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## Activities of red blood cell anti-oxidative enzymes (SOD, GPx) and total anti-oxidative capacity of serum (TAS) in men with coronary atherosclerosis and in healthy pilots

### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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### Background:

Reactive oxygen species (ROS) have been proposed to play important pathogenic roles, especially in harmful oxidative modifications of low-density cholesterol. Redox balance within the organism is largely determined by the activities of anti-oxidative enzymes of red blood cells and by the total anti-oxidative capacity of the serum (TAS).

### Material/Methods:

SOD and GPx activities and TAS in 13 men aged 42–65 years with coronary atherosclerosis (group I) were compared with those of both 15 clinically healthy pilots matched for age and lipid abnormalities (cholesterol and triglycerides) (group II) and 14 age-matched pilots without lipid abnormalities (group III).

### Results:

There were statistically significant differences in SOD and GPx activities and in TAS between the groups.

### Conclusions:

1. SOD and GPx activities and TAS were lower in men with advanced coronary atherosclerosis than in age-matched clinically healthy men with similar dyslipidemia and were even further decreased compared with clinically healthy men without dyslipidemia. 2. The decrease in SOD and GPx activities and TAS in men with advanced coronary atherosclerosis was more pronounced than the degree of hypercholesterolemia or hypertriglyceridemia. 3. If hyperlipidemia and the activity of antioxidative enzymes and TAS were considered without reference to other risk factors of atherosclerosis, it appeared that the decreases in SOD, GPx, and TAS may play a more important role in the development of the atherosclerotic process than isolated increases in free cholesterol or triglyceride levels.

### key words:

**lipid concentration • antioxidative enzymes • coronary artery disease**

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## BACKGROUND

Disturbances in lipid metabolism are considered to be important in the development of atherosclerosis. The results of angiographic studies performed over the last several years have demonstrated that a reduction in LDL-cholesterol (LDL-CH) concentration delays progression or, in some cases, causes regression of atherosclerotic lesions in coronary arteries. In contrast, the lipid profiles of patients with evident stages of atherosclerosis reportedly did not significantly differ from those of control patients [1]. Such an apparent discrepancy occurs especially in those cases when vascular abnormalities are being correlated with a lipidogram performed only one time and/or when hyperlipidemia is considered without reference to other risk factors of atherosclerosis. This may be particularly relevant with regard to those risk factors which are somehow related to dyslipidemia and are not routinely evaluated on clinical examination. In the last few years, reactive oxygen species (ROS) have been proposed to play important pathogenic roles, especially in harmful oxidative modifications of low-density cholesterol. Redox balance within the organism is largely determined by the activities of anti-oxidative enzymes of red blood cells, superoxide dismutase (SOD) and glutathione peroxidase (GPx), and by the total anti-oxidative capacity of the serum (TAS). The latter was shown to be altered in atherosclerosis [2].

Various factors, both intrinsic and extrinsic, can influence the activity of antioxidative enzymes. Our results published earlier indicated that there is a reciprocal relationship between cholesterol concentration (mainly LDL-CH) and SOD and GPx activities and TAS [3]. Therefore, people with high LDL-CH have both decreased activity of antioxidative mechanisms and an excess of substrate, which can undergo abnormal oxidative modifications. Because of the high complexity of the atherosclerotic process, it is not possible to mention all the mechanisms, modifying factors, and relationships involved in its pathogenesis. It is even more difficult to evaluate the degree of involvement of these elements in endothelial damage and the formation of atherosclerotic plaque.

The objective of this study was to compare the activities of SOD and GPx and TAS in male patients with coronarographically documented atherosclerosis with those of age-matched clinically healthy pilots with and without similar abnormalities in total cholesterol and triglyceride (TG) levels.

## MATERIAL AND METHODS

The following groups of patients were studied:

- 13 men (group I), aged 42–65 years (mean  $\pm$  standard deviation: 51.69 $\pm$ 8.05 years) with advanced coronary atherosclerosis confirmed by coronarography (7 patients had atherosclerotic lesions in 3 coronary arteries, 2 had lesions in 2 coronary arteries, the rest had critical stenosis and/or occlusion of 1 coronary artery);
- 15 men (group II) aged 42–51 (46.6 $\pm$ 4.8 years) who were clinically healthy but had abnormalities in concentrations of total cholesterol (T-CH) and triglycerides (TGs) which were comparable to those in group I;
- 14 men (group III) aged 39–51 (43.0 $\pm$ 4.62), clinically healthy, with normal T-CH and TG concentrations.

Groups II and III were chosen (for the purpose of the study) from among 229 pilots of super-sonic aircraft in whom, in addition to lipidograms, SOD and GPx activities, and TAS were determined upon routine periodic medical examinations. All of the pilots and patients were non-smokers and did not take antioxidative vitamin supplements. Table 1 shows the minimal, maximal, and mean values of the components of the lipidograms in all three groups of patients.

As indicated in Table 1, mean concentrations of T-CH and LDL-CH in groups II and I were similar, but appeared to be better in group I. Minimal, maximal, and mean concentrations of HDL-CH appeared to be better in group II. The type and the degree of dyslipidemia were evaluated (according to standards of the Polish Cardiological Society) as follows:

### 1. Mild hyperlipidemia:

T-CH	5.2–6.5 mmol/l;
LDL-CH	3.5–4.5 mmol/l;
TG	2.3–4.6 mmol/l.

### 2. Moderate hypercholesterolemia:

T-CH	>6.5–7.6 mmol/l;
LDL-CH	>4.5–5.5 mmol/l.

### 3. Severe (high) hypercholesterolemia:

T-CH	>7.6 mmol/l;
LDL-CH	>5.5 mmol/l.

The types and prevalence of lipid abnormalities in the two groups of patients studied are shown in Table 2.

One person in each group had a normal T-CH concentration. As indicated in Table 2, qualitative and quantitative burdens of hypercholesterolemia in both groups were very similar, a little higher in pilots (group II: 2 persons more with high hypercholesterolemia). Group II had a higher burden of hypertriglyceridemia. However, it should be noted that this was mild hypertriglyceridemia (TG <4.6 mmol/l). The evaluation of the circulatory system in pilots of super-sonic aircraft (performed upon periodic medical examinations at the Central Military Aeromedical Board) was based on the results of: physical examination, electrocardiogram, echocardiogram, and chest radiogram. T-CH, HDL-CH, and TG concentrations were determined by means of enzymatic methods using reagents from Chiron Diagnostics company (USA) and a CIBA-CORNING Express Plus analyzer. LDL-CH was estimated using Friedewald formula:

$$[\text{LDL} - \text{CH} = \text{T} - \text{CH} - \{(\text{TG}/5) + \text{HDL} - \text{CH}\}]$$

TAS and SOD and GPx activities were determined colorimetrically using a kit from the RANDOX Company (UK). Hemoglobin concentration (for estimation of SOD and GPx activities) was measured in a SERONO-BAKER 9000 hematological analyzer. For all measurements, blood was taken upon fasting from an ulnar vein, either during periodic medical examinations (in pilots) or, in group I patients, 1–2 days before coronarography. Reference values for SOD and GPx activities and TAS were: TAS 1.3–1.7 mmol/l, SOD 1100–1600 U/g Hb, and GPx 27.5–73.5 U/g Hb. Coronarography was done at the Cardiological Clinic of the Central Clinical Hospital of the Military Medical University in Warsaw. Statistical evaluation of the results was carried out by means of the one-sided t-Student's test for unrelated variables, with  $p < 0.05$  denoting significance, utilizing STATISTICA software from Stat-Soft (USA).

**Table 1.** Minimal, maximal and mean values of the components of lipidograms in all three groups of patients.

Parameter mmol/l	Group I N=13			Group II N=15			Group III N=14		
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
T-CH	5.02	10.25	<b>6.96</b>	5.15	8.36	<b>7.03</b>	3.8	5.15	<b>4.48</b>
HDL-CH	0.71	1.24	<b>0.85</b>	0.82	1.89	<b>1.27</b>	0.85	1.63	<b>1.17</b>
LDL-CH	3.08	7.89	<b>4.88</b>	3.5	6.12	<b>4.42</b>	1.99	4.68	<b>2.9</b>
TG	1.1	3.6	<b>2.7</b>	0.66	3.14	<b>1.8</b>	0.55	2.3	<b>1.23</b>

T-CH – total cholesterol; HDL-CH – high density cholesterol; LDL-CH – low density cholesterol; TG- triglycerides.

**Table 2.** Types and prevalence of lipid abnormalities in two groups of patients studied.

Dyslipidemia	Group I N=13		Group II N=15	
Mild hyperlipidemia	2	(15.3%)	2	(13.1%)
Moderate hypercholesterolemia	9	(69.2%)	9	(60%)
Severe (high) hypercholesterolemia	1	(7.6%)	3	(20%)
Mild hypertriglyceridemia	10	(79.6%)	4	(26.6%)

**Table 3.** Minimal, maximal and mean activities of antioxidative enzymes, and TAS, in all groups.

Parameter	Group I N=13			Group II N=15			Group III N=14			p*
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	
SOD U/gHb	346	1002	652.5±204.98	862	1631	1317.33±261.82	1554	2540	1952.78±302.87	<0.05
GPx U/gHb	13.7	26.7	19.13±3.8	21	71.2	42.44±17.68	69.1	98.2	82.55±8.86	<0.05
TAS mmol/l	0.57	1.13	0.97±0.16	0.58	1.69	1.28±0.34	1.35	2.04	1.73±0.2	<0.05

p\* – between all groups.

## RESULTS

In all the patients studied, the activities of antioxidative enzymes and TAS values were decreased. Particularly low SOD activities were noted. In group II, 4 pilots had decreased SOD and GPx activities and TAS and 2 pilots had decreased TAS only. It should be stressed that those were persons having the highest T-CH and LDL-CH concentrations within the group, i.e. 7.1–8.3 (mean: 7.85) mmol/l and 4.94–6.1 (mean: 5.42) mmol/l, respectively. Minimal, maximal, and mean activities of antioxidative enzymes and TAS levels in all groups are shown in Table 3.

As seen in Table 3, both the minimal and maximal values of the respective parameters were decreased in group I, and the mean values were below the reference ranges. In group II, mean SOD and GPx activities and TAS were normal. In group III, the minimal, maximal, and mean activities of SOD and GPx and TAS levels were above the reference ranges. There were statistically significant differences in SOD and GPx activities and TAS between groups I and II, I and III, and also between groups II and III.

## DISCUSSION

According to Steinberg's oxidative theory of atherosclerosis, oxidatively modified LDL-CH molecules are important initiating factors in the atherosclerotic cascade [4]. Under the influence of ROS, produced by endothelial cells, smooth muscle cells, and macrophages, small dense LDL particles that infiltrate the vessel wall become oxidized. LDL oxidation increases their negative charge and their degree of hydration, induces the generation of lipid peroxides, and causes degradation of apolipoprotein B. All the above LDL modifications render it unable to be taken up by cellular receptors for native LDL. Instead, modified LDLs are internalized through so-called scavenger receptors, whose number and activity are not down-regulated by cellular cholesterol content. Macrophages, which take up oxidatively modified LDLs, differentiate into foam cells, which are loaded with free cholesterol. Accumulation of free cholesterol in macrophages causes their disintegration, accompanied by the release of cholesterol into the intercellular space. Simultaneously, oxidatively modified LDLs exert chemotactic influence upon monocytes, thereby increasing their migration into the ves-

sel wall and their differentiation into macrophages. In addition, the motility of macrophages decreases, leading to their accumulation within the vessel wall [5,6]. Therefore it appears that the atherogenic influence of oxidized LDLs may occur at several steps of atherogenesis [7].

The involvement of ROS in the oxidative modification of LDL, an important element of atherogenesis, has drawn attention to the anti-oxidative defense of the organism, including the so-called ROS scavengers. Among natural, so-called primary scavengers, antioxidative enzymes of red blood cells and of the serum play a major role. These are superoxide dismutase, seleno-dependent glutathione peroxidase, glutathione reductase, and catalase [8]. Endothelial damage and increase in polymorphonuclear leukocyte activity which occur during atherogenesis lead to oxidative stress and to an overproduction of reactive forms of oxygen, which in turn exhaust the anti-oxidative pool of the organism [2]. Moreover, the decreased physical activity of coronary disease patients has further detrimental influence on SOD and GPx activities. Additionally, several disturbances in the neurohormonal system (with different grades of intensity) which accompany post-ischemic heart failure intensify oxidative stress [9].

Numerous studies have demonstrated that moderate physical activity has a beneficial effect on the oxido-peroxidative balance. Submaximal physical strain was shown to increase the activity of antioxidative defense enzymes in red blood cells (SOD, GSH-Px, and CAT) with a concomitant decrease in malonyl dialdehyde concentration. The latter indicated a decrease in lipid peroxidation and preservation of anti-oxidative capacity [10–12]. Thus it can be concluded that proper physical training has a highly beneficial influence on mechanisms which defend the human organism against the deleterious influence of reactive oxygen forms. Our results are in agreement with this notion. Considerably decreased physical activity in our group I patients due to advanced coronary disease (class III according to CCS) could be an additional factor lowering SOD and GPx activities and TAS.

In our study, statistically significant differences in SOD and GPx activities and in TAS between groups II and III could be related to the differences in T-CH and LDL-CH between them. The group having increased cholesterol was supposed to have simultaneously lower antioxidative activity. It is worth mentioning that the same kind of abnormalities in TAS values were observed even in a group of children with high lipid concentrations [13]. It was also shown that oxidatively modified (by ROS) low-density lipoproteins (Ox-LDL) and Lp(a)s are more atherogenic than native ones [14]. This is due to their having, in addition to cholesterol esters, high amounts of polyunsaturated fatty acids (PUFAs), which are particularly prone to be modified by various forms of active oxygen. Thus, the more LDL-cholesterol the larger the amount of substrate available for nonenzymatic autooxidation by ROS and the higher the risk of atherosclerosis [15]. However, since cases of clinically advanced atherosclerosis in persons with relatively low hyperlipidemia do exist, the degree of dyslipidemia cannot be the sole factor determining the development of atherosclerosis [1].

Here we demonstrated that in persons with advanced coronary atherosclerosis the activities of SOD and GPx and

TAS are significantly lower compared with clinically healthy persons with similar dyslipidemia. This may mean that the atherosclerotic process (including changes in endothelium and coronary blood flow, ischemia, and others) has its own independent effect lowering SOD and GPx activities and TAS that is greater than the isolated influence of hypercholesterolemia. This is harmonious with the fact that in more than half of group II pilots with increased cholesterol there was normal (within the range of reference values) activity of antioxidative enzymes and normal TAS. However, an unanswered question remains as to whether such a low antioxidative activity in persons with coronary disease (group I) is the cause or merely the effect of underlying atherosclerotic changes.

Since atherosclerosis is a chronic process, single measurements of lipids, SOD, GPx, and TAS as well as other risk factors at a particular stage do not allow inferences of their past influence on the disease's progression. However, the considerable decrease in the activities of SOD and GPx and in TAS level in all group I men suggests that a weakening or destruction of the antioxidative barrier of the organism had been strongly causally related to coronary atherosclerosis and hypercholesterolemia.

Our studies [3] indicate that the activity of antioxidative enzymes is modulated by T-CH concentration, in particular LDL-CH. The activities of SOD and GPx and levels of TAS correlated negatively with T-CH and LDL-CH concentrations. Although the involvement of hyperlipidemia in lowering (exhausting) the enzymatic antioxidative pool in persons with atherosclerosis is clear, it cannot be quantitatively estimated (as a percentage). Among our group of pilots there were persons with very high cholesterol in whom SOD and GPx activities and TAS levels were higher than those of patients with coronary disease and moderately increased LDL-CH (the influence of damaged endothelium). The conditions favoring ROS overproduction with concomitant exhaustion of enzymatic antioxidative pool discussed above and the results presented here point to a major role of the atherosclerotic process (independently of hypercholesterolemia) in lowering the activities of SOD and GPx and levels of TAS. It is difficult to evaluate to what extent a decrease in the respective values can participate in the initiation of the atherosclerotic cascade. Nevertheless it is evident that enzymatic antioxidative activity is decreased in persons with coronary disease, which can facilitate and augment the atherosclerotic process. Whether or not the measurements of antioxidative activities of SOD and GPx and levels of TAS can be used in the diagnosis of coronary atherosclerosis in persons with hypercholesterolemia requires further studies.

## CONCLUSIONS

1. The activities of SOD and GPx and levels of TAS are lower in men with advanced coronary atherosclerosis than in age-matched clinically healthy men with similar dyslipidemia. They are even further decreased compared with clinically healthy men with normal lipidemia.
2. Decreases in SOD, GPx, and TAS levels in men with advanced coronary atherosclerosis are more pronounced than the degree of hypercholesterolemia or hypertriglyceridemia.

3. If hyperlipidemia and the activity of antioxidative enzymes and level of TAS are considered without reference to other risk factors of atherosclerosis, it appears that the decrease in SOD, GPx, and TAS may play a role more important in the development of the atherosclerotic process than isolated increases in free cholesterol or triglycerides.

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