0.009) SOD – sodium dysmutase