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ORIGINAL ARTICLE

Blood metabolic response to a long-term wheelchair rugby training

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Study design: A cross-sectional study with comparison group.
Objectives: To evaluate the effects of long-term wheelchair rugby (WR) training on lipid profile, blood antioxidant status and the brain-derived neurotrophic factor (BDNF) level.
Setting: Academy of Physical Education, Katowice, Poland.
Methods: Thirty-two males with chronic cervical spinal cord injury (SCI) assigned into the physically active 'low-point' (LP, $n=15$) or 'high-point' (HP, $n=8$) WR players groups and the sedentary manual wheelchair users (SED, $n=9$) participated in this study. Fasting blood samples were collected at rest for assessment of activities of antioxidant enzymes, concentrations of reduced glutathione, uric acid, malondialdehyde (MDA), lipid profile measures and BDNF.
Results: No significant differences were found in anthropometric measures and serum lipid profile indices, although a slight tendency toward higher high-density lipoprotein cholesterol level was evidenced in WR players. Significantly lower serum malondialdehyde (MDA) and significantly higher levels of the overall enzymatic antioxidant potential index (EAP) in WR players, compared with SED, may reflect some WR training-induced increase in the blood's antioxidant capacity. There was also a slight tendency toward higher serum BDNF level in WR players compared with the SED group and a significant positive association between years of WR training and the BDNF level.
Conclusion: A voluntary participation in a long-term WR training program has several health promoting outcomes for individuals with chronic SCI. Among the most important are enhancement of the blood antioxidant defense capacity evidenced by lower MDA and higher EAP levels, as well as WR training-induced activation of BDNF signaling.
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INTRODUCTION

Spinal cord injury (SCI) is a devastating condition resulting in a temporary or a permanent loss of normal motor, sensory or autonomic function caused by interruption of pathways from activating centers in the brain to the peripheral sympathetic nervous system and deprivation of neurological activity.[1,2] Among the consequences of SCI are adverse changes in body composition resulting from physical disability, which could lead to higher prevalence of obesity,[3] hypertension, insulin resistance and dyslipidemia, which markedly increases the risk of cardiovascular diseases (CVD) in individuals with paraplegia and tetraplegia.[4,5] The mechanism of accelerated CVD may be partially explained on the basis of the changes in lipid metabolism. The majority of the studies in individuals with SCI have shown an unfavorable lipid profile caused by reduced serum high-density lipoprotein cholesterol (HDL-C) and higher low-density lipoprotein cholesterol (LDL-C).[6] Moreover, a crucial role in pathogenesis of CVD in patients with SCI is attributed to oxidative stress induced by reactive oxygen and/or reactive nitrogen species (ROS/RNS). It appears that individuals with SCI are more prone to oxidative stress due to decreased antioxidant capacity, physiological and metabolic imbalances, multiple organ dysfunction and generalized infection and inflammation.[2,4] An imbalance in prooxidant-antioxidant status may contribute to the increased prevalence of CVD in individuals with SCI. An increase in the physical activity level can be a potential target of therapeutic intervention to improve lipid profile, attenuate oxidative stress and promote functional independence. Moreover, regular physical activity has given positive benefits in cognitive, emotional and motor domains.[7] What is noteworthy, exercise activity is well known as a lifestyle model to improve functional recovery from both brain and SCI by increasing expression of brain-derived neurotrophic factor (BDNF).[8] BDNF is known to affect the survival of motor neurons and remodeling of injured axons and to induce antidepressant effects in behavioral models of depression.[9]

Wheelchair rugby (WR) is a full contact team sport played by tetraplegic athletes with different levels of limitations of movement, strength and control in arms, trunk and legs. According to the degree of these limitations, they may be allocated to one of the seven sport classes, ranging from 0.5 up to 3.5 points, describing, respectively, the most limited (0.5) up to the highest (3.5) level of functional ability, according to the classification system adopted by the International Wheelchair Rugby Federation (IWRF; available at: http://www.iwrf.com/resources/iwrf_docs/IWRF_Classification_Manual_3rd_Edition_-rev-2015_%28English%29.pdf). The typical role of the 'low-point' (LP) players (sport classes 0.5–1.5) is to act as blockers of the

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opponents and the occasional ball handlers. Because of their higher functional abilities, the 'high-point' (HP) players (sport classes 2.0–3.5), with more opportunities for movement on the court, act as ball handlers and fast playmakers.

The present study was aimed on assessment of the effects of long-term WR training on lipid profile, antioxidant status and the BDNF level in WR players with tetraplegia. The research is of relevance to determine the potential effects of exercise activity on metabolic and neurological response.

MATERIALS AND METHODS

Participants

Thirty-two males with cervical SCI, who volunteered to participate in this study, were divided into two groups of inactive (sedentary (SED)) wheelchair users ($n=9$) and WR players ($n=23$) recruited from four teams of the Polish Wheelchair Rugby League (including seven players of the Poland National Wheelchair Rugby Team). All WR players participated regularly in training sessions supervised by professional coaches (once a week for 3 h) since more than 3 years (median 6.0 and 8.5 years for LP and HP players, respectively), which could be categorized as a long-term training. The inclusion criteria were as follows: traumatic cervical SCI at the C4–C8 level; time post injury >3 years; locomotion—manual wheelchair; and signed informed consent. The exclusion criteria were as follows: traumatic cervical SCI at $\leqslant 15$ years; and age $\leqslant 18$ years. Despite a relatively small sample size, the WR players were allocated into the LP ($n=15$, sport classes 0.5–1.5) and HP ($n=8$, sport classes 2.0–3.5) groups, according to the IWRF Classification System. The level of lesion of sedentary wheelchair users and WR players varied from C4 to C6 and from C5 to C7, respectively. According to self-reported information, the most frequent cause of traumatic injury in our subjects was road traffic accident (15 individuals), diving in shallow water (11 individuals) and other causes, such as fall from height. Measurements of body composition included body weight (to the nearest 0.1 kg) using chair medical scale WE150P3 K (MENSOR, Warsaw, Poland), body height to the nearest 0.5 cm while lying supine with straight legs and flexed feet using a non-stretch fiberglass fabric tape, waist circumference (WC) and hip circumference (HC) measured at the narrowest part of the waist after a normal expiration and around the widest portion of the buttocks, respectively. None of the participants had any previous cardiovascular problems, and their basic physical characteristics are presented in Table 1.

The study was performed during the season when the training plan was focused not only on the development of stability of the shoulder and core through concurrent strength and endurance training but also on improvement of technical and tactical game skills, such as ball handling (passing, catching and dribbling), as well as effective wheelchair maneuverability (pushing, starting, stopping, directional changes and tackling, and blocking). It was characterized by frequent short-term efforts of the maximum intensity requiring speed, strength, endurance and coordination.

The present study was conducted according to the Declaration of Helsinki guidelines (Act No 9/2012 of 8 March 2012). The protocol of the study was approved by the Ethics Committee of the Academy of Physical Education. All individuals were informed of the purpose and the nature of the study before giving their written consent to participate.

Analytical procedures

After at least 8 h of fasting, fresh whole-blood samples of all participants collected into heparinized tubes were immediately assayed for reduced glutathione by a colorimetric method with 5,5′-dithiobis-2-nitrobenzoic acid. A portion of heparinized blood was centrifuged for 10 min at 1000*g* at 4 °C to separate plasma and erythrocytes, that were then washed three-times with cold (4 °C) saline and kept frozen at −80 °C until analysis for activities of antioxidant enzymes, that is, superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GPx, EC 1.11.1.9), catalase (CAT, EC 1.11.1.6) and glutathione reductase (GR, EC 1.6.4.2) as previously described in Michalczyk *et al.*[10] The activities of all antioxidant enzymes were measured at 37 °C and expressed per 1 g of hemoglobin as assayed by a standard cyanmethemoglobin method using a diagnostic kit (HG980, Randox, Crumlin, UK). Plasma uric acid concentration was assessed with the Ransod UA230 Kit (Randox). The remaining portion of the fresh whole-blood sample was set to clot and then spun to separate serum.

Concentrations of serum glucose, T-C (total cholesterol), HDL-C and TGs (triglycerides) were assessed by enzymatic methods using commercially available diagnostic kits (GL 2623, CH201, CH203, TR210, respectively) from

Table 1 Characteristics of SCI participants

Variable	*SED* (n = *9*)		*LP* (n = *15*)		*HP* (n = *8*)	
	X ± *s.d.*	*Median (min–max)*	*X* ± *s.d.*	*Median (min–max)*	*X* ± *s.d.*	*Median (min–max)*
Age (years)	30.1 ± 3.6	31.0 (24–36)	32.3 ± 5.9	33.0 (22–42)	33.4 ± 4.5	34.5 (26–39)
Age at time of injury (years)	21.0 ± 3.0	20.0 (17–25)	19.9 ± 4.0	18.0 (15–27)	20.6 ± 2.7	21.0 (16–24)
Years post injury	10.3 ± 3.7	9.0 (5–15)	14.5 ± 5.5	12.0 (8–26)	13.8 ± 3.7	14.0 (9–19)
Training experience (years)	—	—	6.5 ± 2.7	6.0 (3–11)	9.3 ± 4.2	8.5 (4–14)
Lesion level	C4 (*n*=2)		C5 (*n*=9)		C7 (*n*=1)	
	C5 (*n*=2)		C6 (*n*=2)		C5–C7 (*n*=3)	
	C4–C5 (*n*=1)		C5–C6 (*n*=2)		C6–C7 (*n*=4)	
	C4–C6 (*n*=2)		C6–C7 (*n*=2)			
	C5–C6 (*n*=2)					
AIS	A (*n*=4)		B (*n*=9)		B (*n*=2)	
	B (*n*=5)		C (*n*=5)		C (*n*=1)	
			D (*n*=1)		D (*n*=5)	
Body height (cm)	180.0 ± 5.7		181.4 ± 8.5		181.3 ± 4.9	
Body mass (kg)	71.9 ± 8.1		72.0 ± 8.2		78.3 ± 7.3	
BMI (kg m^{-2})	22.2 ± 2.2		21.9 ± 2.7		23.8 ± 2.3	
WC (cm)	94.9 ± 8.4		91.8 ± 7.4		91.5 ± 5.6	
HC (cm)	99.3 ± 10.4		94.9 ± 5.5		89.7 ± 4.9	
WHR	0.96 ± 0.01		0.97 ± 0.06		1.02 ± 0.04	
WHtR	0.54 ± 0.05		0.51 ± 0.05		0.51 ± 0.02	

Abbreviations: AIS, American Spinal Cord Injury Association (ASIA) Impairment Scale; BMI, body mass index; HC, hip circumference; HP, high-point WR players; LP, low-point WR players; SCI, spinal cord injury; SED, sedentary wheelchair users; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Randox. Concentration of serum BDNF (brain-derived neurotrophic factor) was measured using the RayBio Human BDNF ELISA Kit (RayBiotech, Inc., Norcross, GA, USA). Concentrations of LDL-C were calculated using the Friedewald formula. To evaluate risk for vascular disease, the lipid ratios (T-C/HDL-C, LDL-C/HDL-C and TG/HDL-C) and the atherogenic index of plasma (AIP = $\log_{10}$(TG/HDL) with TG and HDL-C expressed in molar concentrations) were calculated.[11] Serum insulin was measured by the immunoradiometric method (Insulin IRMA IM3210, Immunotech SA, Prague, Czech Republic). The homeostasis model assessment (HOMA-IR) was used to estimate insulin resistance using the following formula: HOMA-IR = glucose (mmol l^{-1})*insulin(mlU l^{-1})/22.5. Assessment of lipid peroxidation, as an indicator of tissue injury induced by ROS, was carried out using the thiobarbituric acid assay by reading the absorption at $\lambda = 532$ nm using a multi-mode microplate reader (Synergy 2 SIAFRT, BioTek, Winooski, VT, USA). Standard curve was prepared using water solutions of 1,1,3,3-tetramethoxypropane as standard, and the results were expressed as μmol malondialdehyde (MDA) formed per liter of plasma (μmol l^{-1}).

Data analyses

All data are reported as means ± s.d. A one-way analysis of variance (ANOVA) with group as the main factor, followed, when appropriate, by the Tukey's test, was used to test the significance of between-group differences. Spearman's rank order correlation coefficients were computed to reveal relationships between selected variables. In order to elucidate between-group differences in the capacity of the enzymatic antioxidant defense system in blood, the index of overall enzymatic antioxidant potential index (EAP) was calculated as a sum of standardized values of activities of antioxidant enzymes (SOD, CAT, GPx and GR). Standardized value means that a value is expressed in terms of differences from the mean recorded in the SED group, divided by the standard deviation. The following formula was used:

$$EAP = \sum(SOD_{ST} + CAT_{ST} + GPx_{ST} + GR_{ST})$$

With the aim of interpreting the results by indicating the relative degree to which the variance found in the ANOVA is associated with the group (the main factor), the Eta squared (η^2), as a measure of the effect size in ANOVA, was calculated using the following formula: $\eta^2 = SS_{effect}/SS_{total}$, where SS_{effect} is the sum of squares for whatever effect is of interest, and SS_{total} is the total sums of squares for all effects, interactions and errors in the ANOVA. In all cases, a $P < 0.05$ was considered significant. All statistical analyses were performed with STATISTICA 10.0 (StatSoft, Tulsa, OK, USA) software.

RESULTS

There were no significant between-group differences in any of the anthropometric measures of the participants (Table 1); however, only a marginal tendency toward lower levels of WC, HC and WHtR was observed in both groups of WR players, compared with SED controls with SCI. Despite the lack of significant differences in concentrations of serum lipid profile indices, there was a slight tendency toward higher HDL-C level (Table 2) in both groups of WR players, but the differences did not reach significance ($P = 0.09$, $\eta^2 = 0.15$).

Variables describing the antioxidant status of the blood of all groups of participants are presented in Table 3. Except for a significantly higher GR activity in the LP group, as well as significantly higher mean EAP titers and significantly lower MDA levels in both LP and HP groups, compared with SED, no significant differences were recorded among other assayed measures (Table 3). There was only a marginal

Table 2 Serum lipid profile in SED, LP and HP wheelchair rugby players

Variable	*SED*	*LP*	*HP*	*One-way ANOVA results*
Glucose (mg dl^{-1})	89.5 ± 12.8	91.9 ± 13.3	91.2 ± 6.8	$F = 0.11$, $P = 0.89$, $\eta^2 = 0.01$
T-C (mg dl^{-1})	160.5 ± 22.4	151.6 ± 22.7	171.5 ± 37.8	$F = 1.43$, $P = 0.26$, $\eta^2 = 0.09$
HDL-C (mg dl^{-1})	36.3 ± 6.2	44.1 ± 11.0	47.8 ± 14.7	$F = 2.51$, $P = 0.09$, $\eta^2 = 0.15$
LDL-C (mg dl^{-1})	102.4 ± 23.0	83.4 ± 25.4	99.9 ± 35.9	$F = 1.67$, $P = 0.21$, $\eta^2 = 0.10$
TG (mg dl^{-1})	112.4 ± 27.9	121.7 ± 47.7	100.8 ± 27.1	$F = 0.77$, $P = 0.47$, $\eta^2 = 0.05$
T-C/HDL-C	4.5 ± 0.9	3.8 ± 1.6	3.8 ± 0.9	$F = 1.08$, $P = 0.35$, $\eta^2 = 0.07$
LDL-C/HDL-C	2.9 ± 0.8	2.1 ± 1.2	2.2 ± 0.8	$F = 1.85$, $P = 0.18$, $\eta^2 = 0.11$
TG/HDL-C	3.1 ± 0.6	3.2 ± 2.4	2.4 ± 1.2	$F = 0.54$, $P = 0.59$, $\eta^2 = 0.04$
AIP	0.12 ± 0.1	0.11 ± 0.2	0.09 ± 0.1	$F = 0.09$, $P = 0.91$, $\eta^2 = 0.01$
Insulin (mU l^{-1})	7.4 ± 3.1	6.5 ± 2.9	8.1 ± 3.9	$F = 0.65$, $P = 0.53$, $\eta^2 = 0.04$
HOMA-IR	1.69 ± 0.9	1.5 ± 0.9	1.7 ± 1.1	$F = 0.13$, $P = 0.88$, $\eta^2 = 0.01$

Abbreviations: AIP, atherogenic index of plasma; ANOVA, analysis of variance; HP, high-point WR players; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment; LP, low-point WR players; SED, sedentary wheelchair users; T-C, total cholesterol; TG, triglycerides.

Table 3 Blood antioxidant defense, plasma MDA concentration and serum BDNF level in SED, LP and HP wheelchair rugby players

Variable	*SED*	*LP*	*HP*	*one-way ANOVA results*
SOD (U gHb^{-1})	1070.7 ± 99.2	1148.7 ± 334.0	1294.0 ± 238.2	$F = 1.54$, $P = 0.23$, $\eta^2 = 0.09$
GPx (U gHb^{-1})	206.3 ± 32.5	220.4 ± 41.8	228.9 ± 33.3	$F = 0.12$, $P = 0.89$, $\eta^2 = 0.008$
CAT (U gHb^{-1})	59.5 ± 7.9	61.7 ± 11.8	60.9 ± 11.1	$F = 0.80$, $P = 0.46$, $\eta^2 = 0.05$
GR (U gHb^{-1})	27.9 ± 2.7	35.2 ± 6.4[a]	30.9 ± 6.7	$F = 4.78$, $P = 0.016$, $\eta^2 = 0.25$
GSH (μg gHb^{-1})	2.9 ± 0.7	2.7 ± 0.7	2.3 ± 0.5	$F = 2.16$, $P = 0.13$, $\eta^2 = 0.01$
Uric acid (mg dl^{-1})	5.9 ± 0.6	5.5 ± 1.5	5.9 ± 1.2	$F = 0.59$, $P = 0.56$, $\eta^2 = 0.04$
EAP	0.00	3.7 ± 3.03[a]	3.5 ± 2.7[a]	$F = 6.23$, $P = 0.01$, $\eta^2 = 0.30$
MDA (μmol l^{-1})	4.7 ± 1.3	3.4 ± 0.8[a]	3.3 ± 0.9[a]	$F = 6.19$, $P = 0.01$, $\eta^2 = 0.29$
BDNF (ng ml^{-1})	228.9 ± 32.1	254.5 ± 30.8	251.5 ± 29.1	$F = 2.07$, $P = 0.14$, $\eta^2 = 0.12$

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CAT, catalase; EAP, enzymatic antioxidant potential index; GR, glutathione reductase; GPx, glutathione peroxidase; GSH, glutathione reduced; HP, high-point WR players; LP, low-point WR players; MDA, malondialdehyde; SED, sedentary wheelchair users; SOD, superoxide dismutase.
[a]Significantly ($P < 0.05$) different compared with SED.

Table 4 Spearman's rank correlation coefficients between training experience (years of WR training) and selected variables

Correlation between the selected variables	*WR players (n = 23)*	
	R	P
Years of WR training		
EAP	0.59	0.001
GR	0.52	0.01
MDA	–0.56	0.001
BDNF	0.37	0.05
HDL-C	0.30	0.09

Abbreviations: BDNF, brain-derived neurotrophic factor; EAP, enzymatic antioxidant potential index; GR, activity of glutathione reductase; HDL-C, high-density lipoprotein cholesterol; MDA, malondialdehyde; WR, wheelchair rugby player.

tendency toward higher activities of SOD in both LP and HP groups compared with SED. The next aim of the present study was to find-out whether WR training would affect serum BDNF concentration (Table 3). Although there were no marked differences between the groups, BDNF levels tended to increase in LP and HP players compared with values recorded in the SED group.

In order to reveal whether and how the overall duration of sports activity (years of WR training) affected the blood variables tested (lipid profile measures, antioxidant defense status, insulin resistance), the Spearman rank correlation coefficients for the whole group of WR players (LP and HP) were calculated, and selected statistically significant associations are shown in Table 4.

DISCUSSION

It is well documented that the forced sedentary lifestyle in individuals with SCI is associated with changes in body composition, such as loss of lean body mass and accumulation of adipose tissue in the visceral and subcutaneous abdominal locations in the abdominal region. Excess abdominal fat, especially visceral fat, increases the risk for CVD and diabetes mellitus.[3]

According to de Groot *et al.*[12] the participation in physical activity in early rehabilitation of spinal cord injured individuals has an important role in the improvement of lipid profile, insulin sensitivity and maintenance of the level of physical fitness desirable for their function in daily life. Interestingly, as reported by van Koppenhagen *et al.*[13] wheelchair exercise capacity of individuals with SCI stabilizes between 1 and 5 years after discharge from inpatient rehabilitation. It is worth noting that six out of 23 WR players underwent a training program for more than 5 years (see Table 1). Given that the usual clinical measures of total body fat, such as body mass index (BMI), underestimate the degree of adiposity in individuals with SCI,[6] other simple measures such as WC and waist–to-height ratio (WHtR) are recommended as more sensitive alternative to BMI in this population.[5,14] However, taking the WR players as a whole group compared with sedentary individuals with SCI, there was only a marginal tendency toward lower the levels of WC (91.4 ± 7.1 vs 94.8 ± 8.4 cm, $P = 0.7$), hip circumference (93.4 ± 5.7 vs 99.3 ± 10.4 cm, $P = 0.1$) and WHtR (0.51 ± 0.04 vs 0.54 ± 0.05, $P = 0.6$). Of note, the WC recorded in about 56% inactive wheelchair users from the SED group, but only in 39% of WR players, exceeded the cutoff ($WC \geqslant 94$ cm) for identifying adverse CVD risk in individuals with SCI.[14]

One of the main goals of the present study was to examine whether the long-term WR training would affect the blood lipid profile, insulin sensitivity and the antioxidant defense capacity in WR players. It is well established that physical inactivity leads to metabolic abnormalities related to high T-C, high TG and low HDL-C.[12] In our study, no statistically significant between-group differences in any of the individual lipid profile measures were found, although compared with sedentary individuals, the serum HDL-C content tended to be higher by 21 or 32% in our LP or HP WR players, respectively. Of note, a one-way ANOVA revealed a close to significance effect of WR training on HDL-C ($P = 0.09$, $\eta^2 = 0.15$). Moreover, common lipid ratios such as LDL-C/HDL-C and T-C/HDL-C, considered to be stronger predictors of CVD than HDL-C alone, tended to slightly lower the levels in WR players compared with sedentary disable men, but the differences did not reach significance. However, a somewhat more favorable lipid profile in tetraplegic WR players, and especially a reduction in T-C/HDL-C ratio by about 0.7 unit, may suggest a somewhat lower risk of coronary heart disease in the physically active, compared with the inactive spinal cord disabled men.[6] Similar, although more favorable, changes in lipid profile in tetraplegic rugby players compared with inactive SCI wheelchair users were presented by Hübner-Woźniak *et al.*,[15] which may be attributed to the differences in training frequency applied in our study (one session a week for 3 h) and in that reported by Hübner-Woźniak *et al.*[16] (two sessions a week for 2–3 h). More favorable effects of high intensity, than of low intensity arm exercise training, on lipid profile and insulin sensitivity in individuals with SCI were also reported by de Groot *et al.*[12] Of note, as shown by normal insulin and HOMA-IR levels in most individuals (87% of WR players and 78% of the sedentary controls), a long-term rugby training has not substantially affected insulin sensitivity in our athletes.

One of the well characterized secondary mechanisms occurring early after SCI is the formation of ROS/RNS, a process considered central in the etiology of cellular death and functional loss.[1,17] There are reports that patients with SCI experience increased oxidative stress and reduced antioxidant defense, especially the first year after injury.[18] Therefore, there is an increasing interest in finding efficient, potentially beneficial antioxidant therapies for SCI patients.[17,19]

It is well known that the antioxidant-prooxidant balance may be improved by regular, non-exhaustive physical exercise.[20] Indeed, enhanced blood antioxidant capacity and attenuated exercise-induced oxidative stress have been clearly evidenced in able-bodied subjects,[21] but little is known about these aspects of adaptation to exercise training in individuals with SCI. It has been shown that, in the case of tetraplegics, the most effective is a long-term resistance training, such as WR, focused on improvement of the upper body muscle strength.[22] Positive adaptive response to WR training, characterized by increased activities of red blood cell antioxidant enzymes (CAT and GPx), in both able-bodied and WR players, was recently evidenced by Hübner-Woźniak *et al.*[16] In contrast to this, long-term functional electrical stimulation exercise appeared to be insufficient to change prooxidant/antioxidant status in individuals with SCI.[4]

As regards the effects of WR training on the blood antioxidant status in the present study, no significant differences between activities of antioxidant enzymes, except for GR, were observed between sedentary wheelchair users and WR players. In the latter group, the medium effect of exercise training on GR activity ($P = 0.016$, $\eta^2 = 0.25$) was observed. It should be stressed, however, that the antioxidant defense system in the blood is complex and represents an interaction of different components, including antioxidant enzymes, non-enzymatic antioxidants and repair of oxidatively damaged molecules. Hence, to quantify the capacity of the most important blood enzymatic antioxidant defense system, the index of the overall EAP

index was calculated as a sum of standardized values of activities of all antioxidant enzymes studied (SOD, CAT, GPx and GR). Noteworthy, it appeared that, compared with the sedentary wheelchair users, this index was significantly higher in both groups (HP and LP) of WR players, and the large effect of WR training on this index ($P=0.01$, $\eta^2=0.30$) was revealed by one-way ANOVA. Moreover, the EAP data for the whole group of WR players were positively correlated with duration of their sports activity (see Table 4).

It is well known that attack of ROS on polyunsaturated fatty acids results in the formation of hydroperoxides that are subsequently decomposed to various aldehyde compounds, MDA being the major product.[21] In the present study, plasma MDA level was significantly lower in both groups of WR players than in sedentary disabled wheelchair users. Noteworthy, Spearman's rank order correlation analysis revealed significant inverse interrelationships between the enzymatic EAP index and the MDA level ($r=-0.43$, $P=0.01$), as well as between serum MDA content and duration of athletes' sports activity (years; see Table 4). Our data indicate that higher antioxidant capacity provided by a combined activity of antioxidant enzymes in the blood of WR players, as shown by the EAP index, and substantially reduced blood MDA (marker of lipid peroxidation) might be consequent to positive health outcomes induced by athletes' long-term involvement in WR training activities.

One cannot also ignore the fact that individuals with SCI have several psychological problems such as depression, anxiety and lower quality of life compared with able-bodied persons. The most common psychological issue associated with SCI is depression, affecting about 30% of patients.[23] One of the therapeutic targets for improving psychological status of persons with SCI is sports activity.[24] Participation in sports that involve propulsion, such as wheelchair basketball, rugby or tennis activities, may increase upper body strength, the factor crucial for functional independence. Several recent reports point to the role of BDNF in depression.[9] On the other hand, several human studies have evidenced the beneficial antidepressant effects of aerobic or resistance exercise training in spinal cord injured individuals.[8] One of the interesting findings of the present study is a tendency toward somewhat higher serum BDNF values in WR players compared with the SED group, which may suggest exercise-induced activation of BDNF signaling. Although we did not observe significant differences between physically inactive and active groups, the Spearman rank correlation analysis revealed a significant positive association between years of WR training and the BDNF level, whereas one-way ANOVA demonstrated a medium effect of rugby training ($P=0.14$; $\eta^2=0.12$) on serum BDNF level.

Limitations of the study

The main limitations of this study were its cross-sectional design, relatively small number of participants and the lack of information on their dietary intakes, which will limit the generalizability of results.

CONCLUSIONS

A voluntary participation in a long-term WR training program has several health promoting outcomes for individuals with chronic SCI. Among the most important are enhancement of the blood antioxidant defense capacity evidenced by lower MDA and higher EAP levels, as well as WR training-induced activation of BDNF signaling. On the other hand, only some marginaly favorable changes in body composition (WC and WHtR) and lipid profile (HDL-C) were induced by long-term WR training.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Oyinbo CA. Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. *Acta Neurobiol Exp* 2011; **71**: 281–299.
2 Bedreag OH, Rogobete AF, Sărăndan M, Cradigati A, Păpurică M, Roşu OM *et al.* Oxidative stress and antioxidant therapy in traumatic spinal cord injuries. *RJAIC* 2014; **21**: 123–129.
3 Gater DR Jr. Obesity after spinal cord injury. *Phys Med Rehabil Clin N Am* 2007; **18**: 333–351.
4 van Duijnhoven N, Hesse E, Janssen T, Wodzig W, Scheffer P, Hopman M. Impact of exercise training on oxidative stress in individuals with spinal cord injury. *Eur J Appl Physiol* 2010; **109**: 1059–1066.
5 Cragg JJ, Ravensbergen HJC, Borisoff JF, Claydon VE. Optimal scaling of weight and waist circumference to height for adiposity and cardiovascular disease risk in individuals with spinal cord injury. *Spinal Cord* 2015; **53**: 64–68.
6 Bauman WA, Spungen AM. Coronary heart disease in individuals with spinal cord injury: assessment of risk factors. *Spinal Cord* 2008; **46**: 466–476.
7 Archer T, Josefsson T, Lindwall M. Effects of physical exercise on depressive symptoms and biomarkers in depression. *CNS Neurol Disord Drug Targets* 2014; **13**: 1640–1653.
8 Rojas Vega S, Abel T, Lindschulten R, Hollmann W, Bloch W, Strüder HK. Impact of exercise on neuroplasticity-related proteins in spinal cord injured humans. *Neurosciences* 2008; **153**: 1064–1070.
9 Yu H, Chen Z. The role of BDNF in depression on the basis of its location in the neural circuitry. *Acta Pharmacol Sin* 2011; **32**: 3–11.
10 Michalczyk M, Kłapcińska B, Sadowska-Krępa E, Jagsz S, Pilis W, Szołtysek-Bołdys I *et al.* Evaluation of the blood antioxidant capacity in two selected phases of the training cycle in professional soccer players. *J Hum Kinet* 2008; **19**: 93–108.
11 Dobiášová M, Frohlich J. The plasma parameter log(TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER_{HDL}). *Clin Biochem* 2001; **34**: 583–588.
12 de Groot PCE, Hjeltnes N, Heijboer AC, Stal W, Birkeland K. Effects of training intensity on physical capacity, lipid profile and insulin sensitivity in early rehabilitation of spinal cord injured individuals. *Spinal Cord* 2003; **41**: 673–679.
13 van Koppenhagen CF, de Groot S, Post MWM, van Asbeck FWA, Spijkerman D, Faber WXM *et al.* Wheelchair exercise capacity in spinal cord injury up to five years after discharge from inpatient rehabilitation. *J Rehabil Med* 2013; **45**: 646–652.
14 Ravensbergen HR, Lear SA, Claydon VE. Waist circumference is the best index for obesity-related cardiovascular disease risk in individuals with spinal cord injury. *J Neurotrauma* 2014; **31**: 292–300.
15 Hübner-Woźniak E, Morgulec-Adamiec N, Malara M, Okęcka-Szymańska J. Effect of training on the serum lipid profile in able-bodied and spinal cord injured rugby players. *Biol Sport* 2010; **27**: 269–272.
16 Hübner-Woźniak E, Morgulec-Adamowicz N, Malara M, Lewandowski P, Okęcka-Szymańska J. Effect of rugby training on blood antioxidant defenses in able-bodied and spinal cord injured players. *Spinal Cord* 2012; **50**: 253–256.
17 Hall ED. Antioxidant therapies for acute spinal cord injury. *Neurotherapeutics* 2011; **8**: 152–167.
18 Bastani NE, Kostovski E, Sakhi AK, Karlsen A, Carlsen MH, Hjeltnes N *et al.* Reduced antioxidant defense and increased oxidative stress in spinal cord injured patients. *Arch Phys Med Rehabil* 2012; **93**: 2223–2228.
19 Fatima G, Sharma VP, Das SK, Mahdi AA. Oxidative stress and antioxidative parameters in patients with spinal cord injury: implications in the pathogenesis of disease. *Spinal Cord* 2015; **53**: 3–5.
20 Gomez-Cabrera MC, Domenech E, Viña J. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. *Free Rad Biol Med* 2008; **44**: 126–131.
21 Bloomer RJ, Goldfarb AH. Anaerobic exercise and oxidative stress: a review. *Can J Appl Physiol* 2004; **29**: 245–263.
22 Furmaniak L, Cywińska-Wasilewska G, Kaczmarek D. Influence of long-term wheelchair rugby training on the functional abilities of persons with tetraplegia over a two-year period post-spinal cord injury. *J Rehabil Med* 2010; **42**: 688–690.
23 Arango-Lasprilla JC, Ketchum JM, Starkweather A, Nicholls E, Wilk AR. Factors predicting depression among persons with spinal cord injury 1 to 5 years post injury. *NeuroRehabilitation* 2011; **29**: 9–21.
24 Gioia MC, Cerasa A, Di Lucente L, Brunelli S, Castellano Y, Traballesi M. Psychological impact of sports activity in spinal cord injury patients. *Scand J Med Sci Sports* 2006; **16**: 412–416.