Blood oxidative stress markers in the 32nd Bulgarian Antarctic expedition participants

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Abstract

Antarctic expeditions are associated with significant stress for participants related to long journeys, work in adverse environmental conditions, and social contact changes resulting in oxidative stress (OS) at the molecular level. This study aimed to evaluate the blood serum OS of the participants in the 32nd Bulgarian Antarctic Expedition (2023-2024) and related trace elements iron, copper, zinc, and selenium concentrations, lipid profile, and blood parameters. Blood parameters and trace element concentrations were measured in a clinical laboratory. OS indicators were measured spectrophotometrically using kits. The results showed decreased antioxidant enzyme activities and increased glutathione concentration after the expedition in the blood serum from both females and males. No statistical differences in the values of OS parameters between males and females were observed. However, the mean serum total antioxidant capacity after the expedition in males was two times higher than in females. In males, a statistically significant lower erythrocyte sedimentation rate ($p<0.05$), ferritin ($p<0.001$), and zinc ($p<0.01$) levels, catalase ($p<0.001$) and superoxide dismutase ($p<0.05$) activities were observed after the expedition. No correlation between enzyme activities and the studied trace element concentrations was found. In conclusion, it can be assumed that the expeditioners' OS was higher before the expedition, likely because of psychological and physical stress related to overcoming preparation and logistics problems. After the expedition, OS has subsided. In order to maintain good health and increase physical fitness, it could be recommended that polar expeditioners receive dietary supplements, micronutrients, and vitamins in the setting of rational nutrition, especially before an expedition.

Key words: Antarctics, antioxidants, human, lipid peroxidation, serum trace elements, Livingston Island, Kliment Ohridski Station, Austral summer

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Introduction

The Bulgarian polar expeditions include stays of varying lengths at the "St. Kliment Ohridski" research base on Livingston Island during the Antarctic summer. The participants in these expeditions are subjected to significant psychological and physiological stress associated with a long journey with changing different time zones, away from the family environment, in a closed society, and adverse environmental conditions. Psychological stress in Bulgarian polar explorers has been studied, and the results showed significantly higher levels of perceived stress and situational anxiety at the beginning of the expedition compared to the end (Domuschieva-Rogleva and Iancheva 2017, Domuschieva-Rogleva et al. 2017, Iancheva et al. 2018, 2023). Several publications have shown that participants in such expeditions usually experience sleep disturbances, impaired cognitive abilities, depressed mood, interpersonal tension, and conflicts (Palinkas and Suedfeld 2008, Spinelli and Werner 2022, Johnsen and Gjeldnes 2023). Work in polar conditions is associated with staying outdoors at low temperatures, often covering long distances when exploring different sites and collecting samples. Exposure to cold, even for a short time, reduces physical performance (Stocks et al. 2004). During physical work at low temperatures, there is a decrease in maximal strength, muscle endurance, the efficiency of the cardiovascular system, a significant increase in metabolic costs, and, as a consequence, depletion of energy reserves (Makinen 2007, Oksa et al. 2004, Stocks et al. 2004, Halsey and Stroud 2012, Parent et al. 2018).

The body's adaptation to stressogenic factors of any nature passes through the stages of the so-called general adaptation syndrome formulated by Selye (1950). At the cellular level, stress causes a change in the balance between pro-oxidant and antioxidant processes. Pro-oxidant processes are related to generating reactive oxygen species (ROS). The latter can oxidatively modify biomolecules (lipids, proteins and nucleic acids), disrupting their function and thus leading to pathology (Halliwell and Gutteridge 1999). Under normal physiological conditions, ROS is effectively eliminated by the antioxidant defense system that protects the body from oxidative damage. With excess ROS formation and/or impaired antioxidant protection, the latter's capacity may be insufficient to prevent ROS production or neutralize them, leading to oxidative stress (OS). It should be considered that excessive ROS production may not only have detrimental effects but also be an integral part of a series of signaling events triggered adaptive responses (Margaritelis et al. 2018).

Signs of OS have been found in blood samples from polar expeditions participants'. Mitochondria isolated from leukocytes of participants in long-term Antarctic expeditions show OS-related ultrastructural changes and dysfunctions (Rozova and Moiseyenko 2013, Moiseyenko et al. 2016). A significantly increased level of lipid peroxidation (LPO) and reduced activity of the antioxidant enzyme superoxide dismutase (SOD) were found in the serum of participants in Ukrainian Antarctic expeditions (Moiseyenko et al. 2016). The effectiveness of antioxidant protection is essential for maintaining the pro/antioxidant balance. Some trace elements such as iron (Fe), copper (Cu), zinc (Zn), and selenium (Se) are involved in the catalytic action or the stabilization of the active center of important antioxidant enzymes. Copper and zinc ions are responsible for the activity of SOD, with Cu^{2+} participating in the enzyme catalytic center and Zn^{2+} stabilizing the molecule. Catalase activity is Fe^{2+} dependent. Five out of the eight identified isoforms of the enzyme glutathione peroxidase (GPx) are Se-dependent (Morón and Castilla-Cortázar 2012).

A change in the concentrations of the trace elements iron, copper, and zinc was found in the blood serum of participants in an Antarctic wintering expedition (Moiseyenko et al. 2016). Participants in the Czech Polar Expedition to James Ross Island during the Antarctic summer had significantly increased concentrations of the transferrin (a biomarker of body iron status) at the end of the stay, relative to both pre-departure and mid-stay levels (Žákovská and Zezulová 2019). According to dietary studies of participants in the Australian Antarctic Expeditions from 2004 to 2010, females had dietary iron intakes below recommendations (73±37% of RDI, $p<0.001$) (Iuliano and Ayto 2015). The serum zinc concentration in the Ukrainian expeditions participants increased after the expedition, while the copper content decreased (Moiseyenko et al. 2016). Such changes in the microelements in the blood during the expedition are probably related to the organism's adaptation during an extended stay in the extreme conditions of Antarctica. Maintaining an optimal level of trace elements can increase the body's antioxidant protection and thus improve the health and tolerance to harsh climatic conditions, physical loads, and psychological stress of participants in polar expeditions.

Thus, the present study aimed to evaluate the levels of oxidative stress and the concentration of the trace elements iron, copper, zinc, and selenium in the blood serum of participants in the 32nd Bulgarian Antarctic Expedition (2023-2024) and their changes after the long journey, stay and work in the conditions of the polar research base on Livingston Island. Scientific research obtaining new information about the characteristics and mechanisms of human adaptation in the extreme conditions of the Antarctics is important for expanding knowledge in the field of polar medicine.

Material and Methods

Participants

The studied individuals were participants in the 32nd Bulgarian Antarctic Expedition. There were 26 people (21 males and 5 females). The average age of females and males was 47.4±10.87 years (between 26 and 59 years) and 41.71±7.17 years (between 30 and 55 years), respectively. The participants left by plane from Sofia and, after a different number of transfers, reached Argentina, from where they reached the Bulgarian Antarctic base St. Kliment Ohridski on Livingstone Island (62.6°S, 60.5°W, 17 m above sea level) with the Bulgarian scientific research vessel Sts. Cyril and Methodius (RSV 421) through the Drake Passage for 3 days. The Bulgarian polar base operates seasonally from November to the end of March. With a limited capacity, the base accommodates scientists in rotating groups, with individuals arriving and departing based on the duration and nature of their scientific tasks. For this study, participants were selected from those who spent 30 days at the base between 01 December 2023 and 01 March 2024. The climate of Livingstone Island is marked by average summer temperatures ranging from -3°C to 2°C and almost 100% relative humidity from December to March (Fig. 1). Typical weather conditions include strong winds, overcast skies, frequent fog, and a mix of rain and snow. Fieldwork outside the base premises is largely dependent on weather conditions and the nature of the scientific work. The average duration of fieldwork for participants in this study was 4.5±1.5 hours. Before departure from Bulgaria to Antarctica and no later than three days after their return back, participants had venous blood drawn, and anthropometric measurements were taken. All of them were informed about the aims and objectives of the study and signed an informed consent to participate.

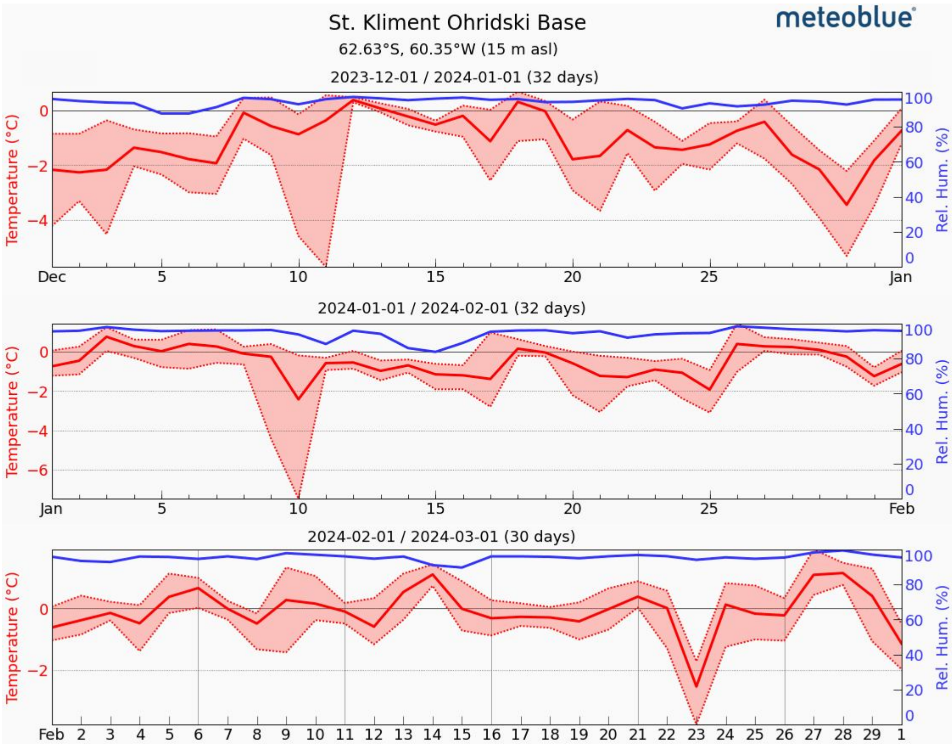

Fig. 1. Air temperatures (mean ± standard deviation) and relative humidity at the Bulgarian Antarctic Base "St. Kliment Ohridski" from December 2023 to March 2024 (*Source of data*: Meteoblue, Switzerland[1]).

Haematological analysis

Peripheral blood samples were collected by a medical laboratory technician. In a certified clinical laboratory, the number of White blood cells (WBC) and Erythrocytes sedimentation (ESR) were analyzed in blood, and C-reactive protein (CRP), Ferritin, Triglycerides (Tg), Low-density lipoprotein cholesterol (LDL), and High-density lipoprotein cholesterol (HDL), as well as the concentration of iron, copper, zinc and selenium were measured in serum. The haematological parameters were analyzed using Sysmex XN 1000 fully automated 5-part differential haematology analyser (Sysmex America, Inc., USA). The biochemical parameters were measured using Cobas® 6000 analyzer (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Trace elements were assessed at MVZ Labor Dr. Limbach (Germany) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (PlasmaQuant MS Elite S, Analytik Jena, Germany).

Oxidative stress assay

The OS markers were measured spectrophotometrically in serum obtained after blood coagulation at room temperature and centrifugation at 3000 rpm/15 min. The following kits manufactured by Elabscience Bionovation Inc. (Houston, Texas, USA) were used: for lipid peroxidation E-BC-K298-M Thiobarbituric Acid Reactants (TBARS) Colorimetric Assay Kit; for total glutathione E-BC-K097-M Total Glutathione (T-GSH)/Oxidized Glutathione (GSSG) Colorimetric Assay Kit; for total antioxidant capacity E-BC-K136-M Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit; for superoxide dismutase activity E-BC-K020-M Total Superoxide Dismutase (T-SOD) Activity Assay Kit (WST-1 Method); for catalase activity E-BC-K031-M Catalase (CAT) Activity Assay Kit; and for glutathione peroxidase activity E-BC-K096-M Glutathione Peroxidase (GSH-Px) Activity Assay Kit. The manufacturer's instructions were strictly followed during the measurements.

Statistical evaluation of the results

Descriptive statistics, Shapiro test for normal distribution, Student's t-test for dependent and independent data, Mann-Whitney and Willcoxon tests, and Correlation statistics were provided by SPSS 26 (IBM, USA). The significance of differences in mean values was assessed based on the results of the Shapiro test of normality. If the data followed a normal distribution, Student's t-test was used. For non-normal distributions, the non-parametric Mann-Whitney or Wilcoxon tests were applied. Average values were presented with ± Standard deviation (SD) in text and with ± Standard Error of measurement (SE) in graphics.

Results

The anthropometric characteristics of the participants showed that females had an average height of 160.80 ± 6.53 cm, average weight of 69.82 ± 18.88 kg, and average BMI of 26.80 ± 6.22 kg.m^{-2}. One female had a BMI greater than 25.0 kg.m^{-2} (overweight), and two had a BMI greater than 30.0 kg.m^{-2} (first degree of obesity). The average height of males was 178.10 ± 5.59 cm, the average weight was 91.8 ± 19.59 kg, and the average BMI was 29.00 ± 6.14 kg.m^{-2}. Seven males had a BMI greater than 25.0 kg.m^{-2} (overweight), and nine had a BMI greater than 30.0 kg.m^{-2} (different degrees of obesity). No statistically significant differences in both sexes before and after the expedition in the mean values of weight (Females 69.1 ± 16.98 kg *vs* 69.05 ± 17.74 kg; Males 91.43 ± 18.79 kg *vs* 92.56 ± 19.71 kg) and BMI (Females 26.55 ± 5.6 kg.m^{-2} *vs* 26.57 ± 6.09 kg.m^{-2}; Males 28.84 ± 5.9 kg.m^{-2} *vs* 29.17 ± 6.07 kg.m^{-2}) were observed.

Table 1 presents the mean values of all investigated indicators for females and males pooled from the pre- and post-expedition measurements, along with the estimation of the distribution according to the Shapiro-Wilk test and the significance of gender differences. Regarding the oxidative stress parameters (the activities of catalase (Cat), glutathione peroxidase (GPx), and superoxide dismutase (SOD), antioxidant capacity (AOC), malondialdehyde (MDA) (as a marker for lipid peroxidation), and total glutathione (GSH) no gender differences were found. The obtained data indicated that the mean value of serum Ferritin concentration in males was almost three times higher than in females (301.95 ± 326.72 ng mL^{-1} *vs* 110.10 ± 84.24 ng mL^{-1}, $p < 0.001$). Although serum concentrations of Zn and Se showed no statistically significant differences between males and females, a significant difference ($p < 0.001$) in serum Cu concentration was found between females and males (1209.8 ± 180.05 µg L^{-1} and 1007.83 ± 134.32 µg L^{-1} respectively). Significant differences were established in Tg ($p < 0.01$) and HDL ($p < 0.001$). While similar reliable differences in HDL concentrations have been observed by other authors (Russo et al. 2015) and most clinical laboratories set different reference values for males and females, for Triglycerides there are standard reference ranges from 0 to 1.71 mmol L^{-1} independent of the sex. For the other indicators included in the analysis (CRP, WBC, ESR, and LDL), for which the reference limits for males and females are equal, no significant differences were found.

Indicator	**Females** (n=10) (Mean ± SD)	**Males** (n=42) (Mean ± SD)	**Distribution**
Cat ($U.mL^{-1}$)	64.62 ± 33.64	88.11 ± 58.92	
GPx (U)	708.94 ± 392.39	770.94 ± 257.07	
SOD (U)	46.56 ± 3.69	47.33 ± 7.62	
AOC ($U.mL^{-1}$)	8.46 ± 7.00	10.86 ± 5.08	Normal
MDA (TBARS $\mu mol.L^{-1}$)	3.02 ± 1.23	3.46 ± 1.68	Normal
GSH ($\mu mol.L^{-1}$)	8.54 ± 0.65	8.31 ± 1.21	Normal
CRP ($mg.L^{-1}$)	2.58 ± 3.22	4.28 ± 11.05	
WBC ($g.L^{-1}$)	5.76 ± 0.81	7.09 ± 2.78	
ESR ($mm.h^{-1}$)	10.9 ± 9.5	7.86 ± 5.96	
Ferritin ($ng.mL^{-1}$)	110.1 ± 84.24	301.95 ± 326.72***	
Zinc ($\mu mol.L^{-1}$)	12.61 ± 1.21	12.96 ± 1.98	Normal
Selenium ($\mu g.L^{-1}$)	93.2 ± 19.49	96.43 ± 9.85	Normal
Copper ($\mu g.L^{-1}$)	1209.8 ± 180.05	1007.83 ± 134.32***	Normal
Tg ($mmol.L^{-1}$)	0.87 ± 0.34	1.51 ± 0.84**	
LDL ($mmol.L^{-1}$)	3.46 ± 0.8	3.65 ± 0.92	Normal
HDL ($mmol.L^{-1}$)	1.99 ± 0.28	1.32 ± 0.27***	Normal

Table 1. Mean values of the studied indicators for females and males from pre- and post-expedition measurements and the significance of gender differences. *Note*: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$ *vs* Female. The distribution assessment was according to the Shapiro-Wilk test. The difference between mean values was verified with the Student's t-test for independent samples in case of normal distributions or with the nonparametric Mann-Whitney test.

Table 2 presents the correlation matrix of the studied parameters. The table presents only the groups of parameters between which significant correlation coefficients were established. The significant differences are marked with asterisks.

n=52	**Age**	**Sex**	**Cat**	**SOD**	**MDA**	**GSH**	**CRP**	**WBC**	**ESR**	**Cu**	**Tg**
Cat	-0.074	0.168	1								
GPx	-0.184	0.087	0.053								
SOD	0.028	0.044	0.386**	1							
GSH	-0.031	-0.084	-0.527**	-0.389**	-0.396**	1					
WBC	0.023	0.206	0.07	-0.043	0.143	-0.036	0.719**	1			
ESR	0.331*	-0.179	0.171	0.172	0.175	-0.301*	0.552**	0.258	1		
Zn	0.121	0.075	0.033	-0.058	0.183	-0.113	-0.169	0.06	-0.123		
Cu	0.384**	-0.492**	-0.068	0.118	0.121	-0.023	0.274*	0.166	0.428**	1	
Tg	0.113	0.320*	0.239	0.051	0.313*	-0.164	0.148	0.326*	0.113	-0.119	1
LDL	0.350*	0.088	0.082	0.196	0.104	-0.075	-0.067	-0.062	0.118	0.376**	0.124
HDL	0.172	-0.709**	-0.17	0.076	-0.162	0.017	-0.105	-0.205	-0.04	0.344*	-0.606**

Table 2. Correlation matrix of tested parameters. *Note*: * – Correlation is significant at the 0.05 level (2-tailed); ** – Correlation is significant at the 0.01 level (2-tailed).

Table 3 presents the average values of the investigated blood indicators in females before and after the Antarctic expedition. There were no significant differences between the studied parameters, probably due to the small number of female participants (n = 5), differences in age, and the type of work engagement during the expedition. However, we should note the increase in the inflammation indicators CRP, ESR, and ferritin, which are also considered inflammatory markers (Kell and Pretorius 2014) in the blood serum samples after the expedition.

Parameter	**Before** Mean ± SD	**After** Mean ± SD	**Distri-bution**	**Significance of differences**$^{\&}$	**Correlation coefficient**$^{\#}$	**Significance of correlation**
CRP (mg.L^{-1})	1.77 ± 1.77	3.40 ± 4.31		0.686	0.502	0.389
WBC (g.L^{-1})	5.74 ± 0.82	5.78 ± 0.89	Normal	0.954	-0.445	0.452
ESR (mm.h^{-1})	8.00 ± 6.04	13.80 ± 12.07	Normal	0.458	-0.463	0.433
Ferritin (ng.mL^{-1})	97.20 ± 67	123.00 ± 104	Normal	0.196	0.997	0.0001***
Zinc (μmol.L^{-1})	12.72 ± 1.00	12.50 ± 1.51	Normal	0.759	0.340	0.575
Selenium (μg.L^{-1})	89.60 ± 17.6	96.8 ± 22.63	Normal	0.230	0.869	0.056
Copper (μg.L^{-1})	1192.0 ± 153	1227.6 ± 221	Normal	0.512	0.887	0.045*
Triglycerides (mmol.L^{-1})	0.81 ± 0.27	0.92 ± 0.42	Normal	0.306	0.873	0.053
LDL (mmol.L^{-1})	3.39 ± 0.72	3.52 ± 0.97	Normal	0.639	0.815	0.093
HDL (mmol.L^{-1})	2.11 ± 0.20	1.88 ± 0.31	Normal	0.173	0.264	0.668

Table 3. Changes in and correlations between measured parameters of female participants (n = 5) before and after the Antarctic expedition. *Note*: * – $p < 0.05$; *** – $p < 0.001$; $^{\&}$ – The distribution assessment was according to the Shapiro-Wilk test. The difference between mean values was verified with the Student's t-test for dependent samples in case of normal distributions or with the nonparametric Willcoxon test. $^{\#}$ – Correlations between measured parameters before and after the expedition.

The mean values of the men's blood indicators before and after the Antarctic expedition, as well as the type of their distributions, significant differences, and correlations, are presented in Table 4. Statistically significant differences were found in the mean values of ESR ($p < 0.05$), Ferritin ($p < 0.001$), and Zn ($p < 0.01$). All these parameters decreased in the samples after the expedition.

Parameter	Before Mean ± SD	After Mean ± SD	Distribution	Significance of differences$^{\&}$	Correlation coefficient$^{\#}$	Significance of correlation
CRP ($mg.L^{-1}$)	5.69 ± 15.30	2.86 ± 3.46	n.e.	0.768	- 0.016	0.946
WBC ($g.L^{-1}$)	7.17 ± 3.53	7.01 ± 1.84	n.e.	0.654	0.748	0.0001***
ESR ($mm.h^{-1}$)	9.71 ± 7.23	6.00 ± 3.63	n.e.	0.022*	0.122	0.599
Ferritin ($ng.mL^{-1}$)	346.70 ± 379	257.21 ± 266	n.e.	0.001***	0.981	0.0001***
Zinc ($\mu mol.L^{-1}$)	13.58 ± 1.71	12.33 ± 2.07	Normal	0.009**	0.460	0.036*
Selenium ($\mu g.L^{-1}$)	95.14 ± 10.16	97.71 ± 9.60	Normal	0.276	0.433	0.050*
Copper ($\mu g.L^{-1}$)	1022.19 ± 136	993.48 ± 133	Normal	0.301	0.580	0.006**
Tg ($mmol.L^{-1}$)	1.64 ± 0.93	1.39 ± 0.73	n.e.	0.135	0.585	0.005**
LDL ($mmol.L^{-1}$)	3.70 ± 0.88	3.60 ± 0.98	Normal	0.474	0.776	0.0001***
HDL ($mmol.L^{-1}$)	1.35 ± 0.27	1.28 ± 0.27	Normal	0.128	0.725	0.0001***

Table 4. Changes in and correlations between measured parameters of male participants (n = 21) before and after the Antarctic expedition. *Note*: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; $^{\&}$ –The distribution assessment was according to the Shapiro-Wilk test. The difference between mean values was verified with the Student's t-test for dependent samples in case of normal distributions or with the nonparametric Willcoxon test. $^{\#}$ – Correlations between measured parameters before and after the expedition; n.e. – not evaluated.

The changes in the OS markers are presented in Fig. 2. In the samples collected after the expedition in females, a large, albeit insignificant, decrease of Cat and GPx activities and AOC was found, and in males, statistically significant decreases were observed in the mean values of Cat ($p < 0.001$), SOD ($p < 0.05$), and GSH ($p < 0.001$) (Fig. 2 A, C, F). There were no statistical differences in the values of OS parameters between males and females. However, the AOC in the samples after expedition in males was two times higher than in females (Fig. 2 D).

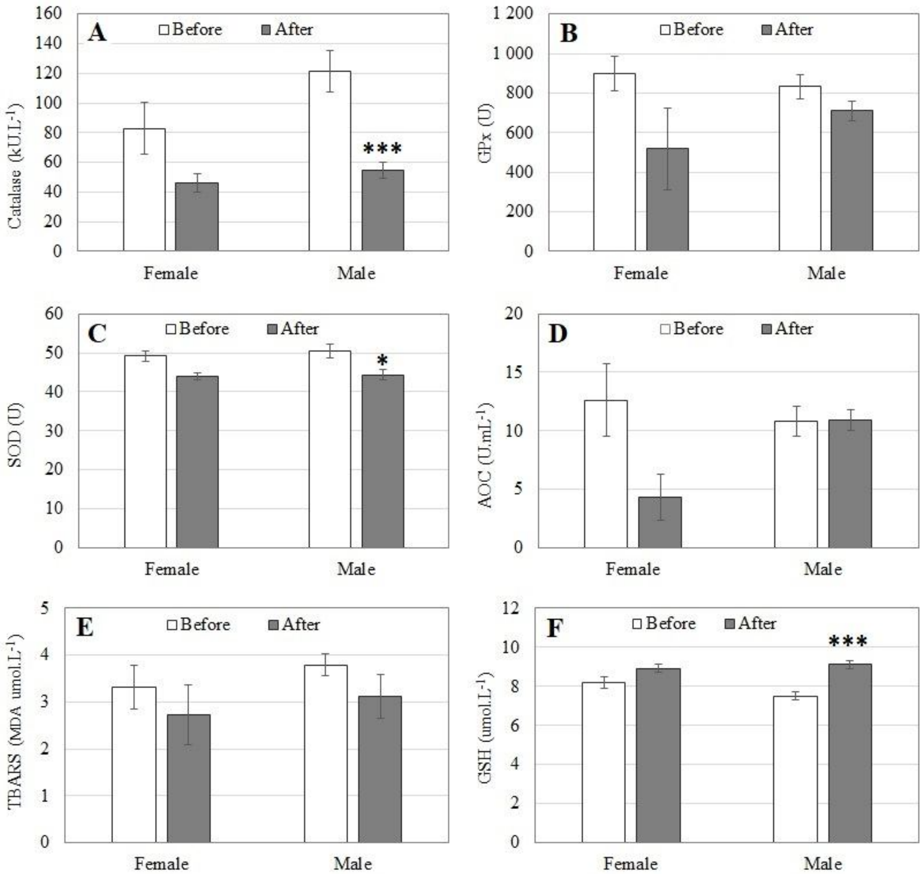

Fig. 2. Changes in oxidative parameters of males and females after participation in Antarctic expedition.

Discussion

The aim of the present study was to evaluate the OS and the concentration of the trace elements (iron, copper, zinc, and selenium) in the blood serum of participants in the 32nd Bulgarian Antarctic Expedition (2023-2024) comparing the pre- and post-expedition levels and differences between females and males. These data are interesting for revealing the human adaptation processes in polar conditions.

A characterization of anthropometric indicators of the participants of the polar expedition was made, and a very high rate of overweight and obesity according to the calculated BMI was found. Over half of females (3 of 5) and males (16 of 21) had weight problems, probably because most polar explorers are professional scientists who often have low physical activity. Premkumar and Sable (2012) reported that only 35% of participants in the 30th Indian Scientific Expedition to Antarctica had a normal BMI, 40% were overweight and 25% were obese. Moreover, a progressive rise in the mean BMI during the expedition has been noted (Premkumar and Sable

2012). Mean BMI over 25 kg.m^{-2} (overweight) and exceeded mean energy intake above the average recommended intake per day of Czech Antarctic expedition were also measured (Žákovská and Zezulová 2016). Given that BMI is a crucial predictor of both general and cardiovascular morbidity, future research should focus on studying and optimizing dietary regimens and physical activity, particularly in the context of pre-expedition preparation and during expeditions. The adipose tissue, especially in the visceral compartment, was considered an active endocrine organ releasing adipocytokines. Based on the complex interplay between adipocytokines, obesity is characterized by chronic low-grade inflammation with permanently increased OS. In turn, the over-accumulation of OS damages cellular structures together with the inhibition of antioxidant enzymes, leading to the development of obesity-related complications (Marseglia et al. 2014).

This study also examined the serum iron and copper levels because of their dual role in redox homeostasis. Both iron and copper are essential for various biological processes but can become harmful when present in excess or in free, unbound form. On the one hand, in excess in the bloodstream or tissues, they can catalyse the formation of reactive oxygen species (ROS) by the Fenton and Fenton-like reaction mechanism, leading to OS. The latter damages cellular components like lipids, proteins, and DNA, and is implicated in various diseases. On the other hand, they are a component of antioxidant enzymes (catalase and superoxide dismutase) and are essential for preventing OS development. Our results, which ascertained significant differences in serum ferritin concentration between males and females (Table 1) are expected since other authors found similar gender differences, and they are close to our reported values of 80.4±1.64 ng.mL^{-1} for females *vs* 201.55±3.6 ng.mL^{-1} for males ($p < 0.0001$) (Han et al. 2014). It should be noted that the mean values were quite high, and some male participants reached extreme values. Given the high mean BMI of the participants, we can assume that the higher intake of iron-containing foods is also likely to be important. Therefore, the reliable decrease of Ferritin concentrations in males after the end of the expedition (from 346.7±379.22 to 257.21±266.1 ng.mL^{-1}; $p < 0.001$) (Table 4) may be due to the rational way of eating at the base. The sex-related differences in Cu concentration (higher in females) (Table 1) have also been reported (Quinn et al. 2011, Nan and Bai 2022). A positive correlation between serum copper concentration and inflammation indicators CRP and ESR (Table 2) was also noted (Bui et al. 2012, Emokpae and Fatimehin 2020, Yuan et al. 2020). In addition, recent studies have linked copper to the regulation of inflammatory processes. Thus, according to Chang and Brady (2023), copper ions can drive inflammation via a mitochondrial signaling pathway that regulates the epigenetic states of immune cells, and this pathway could offer a new route for treating inflammatory diseases. The serum copper significantly correlates positively with the lipid profile indicators LDL-Cholesterol and HDL-Cholesterol (r=0.376 and 0.344) (Table 2). Other authors have explored this correlation in detail. Thus, according to Song et al. (2018), high serum copper was associated with elevated serum concentrations of total cholesterol and HDL cholesterol and increased risks of high LDL cholesterol dyslipidemia. However, our results did not find correlations between the measured iron and copper concentrations in blood serum, enzyme activities, and other OS indicators.

Another result that should be noted is the established significantly higher HDL cholesterol concentration in females than in males (Table 1). This sex-related difference is a long-known fact that partly explains the higher incidence of cardiovascular diseases in males. Similarly, a large-scale population survey examining the association between HDL cholesterol and biological sex in 8,631 females and 10,690 males established a significant gender difference, as the largest one was 0.40 mmol L^{-1} (Davis et al. 1996).

In terms of OS indicators, we measured the MDA level as an indicator of lipid peroxidation (LPO), the GSH concentrations and the antioxidant enzymes SOD, Cat, and GPx. Some correlations between themselves and between them and the other measured biochemical parameters were established. The estimated significant correlation between MDA and one of the indicators of the lipid profile Tg ($r = 0.313$; $p<0.05$) (Table 2) can be explained by the fact that the unsaturated fatty acids in the composition of Tg are the main substrate for the formation of MDA during lipid peroxidation (Salekeen et al. 2022). A similar positive correlation was reported before (Loeper et al. 1983, Ledwozyw et al. 1986). This result was explained by the established inhibition of lipoprotein lipase activity by the lipid peroxides, thereby leading to plasma triglyceride hydrolysis decrease (Wada et al. 1983). The negative correlations of GSH with the activity of the antioxidant enzymes SOD and Cat (Table 2), in our opinion, indicate that a high concentration of GSH is a factor and indicator of a low OS level and reduced induction of enzyme antioxidant protection. This role of GSH is also confirmed by the negative correlation between serum GSH concentration and the lipid peroxidation product MDA. GSH is a molecule that actively participates in counteracting the OS. This tripeptide is present in all tissues, and its synthesis and/or regeneration in both the heart and the liver is essential in preventing or reducing harmful ROS effects in cardiovascular diseases (Matuz-Mares et al. 2021). It has been found that patients with chronic diseases had lower GSH levels than healthy participants. GSH is inversely associated with multimorbidity development and is an essential biomarker of multisystem dysregulation (Pérez et al. 2020). Numerous investigations have suggested that disruption of the GSH system is a critical element in the pathogenesis of myocardial injury. The analysis of the activation strategies of the GSH system identifies promising herbal and synthetic drugs that might be used as new therapeutic approaches (Tan et al. 2023). The decrease in the activity of the antioxidant enzymes CAT and SOD (significant) and GPx (although insignificant) (Fig. 2) can be due, on the one hand, to a drop in the OS level after the expedition, which is also indicated by the significant increase in GSH and the decrease in one of indicators of inflammation - ESR. It is unlikely that the reduced activity of SOD is due to the reduced serum concentration of Zn, since Zn is involved in the stabilization of the SOD molecule and Cu is involved in the catalytic centre of the enzyme.

The design of our study allows us to judge the oxidative stress of the participants only before and after the Antarctic expedition. From the obtained data, we can assume that in the days immediately before the expedition, people were subjected to psychological and physical stress related to overcoming problems of organization and logistics. After the expedition, this stress seems to have subsided, and we observed reduced oxidative stress established through decreased activity of antioxidant enzymes and increased glutathione level. In order to obtain a more accurate picture of oxidative stress in the conditions of polar expeditions, samples should also be taken during the intermediate stages of the expeditions. However, we can argue that GSH is an important indicator of antioxidant protection and a positive indicator of health status. Its serum concentrations can be monitored even before participation in expeditions and can be positively influenced by nutritional supplements such as N-acetylcysteine (NAC) (Tenório et al. 2021) to prevent morbidity away from specialized medical centers. NAC is also used as a sport supplement for its ability to modulate exercise-induced oxidative damage through its antioxidant actions and maintenance of glutathione homeostasis, which makes it a strategy to improve physical performance (Fernández-Lázaro et al. 2023). It could be recommended that participants receive dietary supplements of micronutrients and vitamins in doses equivalent to the RDA especially before an expedition.

In conclusion, it can be assumed that the blood OS of the participants in the 32nd Bulgarian Antarctic expedition was higher before the expedition, likely because of psychological and physical stress related to overcoming preparation and logistic problems. After the expedition, this stress has subsided. To maintain good health and increase physical fitness, it may be recommended that expedition participants take nutritional supplements, minerals, and vitamins, especially before an expedition.

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