Original Article

Non-enzymatic antioxidant blood plasma profile in the period of high training loads of elite speed skaters in the altitude

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ABSTRACT

At the altitude, hypoxia and training load are key factors in the development of oxidative stress. Altitude-induced oxidative stress is developed due to the depletion of antioxidant potential. In the current study, we examined the non-enzymatic antioxidant profile of blood plasma in 7 males and 5 females specializing in speed skating at a 21-day training camp at 1 850 m above sea level. Training included: cycling, roller skating, ice skating, strength training, and special training. At the start point and the endpoint, total hemoglobin mass (tHb-mass), hemoglobin concentration, and circulating blood volume were determined. Antioxidant profiles, hypoxic doses, hypoxic impulses, and training impulses were assessed at 3, 6, 10, 14, and 18 days. Antioxidant profiles consisting of "urate" and "thiol" parts were registered with chemiluminometry. In the training dynamics, antioxidant parameters changed individually, but in total there was a decrease in the "urate" capacity by a factor of 1.6 ($p = 0.001$) and an increase in the "thiol" capacity by a factor of 1.8 ($p = 0.013$). The changes in "urate" capacity positively correlated ($r_S = 0.40$) and the changes in "thiol" capacity negatively correlated ($r_S = -0.45$) with changes in tHb-mass. Both exercise and hypoxic factors affect the antioxidant parameters bidirectionally. They correlated with a decrease in thiol capacity and with an increase in urate capacity. The assessment of the non-enzymatic antioxidant profile can be a simple and useful addition to screening the reactive oxygen species homeostasis and can help choose the personalized training schedule, individualize recovery and ergogenic support.

Introduction

Reactive oxygen species (ROS) play a multivalent role depending mainly on concentration.[1] Excessive ROS are considered toxic and lead to pathological oxidative stress.[2] At low physiological concentrations, ROS act as signaling molecules, providing 'an oxidative interface' in controlling the expression of many genes[3] and adaptation.[4,5] Redox homeostasis is a part of oxidative homeostasis, which is associated with hydrogen peroxide and its reactions with thiol compounds.[6–8] Oxidative and redox balance is a fundamental condition for normal vital functions.[9,10]

The development of oxidative stress during exercise has been proven by numerous studies.[11–16] While moderate aerobic exercise training leads to a decrease in oxidative stress, intense physical activity leads to the opposite result.[17,18] Although elite athletes have a very strong antioxidant defense system, oxidative damage may cause muscle fatigue, injury, and overtraining.[19,20] In training load, changes in ROS homeostasis are very variable, since they are determined by a very large number of factors (type of sport, training regimen, the physical state of an athlete, nutritional support, etc.). In general, exercise-induced oxidative stress is expressed as an increase in carbonylated proteins, biomarkers of lipid oxidative stress, a decrease in reduced glutathione, an increase in total antioxidant capacity, and an increase in pro-inflammatory interleukins.[21] However, the increased ROS level is needed to regulate exercise-related adaptation.[22]

Oxidative reactions are controlled by a complex and heterogeneous antioxidant network.[5] Antioxidant excess ('antioxidative stress') may be also harmful and may develop in healthy people due to excessive intake of antioxidant supplements.[23,24] In sportsmen, antioxidant supplements are an obvious way to neutralize oxidative stress, but they may have a negative effect disrupting metabolic adaptation to physical stress.[25] The general consensus is that the necessary level of exogenous antioxidants can be provided by a balanced, well-varied diet. In high-intensity and/or exhausting exercises, antioxidant supplements should be recommended

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List of abbreviations

ABAP	2,2'-azo-bis(2-amidinopropane) dihydrochloride
arb.u.	arbitrary units
CBV	circulating blood volume
CL	chemiluminescence
CV	coefficient of variation
Hb	hemoglobin concentration
FRAP	Ferric Reducing Antioxidant Power
Hct	hematocrit
HR	heart rate
HYMP	hypoxic impulse
ROS	reactive oxygen species
SD	standard deviation
SpO_2	blood oxygen saturation
TBARS	thiobarbituric acid reactive substances
TEAC	Trolox Equivalent Antioxidant Capacity
tHb-mass	total hemoglobin mass
TRAP	Total Reactive Antioxidant Potential
TRIMP	training impulse
VO_{2max}	maximal oxygen consumption

to prevent or reduce the damaging oxidative effects.[26] The lack of direct recommendations leads to the need to continue systematic research and develop a methodology for making conclusions about the correction of the antioxidant status.

The markers of oxidative stress are of key importance in assessing homeostasis in professional athletes, and the antioxidant status is considered the best indicator of an adequate load.[19] Carbonylated proteins and lipid peroxidation products (isoprostanes, malonic dialdehyde, and thiobarbituric acid reactive substances [TBARS]) can be used as biochemical parameters.[19] Though it is the activity of antioxidant enzymes that primarily lower in exercise-induced oxidative stress at the altitude,[27] it is difficult to use them as screening due to the complicated standardization and harmonization of assays. The water-soluble antioxidant potential of blood plasma is mainly provided by urate, sulfhydryl compounds (albumin and glutathione) and other proteins with non-specific antioxidant effects. To assess the antioxidant potential, a number of methods are developed (*Total Reactive Antioxidant Potential* [TRAP], *Trolox Equivalent Antioxidant Capacity* [TEAC], and *Ferric Reducing Antioxidant Power* [FRAP]).[28] Among them, the TRAP parameter is the most convenient for screening general conditions and monitoring the use of dietary antioxidants.[29]

Altitude training is very popular among elite athletes. Multiple studies have related the application of altitude training to improving both altitude and sea-level athletic performance.[30,31] Several physiological benefits of altitude exposure are also described in the literature in association with sea-level performance, such as hematological adaptations, enhanced glycolysis and buffering capacity, increased oxidative capacity, fiber-selective vasodilation, and others.[32–34] However, the effects of altitude are not always favorable, and the mechanisms of changes remain unclear and far from being proven.[35,36] The specifics of training at altitude require continued systematic research and development of recommendations for specific ergogenic support.[37,38]

Here, we propose the modified TRAP protocol for the analysis of the antioxidant potential of blood plasma, which has two new features. Firstly, instead of the incubation time, we use the signal suppression area, which is proportional to the number of scavenged radicals. Secondly, in addition to the "urate" capacity, our method provides the evaluation of the "thiol" capacity.

In this study, we aimed to answer several questions. Firstly, we studied the dynamics and relationship between the urate and thiol parts of the antioxidant system of blood plasma in exercise-induced oxidative stress. Secondly, we studied the peculiarities of the response of the antioxidant system at the altitude together with hematological parameters of the blood. As a result, we propose our methodology to assess the adaptive reserve of athletes to oxidative stress and the adequacy of their antioxidant support.

Methods

Participants

The current prospective observational study without a control group involves 12 professional athletes (7 males and 5 females) specializing in middle-distance speed skating. The physical characteristics of the participants are presented in Table 1. All athletes were injury and illness-free during the study. The research was conducted in accordance with the Declaration of Helsinki (2013) and approved by the Regional Ethics Committee of the Research Centre for Medical Genetics, Moscow (Approval #5, the 3rd of July 2017) (https://med-gen.ru/nauchnaia-deiatel-nost/komitet-po-biomeditcinskoi-etike/). All participants provided written informed consent.

Study design and participant involvement

The study was conducted during one training camp for 21 days (1 850 m above sea level). According to the preparation phase, all the athletes underwent a similar training program and trained at least 24 h/wk. Training included: cycling, roller skating, ice skating, strength training, and special training. All training loads were controlled using heart rate monitors and training analytics software (TrainingPeks, USA). Distribution of the training load in cycling in the male group was (median [Q1; Q3]): Zone 1–442 (315; 463) min, Zone 2–422 (374; 462) min, Zone 3–183 (156; 233) min, Zone 4–95 (86; 192) min, Zone 5–26 (24; 33) min. In the female group, the distribution in cycling was followed: Zone 1–256 (238; 275) min, Zone 2–315 (281; 350) min, Zone 3–301 (248; 353) min, Zone 4–208 (184; 233) min, Zone 5–15 (7.5; 29) min. Distribution of the training load in ice and roller-skating in the male group was: Zone 1–836 (753; 962) min, Zone 2–320 (270; 379) min, Zone 3–243 (199; 263) min, Zone 4–83 (53; 171) min, Zone 5–0 min. In the female group Zone 1–700 (662; 760) min, Zone 2–366 (320; 370) min, Zone 3–300 (266; 321) min, Zone 4–194 (190; 199) min, Zone 5–18 (14; 19) min.

Total hemoglobin mass (tHb-mass), hemoglobin concentration (Hb), circulating blood volume (CBV), and hematocrit (Hct) were taken twice at the very beginning of training and a month later since they change slowly at day 1 and day 28. Antioxidant profiles, biochemical parameters, iron metabolism indicators, hypoxic doses, hypoxic impulses (HYMPs), and training impulses (TRIMPs) were recorded at 3, 6, 10, 14, and 18 days for each athlete (Fig. 1). The blood sample days were determined by the sports physician and there were the rest days. Iron metabolism indicators were taken twice at the very beginning of training and a month later since they change slowly.

Measurements of tHb-mass and CBV were made right before and immediately after the training camp without any training this day using the technique of rebreathing with carbon monoxide. For 2 min, the athlete breathed through a glass spirometer (Bloodtec, Germany) with a

Table 1
Physical characteristics of the participants, the data are presented as median and quartiles Me (Q1; Q3).

	Age	Height, cm	Weight, kg	Body mass index, kg·m^{-2}	VO_{2max}, mL·min^{-1}·kg^{-1}
Males (n = 7)	26 (24;27)	185 (180;186)	84 (81;86)	24.6 (24.1;25.3)	64 (61;67)
Females (n = 5)	25 (22;32)	164 (161;171)	60 (56;61)	20.8 (20.6;23.2)	57 (55;62)

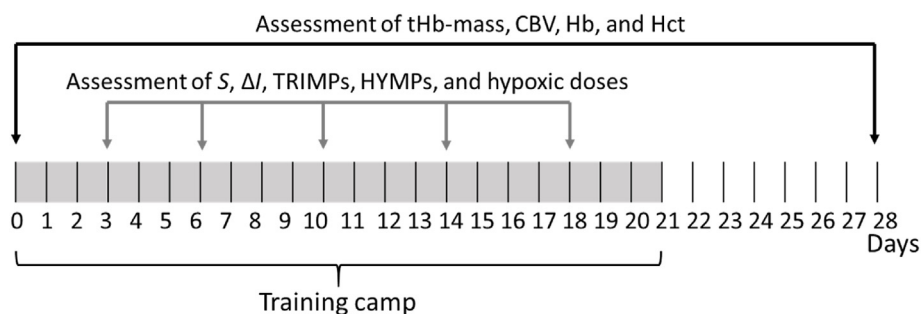VO_{2max}, maximal oxygen consumption.

Fig. 1. The design of the study. Abbreviations: tHb-mass, total hemoglobin mass; Hb, hemoglobin concentration; CBV, circulating blood volume; Hct, hematocrit; HYMP, hypoxic impulse; TRIMP, training impulse; S, "urate" antioxidant capacity; ΔI, "thiol" antioxidant capacity.

mixture containing CO (1 ml·kg^{-1} for males and 0.8 ml·kg^{-1} for females). To determine hemoglobin CO saturation (% HbCO), capillary blood (210 μL) was taken before the procedure, at the 6th and 8th min of return breathing. Blood samples were analyzed by a CO-oximeter (ABL80 FLEX COOX Radiometr, Denmark). The calculation of the amount of CO before and 4 min after the start of the procedure was measured for the amount of SO$_2$ in exhaled air using an analyzer (Drager Pac 800, Germany). The calculations were performed using the developer software (Bloodtec, Germany).[39] Hb and Hct were determined from capillary blood samples using a Hemo Control device (EKF, Germany).

Hypoxic exposure was calculated using two methods. The first method was based on the "saturation hours" index and the second one on the "kilometer hours" index. We measured blood oxygen saturation (SpO$_2$) every 2 h during the day, before bedtime, and after waking up, using fingertip pulse oximeters (Choicemmed MD 300C^{31}8, China). During training, blood oxygenation was measured continuously. Hypoxic impulses (HYMPS) were calculated as "saturation hours" using the formula proposed by the authors[40]:

$$\%h = (98/s - 1) \times h \times 100,$$

where %h is hypoxic impulse HYMP (HYpoxyc iMPulse) or "saturation hours", s is arterial oxygen saturation, and h is hours of hypoxic exposure.

The hypoxic dose was calculated as[41]:

$$km\cdot h = (km/1\ 000) \times h,$$

where $km\cdot h$ is "kilometer hours"; km is the altitude above sea level, h is hours of hypoxic exposure.

TRIMP (TRaining IMPulse) (a quantitative index of aerobic training load) was calculated as described elsewhere[42]:

$$TRIMP = duration\ of\ training\ (min) \times \Delta HR\ ratio \times Y$$

where ΔHR ratio $= (HR_{ex} - HR_{rest})/(HR_{max} - HR_{rest})$; HR_{ex} is an average heart rate of the exercise session, HR_{rest} is resting heart rate, and HR_{max} is maximal heart rate; $Y = 0.64e^{1.92\Delta HR}$ for males, $Y = 0.86e^{1.67\Delta HR}$ for females.

Participants were not involved in the study, since the study was aimed at studying objective laboratory indicators of homeostasis.

Nutritional profiles were controlled by a sports dietitian using a questionnaire, food diary, and nutritional analysis software (Cronometer, Canada). The diet was well-balanced and contained cereals, legumes, meat, fish, dairy products, fruits, and others balanced in proteins/fats/carbohydrates. In the male group, it contained 24 (22; 25)% proteins, 17 (16; 18)% fats, 61 (58; 62) %carbohydrates, and 3 205 (2 777; 3 355) kCal/day; in the female group, 23 (21; 24)% proteins, 18 (17; 19)% fats, 58 (57; 62) %carbohydrates, and 2 001 (1 980; 2 010) kCal/day. The data are presented as median (Q1; Q3). Every athlete was using a recovery protein shake (SiS REGO Rapid Recovery Powder, UK), every first day of the microcycle in the long cycling training nitrate supplementation (400 mg of dietary nitrate in a pocket-sized 70 mL) was used (Beet It Sport Nitrate 400 Shot, UK). The athletes got Fero-Grad Vitamine C 500

(ferrous sulfate 105 mg, sodium ascorbate 500 mg) (Teofarma SRL, Italy) once a day during all training camps.

For more control of the study, the biochemical parameters were taken on the same day as the antioxidant capillary blood sample on the day after the overnight fast. In this paper, we present the data just from the first and last measurements.

Antioxidant profile assay

Capillary blood samples for the antioxidant profile analysis were taken on the day in the morning after overnight fasting and before breakfast.

The antioxidant profile was assessed with luminol-enhanced chemiluminescence protocol based on TRAP but using new analytical signals. Concentrations of reagents and operation conditions were developed previously.[43] A Lum-1200 chemiluminometer (DISoft, Russia) was used. A luminol solution (1 mM; Sigma, USA) and 2,2′-azo-bis(2-amidinopropane) dihydrochloride (ABAP; Fluka, Germany) solution (50 mM) were prepared by dissolving weighed samples in a phosphate buffer solution (100 mM KH$_2$PO$_4$, pH 7.4, Sigma, USA). Plasma samples were stored at $-20°$ and 10-fold diluted with distilled water immediately before testing. A mixture of ABAP and luminol (final concentrations, 2.5 mM and 2 μM, respectively) was added to a buffer solution at 37 °C. The chemiluminescence was recorded until a stationary level had been achieved, and then an aliquot of the diluted blood plasma was added. The registration was performed until the new steady-state level was achieved (Fig. 2). Two parameters were calculated: the area of depression of chemiluminescence (S), which characterizes the capacity of uric acid (the "urate" antioxidant capacity), and the difference in the initial and final
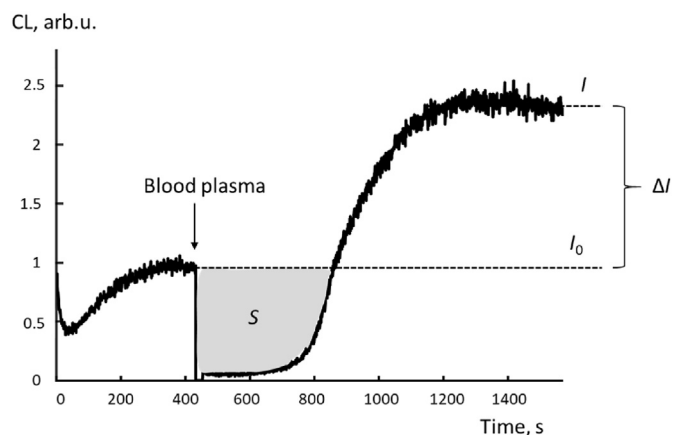

Fig. 2. The antioxidant profile of blood plasma of a healthy donor; the arrow indicates the time when the sample was added. Here I_0 is the initial steady-state chemiluminescence intensity, I is the final steady-state chemiluminescence intensity S id the "urate" antioxidant capacity, ΔI is the "thiol" antioxidant capacity; CL, chemiluminescence; arb. u., arbitrary units.

stationary levels of chemiluminescence (ΔI), which characterizes the number of unoxidized thiol groups of albumin (the "thiol" antioxidant capacity). The area of depression of chemiluminescence (S) and ΔI were calculated using PowerGraph 3.0 Software (DISoft, Russia) supplied with the chemiluminometer.

Reproducibility has been examined for the 12 parallel simultaneous measurements provided by the chemiluminometer. For a reference solution of uric acid (Sigma, USA) ($c = 3.0$ mM), mean $S = 322$ arb. u., standard deviation (SD) $= 10$. For a reference solution of human serum mercaptoalbumin (Sigma, USA) ($c = 0.66$ μM), mean $\Delta I = 1.48$ arb. u., $SD = 0.04$. For all detected S and ΔI values, the coefficients of variation were $CV \leq 0.03$.

Previously, we have performed a special study to determine the reference intervals for the developed protocol. The control group included 120 healthy donors (60 males and 60 females) aged 18–60 years (all of them were blood donors). The participants were divided into age groups according to WHO recommendations: 18–44 years old (young age) and 45–59 years old (middle age). Exclusion criteria: any acute or chronic diseases, any congenital genetic diseases, allergies, malignant process, acute respiratory disease within the previous six months, pregnancy, burns, mechanical and other injuries within six months, any drug therapy, and additional nutritional support. Comparison of data across groups showed no differences in sex ($p = 0.89$ for the young age, $p = 0.92$ for middle age, Mann–Whitney U test) and age ($p = 0.85$ for males, $p = 0.67$ for females, Mann–Whitney U test), which gave us the basis to calculate reference intervals for the total group: S [195–455] and ΔI [1.0–2.2]. As a reference interval, we took the interval that included the data of 95% of the participants (114 people). Decreases in S and ΔI reflect the states of "urate" and "thiol" oxidative stress, respectively. An increase in S and ΔI indicates a state of excess antioxidants.

Statistics

Statistical data processing was performed using the STATISTICA 10 software (StatSoft, USA). Since the distribution differed from normal (Shapiro–Wilk test, $p > 0.05$), the median and interquartile range were used to characterize the samples. To find correlations, Spearman coefficients were calculated. The Mann–Whitney test was used to compare independent samples ($p = 0.05$).

Results

Antioxidant profiles and hematological parameters

The start-point values of tHb-mass, Hb, CBV, Hct were determined at day 1, and antioxidant indices (S and ΔI) along with biochemical parameters were determined at day 3 for each athlete. From these data, it follows that before the start of training, "urate" antioxidant capacity in all athletes was within the reference interval for practically healthy non-athletes; in two athletes (both are males), this indicator exceeded the reference interval (excess 15% and 35%). The "thiol" antioxidant capacity, on the other hand, was significantly below the lower limit of the reference interval in 11 of 13 athletes (85%). Only two athletes (both are males) had the "thiol" antioxidant capacity within the reference range.

The endpoint values of tHb-mass, Hb, CBV, Hct were determined at day 28, and antioxidant indices (S and ΔI) were determined at day 18. The data shows that at the end of the training camp, the "urate" antioxidant capacity in two athletes was lower than the reference interval; for the rest of the athletes, this indicator was within the reference interval. The "thiol" antioxidant capacity, on the other hand, increased. Only four athletes had this parameter lower than the reference interval.

For antioxidant parameters, no significant differences were found between males and females using the Mann–Whitney test ($p = 0.25$ for S and $p = 0.91$ for ΔI). Descriptive statistics and p-values according to the Mann–Whitney test are presented in Table 2.

To sum, at the end of the training camp, the "urate" antioxidant

Table 2
Descriptive statistics of antioxidant profile indices and hematological parameters and p-values according to the Mann–Whitney test, the data are presented as median and quartiles Me (Q1; Q3).

Parameter	At the start	At the end	p-value
S (total), arb.u	398 (339; 426)	250 (190; 287)	0.001
S (males), arb.u	407 (367; 485)	281 (233; 326)	0.001
S (females), arb.u	388 (327; 412)	198 (190; 238)	0.001
ΔI (total), arb.u.	0.67 (0.18; 0.95)	1.19 (0.89; 1.38)	0.013
ΔI (males), arb.u.	0.61 (0.35; 0.98)	1.25 (0.86; 1.66)	0.008
ΔI (females), arb.u.	0.72 (0.13; 0.93)	1.17 (0.89; 1.31)	0.025
tHb-mass (total), g	956 (667; 1 054)	971 (665; 1 082)	0.184
tHb-mass (males), g	1 021 (998; 1 210)	1 069 (1 008; 1 155)	0.898
tHb-mass (females), g	639 (595; 677)	619 (595; 681)	0.916
CBV (total), mL	7 092 (5 097; 7 861)	6 591 (4 809; 7 613)	0.406
CBV (males), mL	7 734 (7 441; 8 505)	7 581 (6 799; 8 291)	0.523
CBV (females), mL	5 054 (4 990; 5 112)	4 704 (4 567; 4 844)	0.143
Hb (total), g·L^{-1}	148 (141; 155)	166 (154; 168)	0.020
Hb (males), g·L^{-1}	155 (146; 156)	168 (166; 176)	0.010
Hb (females), g·L^{-1}	135 (132; 144)	155 (136; 160)	0.210
Hct (total)	45 (42; 46)	49 (46; 49)	0.022
Hct (males)	46 (43; 46)	49 (49; 52)	0.002
Hct (females)	42 (39; 46)	46 (40; 47)	0.601

tHb-mass, total hemoglobin mass; Hb, hemoglobin concentration; CBV, circulating blood volume; Hct, hematocrit; S, "urate" antioxidant capacity; ΔI, "thiol" antioxidant capacity.

capacity S significantly decreased for both males and females ($p = 0.001$), "thiol" antioxidant capacity ΔI significantly increased ($p = 0.013$) like Hb ($p = 0.020$) and Hct ($p = 0.022$). For hematological indices, a significant correlation was found for S_{end}–S_{start} and tHb-mass$_{end}$ – tHb-mass$_{start}$ ($r_S = 0.40$) as well as for ΔI_{end}– ΔI_{start} and tHb-mass$_{end}$ – tHb-mass$_{start}$ ($r_S = -0.45$).

Antioxidant profiles and hypoxic doses in training dynamics

The total parameters of the training load and hypoxic doses and changes in the antioxidant profile are presented in Table 3.

The changes in the antioxidant profile dynamics were individual for athletes (see several examples in Fig. 3). Since all athletes were in the same housing, nutrition, and training conditions, lifestyle factors that could affect the result were excluded. It can be assumed that such a dynamic is due to internal homeostatic factors.

We calculated the correlation coefficients between S and ΔI and between TRIMPs or HYMPs values and S or ΔI for each participant (Table 4) and for the total cohort (Table 5).

A correlation was found between changes in the antioxidant profile parameters for TRIMPs and $|S_{start}$–$S_{end}|$: $r_S = 0.39$. The higher the training load was, the more significant the change in the "urate" antioxidant capacity was. It follows from the data that the assessment of the hypoxic dose in "saturation hours" is less informative than the HYMP index, therefore, for further assessments, we used HYMPs.

The calculated correlation coefficients differ significantly, but it can be assumed that a decrease in "thiol" antioxidant capacity is primarily associated with training loads (the correlation coefficients between TRIMPs and ΔI are strongly or moderately negative in 7 of 11 cases). Simultaneously, an increase in loads leads to increased "urate" antioxidant capacity (the correlation coefficients between TRIMPs and S are positive in 8 of 11 cases). HYMPs are negatively correlated with the "urate" antioxidant capacity in 8 of 12 cases.

In all cases, the correlation between "urate" and "thiol" antioxidant capacity was negative. That is, a decrease in "urate" capacity was accompanied by an increase in "thiol" capacity. This can be seen from the dynamics in Fig. 3.

Correlation coefficients for the total cohort at the first and last time points are presented in Table 5. These data were calculated from a larger sample volume and specify the preliminary results presented in Table 4. Moderate correlations were obtained for all pairs except HYMPs vs. ΔI.

Table 3

The total parameters of the training load and changes in the antioxidant profile.

Subject #	Total TRIMPs	Total HYMPs	Total saturation hours	S_{end}–S_{start}	$\Delta I_{end} - \Delta I_{start}$
1 (male)	1 074.1	369.4	219.3	−207	0.35
2 (male)	1 144.5	336.1	221.3	−207	0.64
3 (male)	905.2	512.0	221.2	−40	1.60
4 (male)	1 243.2	489.1	221.2	−160	0.02
5 (male)	1 014.0	573.1	221.3	−276	0.99
6 (male)	1 145.0	425.4	221.4	−274	0.40
7 (male)	932.4	443.4	221.3	−49	−0.31
8 (female)	1 005.0	310.7	221.6	−265	1.25
9 (female)	958.0	184.7	221.6	−137	1.20
10 (female)	935.0	385.9	221.6	−137	0.17
11 (female)	714.0	285.2	220.8	−214	0.22
12 (female)	809.0	374.4	221.6	−60	−0.10

HYMP, hypoxic impulse; TRIMP, training impulse; S, "urate" antioxidant capacity; ΔI, "thiol" antioxidant capacity; 'start' and 'end' subscripts denote the start point and endpoint of the training camp, respectively.
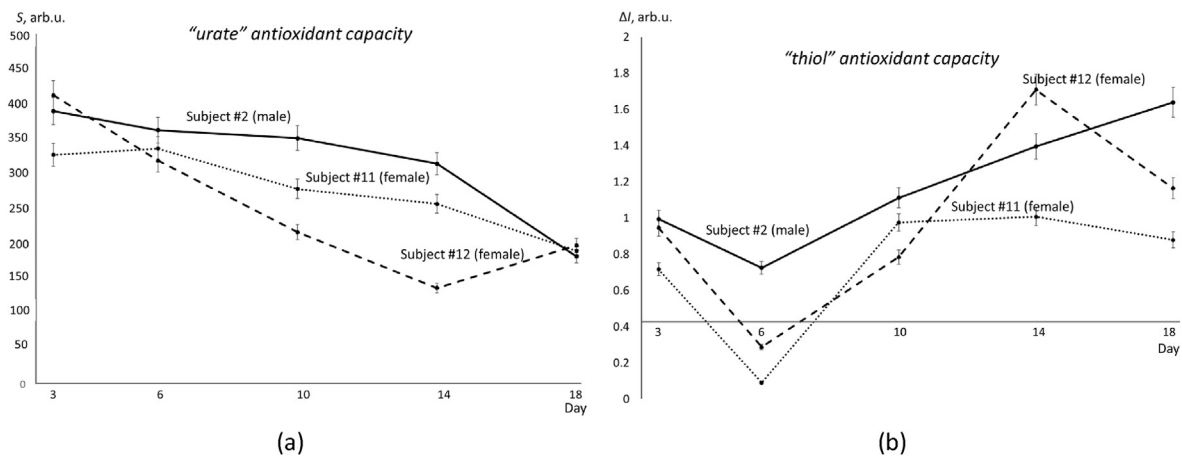

Fig. 3. The individual dynamics of "urate" (a) and "thiol" (b) antioxidant capacities during training camp for subjects #2 (male), #11 (female), and #12 (female); the general trend for both males and females is that by the end of training camp, "urate" capacity decreased and "thiol" capacity increased. S is the "urate" antioxidant capacity, ΔI is the "thiol" antioxidant capacity; arb. u., arbitrary units.

Table 4

The correlation coefficients r_S between S and ΔI and between TRIMPs or HYMPs values and S or ΔI in the training dynamics.

Subject #	TRIMPs vs. S	HYMPs vs. S	TRIMPs vs. ΔI	HYMPs vs. ΔI	S vs. ΔI	Antioxidant capacity at the endpoint
1 (male)	−0.44*	−0.37*	0.13	0.75**	−0.46*	Decreased "urate" capacity
2 (male)	0.50*	0.29	−0.78**	−0.18	−0.85**	Decreased "urate" capacity
3 (male)	0.66*	−0.47*	−0.41*	0.72**	−0.95**	Both parameters are within the reference interval
4 (male)	−0.49*	−0.62*	0.58*	0.43*	−0.61*	Both parameters are within the reference interval
5 (male)	0.73**	−0.31*	−0.84**	0.02	−0.82**	Both parameters are within the reference interval
6 (male)	0.23	0.13	0.16	−0.28	−0.35*	Decreased "thiol" capacity
7 (male)	−0.18	0.52*	0.04	0.03	−0.71**	Decreased "thiol" capacity
8 (female)		−0.24		0.14	−0.97**	Decreased "urate" capacity
9 (female)	0.17	0.50*	−0.31*	−0.32*	−0.97**	Both parameters are within the reference interval
10 (female)	0.55*	−0.11	−0.61*	−0.61*	−0.63*	Decreased "thiol" capacity
11 (female)	0.70**	−0.30*	−0.98**	−0.40*	−0.61*	Both parameters are within the reference interval
12 (female)	0.35*	−0.27	−0.61*	0.16	−0.14	Decreased "thiol" capacity

HYMP, hypoxic impulse; TRIMP, training impulse; S, "urate" antioxidant capacity; ΔI, "thiol" antioxidant capacity.
'*' denotes moderate correlation ($|0.3| \leq r_S < |0.7|$).
'**' denotes strong correlation ($r_S \geq |0.7|$).

The data indicate that both the factor of exercise and hypoxia act differently on the studied parameters. These factors are associated with an increase in the "urate" capacity and a decrease in "thiol" capacity.

Discussion

Here, we studied changes in blood plasma antioxidant profiles in professional speed skaters training at altitude. We used an original technique that allows us to assess two parts of the water-soluble antioxidant potential: the part provided by uruc acid (the "urate" capacity) and that provided by mercaptoalbumin (the "thiol" capacity). The working conditions for the implementation of the protocol on our equipment were previously determined.[43] However, there we used the induction time, as it should be in the originally developed TRAP method.[44] Here, we used a new analytical signal, the suppression area (Fig. 2), which is directly proportional to the amount of scavenged free radicals. This suppression is provided mainly by urate acid, but also by other strong plasma antioxidants (e.g., ascorbate). We also used for the

Table 5
The correlation coefficients for S *vs.* ΔI and for TRIMPs or HYMPs values *vs.* S or ΔI for the total cohort.

Day	TRIMPs *vs.* S	HYMPs *vs.* S	TRIMPs *vs.* ΔI	HYMPs *vs.* ΔI	S *vs.* ΔI
3	0.62*	0.53*	−0.44*	−0.18	−0.53*
18	0.31*	0.60*	−0.31*	−0.24	−0.57*

HYMP, hypoxic impulse; TRIMP, training impulse; S, "urate" antioxidant capacity, ΔI; "thiol" antioxidant capacity.
'*' denotes moderate correlation ($|0.3| \leq r_S < |0.7|$).

first time an increase in the steady-state chemiluminescence, which is characteristic of blood plasma but not of saliva.[45] Recently, we have performed a detailed study of this phenomenon and proved that this increase is proportional to the amount of reduced sulfhydryl groups of albumin and glutathione ("thiol" capacity) (data are being prepared for publication). Thus, one chemiluminogram provides integral information about two parts of the water-soluble antioxidant system of the blood, where the "thiol" capacity determines the redox potential of blood plasma. The information about the relationship between the "urate" and "thiol" parts of the antioxidant system in athletes during exercise is of particular novelty and interest.

Since ROS homeostasis is the primary regulation system, changes in the antioxidant profile indicate changes in homeostasis in general. On the other hand, this change can be used to judge the development and compensation (or decompensation) of oxidative stress. Total hemoglobin mass was determined, which is considered a more stable indicator than Hb and Hct since hemoglobin concentration strongly depending on the circulating blood volume,[46,47] and the hematocrit depends on blood plasma volume.[48] Exercise and hypoxic parameters were assessed as TRIMPs, HYMPs, and hypoxic doses.

The main results are as follows: (1) In the dynamics of training, there is a change in antioxidant parameters individually for each athlete; negative correlation coefficients between S and ΔI indicate that the changes between the "urate" and "thiol" capacity are multidirectional; (2) for most athletes, at the endpoint, there is a decrease in the "urate" capacity and an increase in the "thiol" capacity, and in general balancing the antioxidant profile, especially characteristic of athletes showing high results; (3) the changes in the antioxidant profile are associated more with changes in tHb-mass, rather than CBV, Hb, and Hct; (4) high training loads are associated with a decrease in the "thiol" antioxidant capacity and an increase in the "urate" antioxidant capacity; and (5) two-thirds of cases, HYMPs are negatively correlated with the "urate" antioxidant capacity.

A decrease in the "urate" antioxidant capacity at the end of training can respond to oxidative training-induced stress. The "uric" acid part is the system of rapid response to oxidative stress, designed to maintain ROS homeostasis including during exercise.[49] There is evidence that ROS levels rise during exercise and are subsequently reduced to basic levels.[13,50] The researchers demonstrated oxidative changes in proteins, an increase in lipid peroxidation markers, and an apparent compensatory increase in the total antioxidant capacity.[20] The rapid return of oxidative stress markers to basic values in professional triathletes after a competition and the absence of chronic oxidative stress has been shown elsewhere.[51] Other researchers confirm that improved plasma antioxidant status is characteristic of professional athletes.[52] Following the hormesis principle, low-grade oxidative stress appears necessary for various physiological adaptations.[53] Thus, continuing exercise and enhanced ROS production lead to an upregulation in the body's antioxidant defense system and the associated shift in redox balance in favor of a more reducing environment, providing adaptive protection from ROS during subsequent training sessions. It seems that there is a U-shaped association between ROS/inflammation and performance: if ROS/inflammation increases so does the performance, but only up to a limit, after which, as ROS/inflammation continues to increase, the performance decreases.[54] In our study, for most athletes, the "urate" capacity remained at the end

of training within the reference interval, which indicates correctly selected training loads. Note that the most successful athletes generally had a balanced antioxidant profile by the end of the training process.

The "thiol" part of the antioxidant capacity is associated with the glutathione redox system. In pathological conditions, its capacity decreases in many cases. An increase in the "thiol" capacity for most athletes by the end of training contradicts some data.[55] Tong et al. studied the effect of training at 1 700 m in professional adolescent athletes and showed a decrease in reduced glutathione and in the activity of antioxidant enzymes (catalase and glutathione peroxidase). In contrast to this, in elite basketball players during a long-term intensive load, the levels of TBARS, carbonylated proteins and reduced glutathione increased at the beginning of the training load and decreased at the end of it.[56] This study confirms our results that compensation for oxidative stress occurs quite quickly and ROS homeostasis returns basal parameters by the end of the training process. Glutathione disbalance has been shown along with an increase in hydrogen peroxide and malondialdehyde in professional female water polo athletes,[57] while the total antioxidant capacity and the activity of antioxidant enzymes (catalase and superoxide dismutase) are increased. Based on a comparison of data for water polo and football, the authors concluded that the mechanisms of ROS homeostasis imbalance and its adaptation depend on the sport.

For training at the altitude, oxidative stress is resulted from two independent factors. First, microdamage to muscles during physical exertion leads to an inflammatory reaction, the formation of ROS, and a change in the redox balance towards oxidative stress.[58] Hypobaric hypoxia is the second oxidative stress factor.

The main targets of hypobaric hypoxia are ROS and iron homeostasis.[59] In ROS metabolism, altitude-induced oxidative stress affects primarily lipids and the glutathione system.[60] The severity of oxidative stress depends on altitude, while the pattern of oxidative damage resembles ischemia/reperfusion.[61] Physiologically, the response to altitude-induced hypoxia is a compensatory enhancement of the antioxidant system[62] and above all, antioxidant enzymes.[63] Altitude-induced oxidative stress leads to transient tissue damage followed by adaptation.[64] It is obvious that the simultaneous effect of intense physical exercises and hypoxia leads to more pronounced oxidative stress.[65] Leon-Lopez et al. studied the impact of various training regimens on the prooxidant/antioxidant status of elite athletes. The general conclusion was that oxidative stress at altitude is associated precisely with the depletion of antioxidant systems and, above all, the glutathione system.[66] Thus, screening of the antioxidant system is necessary for assessing adaptation at the altitude and prescribing antioxidant nutritional support. On the other hand, for well-trained cyclists, there were no differences in the rates of oxidative stress at altitude and sea level.[27] Córdova Martínez et al. reported that in well-trained cyclists, the recovery period between stages is enough to normalize the redox state.[67] They reported a reduction in reduced glutathione and an increase in oxidized glutathione levels in erythrocytes and suggested that muscle capitation of reduced glutathione could explain its decreases in plasma. In our case, we did not see the harmful effects of altitude on the antioxidant profile indicators in training dynamics.

The individuality of changes in the antioxidant profile can serve as the basis for a personalized approach to training planning and the basis for the appointment of antioxidant support. This issue is discussed in the literature, but no consensus has been achieved. There is evidence that antioxidants do not prevent the development of oxidative stress.[68] Koivisto et al. showed that the administration of antioxidants in elite athletes does not affect oxidative status but reduces the level of pro-inflammatory cytokines.[69] Dosek et al. proved that antioxidant supplements lead to accelerated adaptation to oxidative stress.[61] Vani et al. demonstrated that antioxidant supplements contribute to the stability of erythrocyte membranes and increase the activity of glutathione peroxidase red blood cells.[70] Among antioxidants, the Q10 coenzyme is especially beneficial as it has antioxidant and cardiometabolic effects.[71]

To sum up, compensated oxidative stress contributes to the activation

of body reserves and ultimately leads to improvements in health and/or human performance. An antioxidant overload could delay the adaptation and negatively interfere with training. However, for specific situations in which there is decompensated ROS homeostasis impairment, such supplements may be helpful. In continuation of the research, we plan to study antioxidant intake during training on the antioxidant profile indicators.

Conclusions

The assessment of the antioxidant profile of blood plasma by the rapid and simple chemiluminometric method can be a useful addition for screening the ROS homeostasis of athlete bodies along with standard tests. Two parameters characterizing the water-soluble antioxidant capacity provided by uric acid and the glutathione system changed individually in training dynamics. Both exercise and hypoxic factors affect the antioxidant parameters bidirectionally. They correlated with a decrease in the thiol capacity and with an increase in urate capacity. Towards the end of the training camp, the "urate" part decreased in response to exercise-induced oxidative stress but remained within the reference range, and the "thiol" part even increased. These antioxidant profile parameters can help select the personalized training schedule and nutritional support.

Submission statement

This article has not been published previously and it is not under consideration for publication. This publication is approved by all authors and by the responsible authorities where the work was carried out. After accepted, this article will not be published elsewhere including electronically in the same form, in English or any other language, without the written consent of the copyright-holder.

Ethical approval statement

The research was conducted in accordance with the Declaration of Helsinki (2013) and approved by the Regional Ethics Committee of the Research Centre for Medical Genetics (Approval #5, 3rd of July 2017) (https://med-gen.ru/nauchnaia-deiatel-nost/komitet-po-biomeditci nskoi-etike/). All participants provided written informed consent.

Authors' contributions

Elena Proskurnina: Conceptualization, Data curation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. Dmitry Martynov: Investigation, Validation. Andrey Yakushkin: Investigation, Validation. Irina Zelenkova: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – review & editing.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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