SOD2 gene polymorphism and muscle damage markers in elite athletes

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ABSTRACT

Exercise-induced oxidative stress is a state that primarily occurs in athletes involved in high-intensity sports when pro-oxidants overwhelm the antioxidant defense system to oxidize proteins, lipids and nucleic acids. During exercise, oxidative stress is linked to muscle metabolism and muscle damage, because exercise increases free radical production. The T allele of the Ala16Val (rs4880 C/T) polymorphism in the mitochondrial superoxide dismutase 2 (*SOD2*) gene has been reported to reduce SOD2 efficiency against oxidative stress. In the present study we tested the hypothesis that the *SOD2* TT genotype would be underrepresented in elite athletes involved in high-intensity sports and associated with increased values of muscle and liver damage biomarkers. The study involved 2,664 Caucasian (2,262 Russian and 402 Polish) athletes. *SOD2* genotype and allele frequencies were compared to 917 controls. Muscle and liver damage markers (creatine kinase (CK), creatinine, alanine transaminase, aspartate transaminase, alkaline phosphatase) were examined in serum from 1,444 Russian athletes. The frequency of the *SOD2* TT genotype (18.6%) was significantly lower in power/strength athletes (n = 524) compared to controls (25.0%, $p = 0.0076$) or athletes involved in low-intensity sports (n = 180; 33.9%, $p < 0.0001$). Furthermore, the *SOD2* T allele was significantly associated with increased activity of CK (females: $p = 0.0144$) and creatinine level (females: $p = 0.0276$; males: $p = 0.0135$) in athletes. Our data show that the *SOD2* TT genotype might be unfavorable for high-intensity athletic events.

Keywords: gene polymorphism; MnSOD; muscle damage; biomarkers; creatinine; creatine kinase

INTRODUCTION

Exercise-induced oxidative stress is a state that primarily occurs in athletes involved in high-intensity sports when pro-oxidants overwhelm antioxidant defenses to oxidize proteins, lipids and nucleic acids [1,2]. In trained male athletes, moderate aerobic exercise [3] induces only relatively small increases in oxidative stress and inflammation, contrary to intensive training [4]. During exercise, oxidative stress is linked to muscle metabolism and muscle damage, because exercise increases free radical production [5]. Strenuous exercise that damages skeletal muscle cell structure at the level of the sarcolemma and Z-disks [6] results in an increase in total creatine kinase (CK) [7]. A high CK value in the athlete can serve as an indicator of severe muscle injury or the development of chronic fatigue, which may lead to overtraining syndrome if the training loads are not decreased [8,9]. Furthermore, healthy subjects performing intensive exercise may exhibit altered liver function (elevations of aspartate aminotransferase and alanine aminotransferase) [10].

Antioxidant defenses are provided by several factors including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and numerous non-enzymatic antioxidants (vitamins A, E and C, glutathione, ubiquinone, and flavonoid) [1]. In particular, manganese superoxide dismutase (MnSOD; encoded by the *SOD2* gene) catalyzes the dismutation of superoxide radicals in mitochondria by converting anion superoxide into hydrogen peroxide and oxygen. Experimental results indicate that MnSOD prevents the disruption of mitochondrial membrane potential [11]. The inhibition of MnSOD causes the accumulation of superoxide radicals, and leads to free radical-mediated damage to mitochondrial membranes and the apoptosis of cells [12].

Antioxidant capacity varies greatly from one individual to another [13], which can be determined, in part, by genetic polymorphisms. A number of polymorphisms in the *SOD2* gene have been described, and one polymorphism in the mitochondrial targeting sequence (rs4880 C/T; it causes an amino acid change from Ala (A) to Val (V)) has been demonstrated to have functional significance [14]. Indeed, the T allele of the Ala16Val polymorphism in the *SOD2* gene has been reported to decrease MnSOD efficiency against oxidative stress [15], which is supported by the observation that

the T-variant decreases formation of the active MnSOD protein in the mitochondrial matrix [14]. Recently, Akimoto et al. [16] demonstrated that athletes with the *SOD2* TT genotype had an increased CK value after racing, suggesting that the CC genotype is associated with lower muscle damage. Interestingly, Ben-Zaken et al. [17] found that the frequency of the *SOD2* C allele was significantly higher in Israeli athletes (46 vs. 29%) compared with controls.

We thus tested the hypothesis that the *SOD2* TT genotype would be underrepresented in elite athletes involved in high-intensity sports and associated with increased levels of muscle and liver damage biomarkers. We addressed this question by examining a large sample of Caucasian athletes of Eastern European descent with respect to their specialty and level of achievement.

The aim of the present study was to investigate the possible associations between the *SOD2* genotypes and muscle/liver damage biomarkers.

METHODS

Participants

The study involved 2,664 Caucasian (2,262 Russians and 402 Poles; 1540 male and 1124 female) international-level athletes stratified into 7 groups with similar physiological characteristics of the training according to type, intensity, and duration of exercise [18, 19] (Table 1). The first group (‘group I’; n = 180) comprised athletes involved in low-intensity sports. The next three groups included very long distance athletes (‘group II’; n = 235), long distance athletes (‘group III’; n = 187) and middle distance athletes (‘group IV’; n = 86). The fifth group (‘group V’ or ‘mixed group’; n = 1,452) comprised athletes whose sports require mixed anaerobic and aerobic energy production. The sixth group (‘group VI’ or ‘power group’; n = 321) included sprint athletes with predominantly anaerobic energy production. The seventh group (‘group VII’ or ‘strength group’; n = 203) included strength athletes (Table 1).

Controls were 917 (558 males and 359 females) healthy, unrelated citizens of Russia (n = 489) and Poland (n = 428) without any competitive sport experience. The athletes and controls were all Caucasians of Eastern European descent.

The procedures followed in the study were conducted ethically according to the principles of the World Medical Association Declaration of Helsinki and ethical standards in sport and exercise science research. The Ethics Committees of the All-Russian Research Institute of Physical Culture and Sport and the Pomeranian Medical University approved the study (#02.522). All participants were given a consent form and a written information sheet concerning the study, providing all pertinent information (purpose, procedures, risks, benefits of participation). The experimental procedures were conducted in accordance with the set of guiding principles for reporting the results of genetic association studies defined by the STrengthening the REporting of Genetic Association studies (STREGA) Statement.

Methods

Four ml of venous blood from Russian cohorts were collected in tubes containing EDTA (Vacuette EDTA tubes, Greiner Bio-One, Austria). DNA extraction and purification was performed using a commercial kit according to the manufacturer's instructions (Technoclon, Russia) and included chemical lysis, selective DNA binding on silica spin columns and ethanol washing. Extracted DNA quality was assessed by agarose gel electrophoresis at this step. Since genetic analyses were performed in different laboratories, *SOD2* genotyping was conducted using the genotyping technique available in the individual laboratory. HumanOmni1-Quad BeadChips (Illumina Inc, USA) were used for genotyping of the *SOD2* Ala16Val polymorphism in 914 subjects. The assay required a 200 ng DNA sample. Exact concentrations of DNA in each sample were measured using a Qubit Fluorometer (Invitrogen, USA). All further procedures were performed according to the instructions of Infinium HD Assay. In total, 1444 samples were genotyped by real-time PCR assays using a DT-384 amplifier (DNA-Technology JSC, Russia). The others were genotyped by PCR and restriction enzyme digestion,

as previously described [20]. All samples were genotyped in duplicate, with a further 10% of samples genotyped in a separate run as a quality assurance measure, with 100% agreement between runs. Furthermore, 227 samples were genotyped using 2 methods (microarray and real-time PCR) with 100% agreement.

The buccal cells donated by the Polish subjects were collected in Resuspension Solution (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma, Germany) using sterile foam-tipped applicators (Puritan, USA). DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's protocol. All samples were genotyped in duplicate using an allelic discrimination assay on a StepOne Real-Time Polymerase Chain Reaction (PCR) instrument (Applied Biosystems, USA) with TaqMan® probes. To discriminate *SOD2* C (Ala) and T (Val) alleles (rs4880), a TaqMan® Pre-Designed SNP Genotyping Assay was used (Applied Biosystems, USA) (assay ID: C___8709053_10), including primers and fluorescently labeled (FAM and VIC) MGB™ probes to detect both alleles. Genotypes were assigned using all of the data from the study simultaneously.

Muscle and liver damage markers (creatine kinase (CK), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP)) were examined in serum from 1,444 Russian athletes (787 males and 657 females; 688 elite and 756 sub-elite athletes). For these purposes, 10 ml of venous blood was collected the morning after an overnight fast and sleep into EDTA vacutainer tubes and kept at 4°C until processing. For each sporting discipline (all athletes were members of the same sport team and trained in the same sport center or training camp) blood was collected on the same day. Biomarkers were analyzed using enzyme immunoassay (Alkor-Bio, Russia) and commercial test systems on a Benchmark Plus Microplate Spectrophotometer (Bio-Rad, France).

Statistical analysis

Genotype distribution and allele frequencies between groups of athletes and controls were compared using χ^2 tests. Spearman's (non-parametric) correlations were used to assess the relationships between

the biochemical parameters. Differences in the level or activity of biomarkers between different genotype groups were analyzed using ANOVA. All values are means (SD). *P* values < 0.05 were considered statistically significant. Bonferroni's correction for multiple testing was performed by multiplying the *P* value with the number of tests where appropriate. It has been estimated that the sample size required in this study to obtain a statistical power of 80% was sufficient [21]. Statistical analyses were conducted using GraphPad InStat and StatistiXL v. 1.8 software.

RESULTS

As shown in Table 2, female athletes were younger than male athletes (22.8 (5.7) vs 23.8 (5.4) years; $p = 0.0007$), and sub-elite athletes were younger than elite athletes (20.5 (3.7) vs 26.4 (5.6) years; $p < 0.0001$). Mean values of CK, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) of 1,444 Russian athletes in relation to their specialty, sex and level of achievement are shown in Table 3. In general, male athletes of all groups had significantly higher values of damage biomarkers than female athletes. Furthermore, in most cases elite athletes had significantly lower activity of CK and ALP in comparison with sub-elite athletes. As expected, with increases in training intensities in sporting groups, the values of damage biomarkers significantly increased (CK: females ($p < 0.0001$), males ($p < 0.0001$); creatinine: females ($p = 0.0374$), males ($p < 0.0001$); ALT: females ($p < 0.0001$), males ($p < 0.0002$); AST: females ($p < 0.0001$), males ($p = 0.0004$); ALP: females ($p < 0.0001$), males ($p < 0.0001$)).

We also examined the relationship between the levels of damage biomarkers (Table 4). With some gender-specific exceptions, all biochemical parameters correlated with each other (data not shown). Only creatinine level correlated with ALP negatively, while other markers had a positive relationship.

The frequencies of the *SOD2* genotypes and alleles did not differ between Russian and Polish controls or athletes (data not shown), supporting our previous observations that Russian and Polish

populations have similarities in their genetic profile [22-29]. Therefore, for the main analyses we used the combined data (i.e. combined groups of Caucasians, independent of precise ethnicity).

SOD2 genotype distributions in the control group and athletes were in Hardy-Weinberg equilibrium (χ^2 = 1.66, p = 0.436; χ^2 = 2.96, p = 0.228, respectively). The genotype distribution and allele frequency amongst the whole cohort of athletes was similar to that amongst controls (Table 5). We found that the frequencies of the T allele in low-intensity and power athlete groups were different in comparison with controls. However, after Bonferroni correction for multiple testing these associations became non-significant.

We next compared TT genotype and T allele frequencies of the *SOD2* gene between athletes involved in high-intensity sports (i.e. the combined group comprising power and strength athletes, *n* = 524), the low-intensity group and controls. The frequency of the TT genotype was significantly lower (18.7%) in high-intensity groups compared with controls (25.0%, p = 0.0076) and the low-intensity group (33.9%, p < 0.0001). Compared with TT genotype carriers, the odds ratio (OR) of being a sprinter/strength athlete in C allele carriers was 1.45 (95% confidence interval (CI): 1.109-1.887, *P* = 0.0076). Furthermore, the frequency of the T allele was significantly lower in high-intensity groups (45.4%) compared with the low-intensity group (55.0%, p = 0.0017). Interestingly, we found a decreasing linear trend of TT genotype with the component of increasing intensity of physical performance (p = 0.0001 for linear trend) (Figure 1).

The *SOD2* Ala16Val polymorphism was associated both with CK activity (females: CC – 214 (224) U/l, CT – 288 (308) U/l, TT – 231 (243) U/l, p = 0.022; males: CC – 340 (392) U/l, CT – 392 (381) U/l, TT – 402 (455) U/l, p = 0.284) in groups III-VII (groups I and II were excluded since they had the lowest values of CK) and creatinine (females: CC – 83.2 (12.9) μmol/l, CT – 84.7 (20.2) μmol/l, TT – 87.3 (20.4) μmol/l, p = 0.0276; males: CC – 97.2 (14.4) μmol/l, CT – 100.8 (17.3) μmol/l, TT – 101.2 (15.9) μmol/l, p = 0.0135) in the overall groups of athletes, indicating that *SOD2* T allele carriers exhibit higher values of muscle damage markers. No association was found between the *SOD2* Ala16Val polymorphism and other biomarkers.

DISCUSSION

To the authors' best knowledge, this is the first study aimed at investigating the association between the *SOD2* genotype and muscle and liver damage biomarkers in a large cohort of competitive athletes. We found that the *SOD2* TT genotype was under-represented in athletes involved in high-intensity sports (power and strength) in comparison with controls and athletes of low-intensity. This confirms our primary hypothesis. Power and strength athletes are characterized by performing dynamic/isometric resistance or sprint exercises which have the potential to result in increased oxidative stress. The majority of studies subsequent to these types of exercises have noted an increase in lipid peroxidation following exercise, as well as changes in the glutathione redox status, decreased antioxidant capacity and DNA damage [30]. Oxidative stress can lead to damage or destruction of cellular macromolecules such as lipids, proteins, and nucleic acids. Therefore, oxidative stress has been associated with decreased physical performance, muscular fatigue, muscle damage, and overtraining [1,2,5].

We also found that the *SOD2* TT genotype was associated with increased activity of CK (a muscle damage marker) in athletes involved in sports with moderate and short-term physical activities. We therefore confirmed the results of the previous study by Akimoto et al. [16], where *SOD2* TT genotype was shown to be associated with increased CK values of trained runners after racing. The possible mechanisms underlying the association of the *SOD2* Ala16Val polymorphism with muscle damage markers might be explained by the findings that the T allele, by altering the function of MnSOD, significantly reduces its efficiency against oxidative stress [14,15,31]. Recently, Bresciani et al. [32] found that males with the *SOD2* CC genotype demonstrated increased MnSOD mRNA expression and enzyme activity 1 hour after a bout of intense exercise. Conversely, MnSOD mRNA expression did not change, but protein thiol content (an indicator of antioxidant defense deficiency) decreased significantly after the bout of exercise in TT carriers. Furthermore, Montano et al. [33]

demonstrated that the *SOD2* TT genotype was associated with increased production of proinflammatory cytokines. Importantly, several studies have shown that the *SOD2* T allele was associated with increased risk of various diseases [20, 34, 35], although contradictory results exist. The use of the HaploReg (version 2) online program predicted the change of transcription factor-binding motifs in the rs4880 locus of the *SOD2* gene for chromodomain helicase DNA binding protein 2 (CHD2). CHD2 is known to be involved in the later stage of the DNA damage response pathway by influencing the transcriptional activity of several genes [36].

We therefore speculate that the *SOD2* TT genotype, due to its low antioxidant and anti-damage effects, might be unfavorable for power and strength athletes. Indeed, Ben-Zaken et al. [17] have reported a lower proportion of TT genotype in a combined group of Israeli endurance and power athletes compared to controls. There are already other genetic variants that have been reported to be disadvantageous for power and strength exercise [37], and we strongly suspect that many additional common polymorphisms, and probably rare mutations as well, will be shown to be associated with exercise-induced oxidative stress in athletes. Nevertheless, the preliminary data suggest an opportunity to use the analysis of the *SOD2* Ala16Val polymorphism along with standard biochemical assessment in predicting exercise-induced oxidative stress and damage of tissues, although more replication studies are needed.

Our findings may also have broader implications for the development of nutritional strategies in sports medicine. Indeed, antioxidant supplementation may be one intervention to reduce exercise-induced oxidative stress [1]. One might speculate that *SOD2* TT genotype carriers would benefit in their recovery after strenuous exercise through antioxidant supplementation. Future studies aiming to analyze the effect of some antioxidant supplements on oxidative stress outcomes modulated by the *SOD2* genotype would be of interest.

We also found that elite Russian athletes of different groups, with some exceptions, had significantly lower values of CK and ALP than sub-elite athletes. One explanation of this finding could be the notion that well-trained athletes are more adapted to exercise-induced oxidative stress than less

trained subjects, as shown previously [38]. On the other hand, sub-elite athletes in our study were significantly younger than elite athletes, and the observed differences could be linked to the age of subgroups of athletes. At least ALP was shown to decrease with age in healthy subjects [39]. The latter may also partly explain the negative correlation between creatinine level and ALP observed in our study, since muscle mass (which is positively correlated with creatinine) increases with age of athletes. Additional studies with different designs are warranted to clarify this point.

Conclusion

In conclusion, our data show that the *SOD2* TT genotype might be unfavorable for high-intensity athletic events.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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Table 1. Stratification of athletes involved in the study according to type, intensity, and duration of exercise

I. Low-intensity (n=180)	II. Long-endurance (n=235)	III. Middle-endurance group (n=187)	IV. Short-endurance group (n=86)	V. Mixed group (n=1452)	VI. Power group (n=321)	VII. Strength group (n=203)
Curling players (16), equestrian athletes (10), sailors (50), shooters (69), rhythmic gymnasts (7), divers (17), chess players (11)	5-25 km swimmers (30), cross-country skiers (79), biathletes (61), marathon runners (9), race walkers (8), road cyclists (28), triathletes (20)	3-10 km runners (20), 5-10 km skaters (3), 800-1500 m swimmers (24), rowers (140)	200-400 m swimmers (22), 800-1500 m runners (25), 1500-3000 m skaters (17), 500-1000 m kayakers (10), 1500-3000 m short trackers (7), 500-1000 m canoeists (5)	Badminton players (24), basketball players (109), baseball players (38), boxers (143) handball players (92), ice hockey players (111), karate athletes (22), taekwondo athletes (18), field hockey players (19), synchronized swimmers (27), fencers (64), freestyle skiers (11), figure skaters (76), archers (24), Nordic combined athletes (10), snowboarders (33), football players (36), pentathletes (23), softball players (31), rugby players (48), table tennis players (11), volleyball players (115), mini-football players (9), water polo players (59), whitewater slalomists (5), wrestlers (294)	50-100 m swimmers (53), 100-400 m runners (57), 500-1000 m speed skaters (15), alpine skiers (26), track-and-field jumpers (37), ski jumpers (7), throwers (18), 200 m kayakers (26), 500-1000 m short trackers (16), 200 m canoeists (24), sprint cyclists (31), decathletes (8), heptathletes (3)	Artistic gymnasts (63), powerlifters (35), weightlifters (105)

Table 2. Characteristics of Russian athletes involved in muscle/liver damage markers study.

	Athletes		
	Males	Females	All
All, *n*	787	657	1444
Age, years	23.8 (5.4)*	22.8 (5.7)	23.3 (5.5)
Elite, *n*	377	311	688
Age, years	26.8 (5.6)**	25.8 (5.6)**	26.4 (5.6)
Sub-elite, *n*	410	346	756
Age, years	21.0 (3.3)	20.0 (4.1)	20.5 (3.7)

Values are means (SD). *P=0.0007, statistically significant differences between males and females. **P<0.0001, statistically significant differences between elite and sub-elite athletes in both sexes.

Table 3. Serum biochemical parameters of Russian athletes in relation to their specialty, sex and level of achievement.

Group	*n*	CK, U/l	Creatinine, μmol/l	ALT, U/l	AST, U/l	ALP, U/l
I. Low-intensity group	156					
Females elite	32	103 (56.4)	80.9 (7.2)	15 (6.5)	19.4 (5.5)	77.8 (32)
Females sub-elite	27	116 (79.4)	82.2 (10.4)	17 (11.5)	21.3 (8.5)	79.4 (36)
All females	59	109 (67)*	81.5 (8.7)	15.9 (9.1)	20.3 (7.0)	78.5 (34)
Males elite	52	162 (97)	96.2 (12.2)	28.4 (18.7)	25.1 (9.6)	96.6 (49.1)
Males sub-elite	45	194 (135)	96.9 (14.9)	21.0 (7.9)	23.8 (6.2)	113.6 (49.3)
All males	97	177 (117)*	96.5 (13.5)	24.9 (15.1)	24.5 (8.2)	104.5 (49.7)
II. Long-endurance group	124					
Females elite	22	130 (56)	81.6 (8.6)	17.7 (8.1)	21 (6.4)	60.9 (19.7)
Females sub-elite	34	208 (149)	84.5 (11.1)	19.2 (8.4)	25.9 (8.2)	79.9 (23.6)
All females	56	177 (126)*	83.4 (10.2)	18.6 (8.2)	23.9 (7.8)	72.3 (23.9)
Males elite	30	206 (156)	95.3 (10.4)	22.8 (9.5)	24.7 (9.5)	62.3 (13.2)
Males sub-elite	38	306 (293)	91 (11.9)	21.4 (6.9)	30.6 (17)	121.4 (60.6)
All males	68	262 (246)*	92.9 (11.4)	22 (8.1)	28.0 (14.4)	95.3 (54.5)
III. Middle-endurance group	83					
Females elite	15	150 (119)	80.1 (15.2)	21.8 (10.2)	24.1 (4.9)	74.7 (25.5)
Females sub-elite	28	264 (308)	82.6 (12.3)	16.5 (5.4)	26.8 (8.4)	97.3 (46.7)
All females	43	224 (262)*	81.7 (13.2)	18.4 (7.7)	25.9 (7.4)	89.4 (41.7)
Males elite	15	170 (88)	97.4 (10.8)	23.1 (11.8)	23.3 (10.0)	78.3 (26.7)
Males sub-elite	25	262 (167)	92.5 (11.9)	22 (15.7)	34.5 (52.7)	97.9 (35.9)
All males	40	239 (147)*	94.4 (11.6)	22.4 (14.2)	30.3 (42.1)	90.5 (33.8)
IV. Short-endurance group	60					
Females elite	15	137 (73)	83.5 (10.1)	20.3 (8.2)	25.2 (7.7)	67.9 (30.7)
Females sub-elite	6	272 (169)	87.2 (17.8)	14.5 (2.4)	22.2 (5.1)	107.8 (68.1)
All females	21	176 (121)*	84.6 (12.4)	18.6 (7.5)	24.3 (7.0)	79.3 (46.5)
Males elite	24	181 (79)	95.3 (17.9)	21.3 (9.3)	23.9 (6.9)	85.7 (36.8)
Males sub-elite	15	240 (116)	99.1 (23.2)	22.3 (14.5)	22.7 (8.8)	95.3 (31.6)
All males	39	204 (98)*	96.8 (19.9)	21.7 (11.2)	23.5 (7.6)	89.4 (34.8)
V. Mixed group	757					
Females elite	166	252 (252)	88.8 (14.2)	17.9 (7.7)	25.1 (10.6)	57 (21.3)
Females sub-elite	201	220 (204)	85.0 (26.9)	17.6 (6.4)	24.2 (8.0)	86.4 (53.2)
All females	367	234 (227)*	86.7 (22.1)	17.7 (7.0)	24.6 (9.3)	73.1 (44.4)
Males elite	167	338 (298)	105 (14)	28.6 (18.4)	29.5 (15.9)	72.3 (25.4)
Males sub-elite	223	419 (428)	99.9 (12)	24.2 (12.3)	29.7 (18.1)	95.5 (42.4)
All males	390	384 (380)*	102.1 (13.2)	26.1 (15.4)	29.6 (17.2)	85.6 (37.9)
VI. Power group	171					
Females elite	34	197 (128)	78.2 (17.7)	22.3 (13.3)	25.0 (8.5)	84.7 (37.3)
Females sub-elite	32	158 (94)	82.2 (13.8)	14.0 (5.4)	20.1 (5.3)	95.5 (50.5)
All females	66	178 (113)*	80.2 (15.9)	18.3 (11.0)	22.6 (7.5)	89.9 (44.2)
Males elite	72	350 (296)	100.7 (16.9)	27.9 (15.2)	29.5 (15.1)	92.8 (41.5)
Males sub-elite	33	311 (282)	92.5 (16.1)	23.8 (15.9)	26.2 (8.7)	110.2 (55.3)
All males	105	338 (291)*	98.1 (17.1)	26.6 (15.5)	28.5 (13.4)	98.2 (46.7)
VII. Strength group	93					
Females elite	26	705 (589)	92.3 (17.5)	32.4 (20.2)	40.7 (17.5)	108.7 (58.4)
Females sub-elite	19	508 (403)	84.4 (10.4)	29.9 (11.1)	37.4 (13.4)	262 (130)
All females	45	620 (521)*	88.9 (15.2)$^{\&}$	31.4 (16.7)*	39.3 (15.8)*	175 (122)*
Males elite	17	587 (520)	106.8 (23.9)	29.3 (22.9)	35.2 (13.9)	110.7 (75.4)
Males sub-elite	31	831 (669)	112.6 (36.6)	37.9 (22.6)	39.9 (15.3)	202.4 (164)
All males	48	748 (627)*	110.5 (33)*	34.8 (22.8)$^{\Delta}$	38.3 (14.8)$^{\Delta}$	170 (145)*

Values are mean (SD). Comparisons between all subgroups of athletes were performed by ANOVA.

*$P < 0.0001$, $^{\&}P \leq 0.05$, $^{\Delta}P \leq 0.001$.

Table 4. Relationships between biochemical parameters in male and female athletes (n = 1,444).

	Creatinine	**ALT**	**AST**	**ALP**
CK	Males: r = 0.144, P < 0.0001. Females: r = 0.122, P = 0.0016.	Males: r = 0.425, P < 0.0001. Females: r = 0.474, P < 0.0001.	Males: r = 0.692, P < 0.0001. Females: r = 0.658, P < 0.0001.	Males: r = 0.13, P = 0.0002. Females: P > 0.05.
Creatinine		Males: r = 0.1, P = 0.0042. Females: r = 0.10, P = 0.0094.	Males: P > 0.05. Females: r = 0.099, P = 0.01.	Males: r = -0.21, P < 0.0001. Females: r = -0.28, P < 0.0001.
ALT			Males: r = 0.64, P < 0.0001. Females: r = 0.66, P < 0.0001.	Males: P > 0.05. Females: r = 0.09, P = 0.0163.
AST				Males: r = 0.134, P = 0.0002. Females: r = 0.153, P < 0.0001.

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase. P < 0.05, statistically significant relationships (by Spearman's rank correlation).

Table 5. *SOD2* genotype distribution and frequencies of *SOD2* gene T allele in athletes stratified by type, intensity, and duration of exercise.

Group	n	Genotypes (n)			*P*	T allele, %	*P*
		CC	CT	TT			
I. Low-intensity group	180	43	76	61	0.0459*	55.0	0.042*
II. Long-endurance group	235	58	116	61	0.815	50.6	0.594
III. Middle-endurance group	187	43	98	46	0.505	50.8	0.594
IV. Short-endurance group	86	28	41	17	0.394	43.6	0.192
V. Mixed group	1452	382	735	335	0.494	48.4	0.621
VI. Power group	321	95	166	60	0.071	44.5	0.045*
VII. Strength group	203	51	114	38	0.0844	46.8	0.395
All athletes	2664	700	1346	618	0.455	48.5	0.641
Controls	917	245	443	229	1.000	49.1	1.000

*$P<0.05$, statistically significant differences. Comparison with controls was by χ^2 test

CC wild-type homozygote; CT heterozygote; TT mutant homozygote

Fig. 1. *SOD2* TT genotype frequency amongst athletes of different groups is shown. TT genotype frequency in the low-intensity group (I) was 33.9%. By comparison, it was 26.0, 24.6, 19.8, 23.1, 18.7 and 18.7% for groups II, III, IV, V, VI and VII, respectively (P = 0.0001 for linear trend).