RELATIONSHIP BETWEEN AEROBIC EFFICIENCY OF THE ATHLETES AND BLOOD LIPID PEROXIDATION

Albena Alexandrova
Department of Physiology and Biochemistry, Coaching Faculty, National Sports Academy "Vassil Levski", Sofia, Bulgaria, a_alexandrova_bas@yahoo.com

Abstract: Inhaled O_2 is necessary for respiratory chain functioning and ATP synthesis. Although most of the O_2 utilized by the mitochondria is converted to harmless H_2O, 2 to 5% of it forms reactive oxygen species (ROS) under physiological conditions. Intense physical activity leads to increase of O_2 consumption about 10 to 20 times from the consumption at rest. Therefore, the intensification of oxidative phosphorylation in response to physical activity will be accompanied by increased production of ROS. As highly reactive the ROS are able to damage all biological molecules and lipids are the most vulnerable to their action. The oxidative modification of the molecules could lead to alterations in the function of cell structures and consequently to cell dead. However ROS could trigger different cell signaling pathways and play a beneficial role in adaptation to physical exercises. It is supposed that regular training leads to a gradual increase in the level of adaptation by repeated activation of antioxidant genes expression and antioxidant enzyme activity. Thus, the purpose of this study was to establish the possible relationship between athletes' working capacity and blood oxidative status. Twelve wrestlers (9 males and 3 females) took part in this study. They conducted a single treadmill test according to a following protocol: the duration of step was 1 min 30 sec, the initial speed was 6 km/h, and the acceleration of each step of treadmill was 1.2 km/h with a constant slope 2.5%. The maximum oxygen (VO_2max) uptake was determined and the LPO after the exercise was measured spectrophotometrically in erythrocyte suspension and plasma. The results showed good aerobic efficiency of the tested athletes with maximum oxygen consumption between 45.8 to 58.9 ml/min /kg. Only two of the tested male wrestlers had maximum oxygen consumption less than 50 ml/min/kg. The three tested women also showed good aerobic efficiency with VO_2max of 46.8, 47.7 and 45.8 ml/min/kg. It was found that the alteration of LPO level after a single treadmill test conduction depended on the training level of the respondents. In athletes with better training level, a lower LPO level was observed in both erythrocyte suspension and plasma. In men with maximum oxygen consumption over 50 ml/min/kg the LPO in erythrocytes was 3.78 ± 0.58 μmol/gHb and in plasma was 19.06 ± 3.11 μmol/L in comparison to men with maximum oxygen consumption less than 50 ml/min/kg where the LPO in erythrocytes was 5.17 ± 0.07 μmol/gHb and in plasma 25.60 ± 0.11 μmol/L. It could be concluded that the occurrence of LPO in athletes depends on their training level. Therefore the extent of changes in the oxidative status and in particular in blood LPO level can serve as an indicator of adaptation processes occurring in the body of the athlete as a result of the training process.
Keywords: aerobic efficiency, lipid peroxidation, sport

1. INTRODUCTION

The first assumption that reactive oxygen species (ROS) are generated in tissues during physical exercise arises in 1978 when Dillard et al. demonstrated a 1.8-fold increase in exhaled pentane as a lipid peroxidation marker after 60 minutes of work on a bicycle with a load of 25-75% of the maximal oxygen consumption (VO_2max) (Dillard et al., 1978). Since then, numerous studies have shown increased generation of ROS in intensive aerobic (Vollaard et al., 2005) and anaerobic loads (Bloomer and Goldfarb, 2004). For detection of ROS and the disruption of the fine balance between pro and anti-oxidative processes in cells (so-called oxidative stress) a variety of biomarkers are used. Most often they are oxidatively modified biomolecules. Because of their high reactivity ROS are able to attack lipids, protein, or DNAs. Common measures of biooxidation include protein carbonyls as an indicator of protein oxidation, malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS) and 4-hydroxyl-2-nonenal as markers of lipid peroxidation; and 8-hydroxy-2'-deoxyguanosine (8-OH-dG) as a sign of DNA oxidation. The most vulnerable molecules to ROS are lipids, in particular the polyunsaturated fatty acids, because of the presence of double bonds in the hydrocarbon chain. Increased lipid peroxidation is considered to be the most significant indicator for oxidative stress occurrence. Early theories of oxidative stress in the 1980s and 1990s created the belief that the generation of ROS as a consequence of intense physical exertion had a predominantly negative effect on the human organism. Nowadays, with the deepening of biochemical knowledge, it has been found that ROS play a key role in regulating many intracellular mechanisms and contribute significantly to the induction of different cell signaling pathways involved in adaptation of body to physical exercise. Of interest are the lipid peroxidation

products, in particular 4-hydroxynonenal (HNE), which has been shown to induce DNA damage (Eckl et al., 1993), but which is involved in the regulation of cell proliferation and growth as well as necrotic or apoptotic cell death by its ability to modulate some major pathways of cell signaling and hence gene expression (Poli et al., 2008). In a growing number of studies and in a number of review articles, it is shown that controlled generation of ROS contributes to mitochondrial biogenesis, angiogenesis, skeletal muscle hypertrophy and proper functioning of the immune system (Ji et al., 2006; Jackson 2002; Powers et al., 2010, 2011). However, the beneficial effects seem to depend on the duration of the load. It is assumed that single physical exercise leads to poor adaptive response while regular training leads to a gradual increase in the adaptation level by repeated stimulation of antioxidant genes expression and antioxidant enzymes, activity.

Thus, the purpose of this work is to establish a possible relationship between the athletes' physical fitness level and changes in lipid peroxidation level in blood. The presence or absence of these may serve as an indicator of the adaptation processes occurring in the body as a result of the training process. Measurements of the oxidative markers in the blood can provide information about the current tolerance of the body to physical effort and, thus, to regulate the training process, which will lead to achieving higher sports results

2. METHODS

Statement

The study was performed in accordance with the Declaration of Helsinki for Human Researches (WMA, 2016).

Participants

In this study 9 males and 3 females international level wrestlers took part. Their mean age was 21.0 ± 0.85 years, mean height 169.0 ± 7.42 cm and mean weight 74.0 ± 11.29 kg.

All participants received detailed information about the objectives and conduct of the study and signed an informed consent form.

Study protocol

After medical examination by a physician the wrestlers conducted a single treadmill test. Before and immediately after the exercise venous blood samples were taken in heparin vacutainers (Vacuette/Li Hep) by authorized nurse.

VO_2max testing

The maximal exercise test was performed on a treadmill (H/P/Cosmos, Germany) according to following protocol: initial speed - 6 km/h, acceleration of each step - 1.2 km/h, duration of the step - 1 min 30 sec, 2.5% constant slope. The gas exchange was analyzed in real time, using a metabolic device Jaeger-Pro, Germany. The O_2 and CO_2 analyzers were calibrated before each test using a reference gas (12% O2; 5% CO2; nitrogen balance).

Biochemical analysis

Blood processing

The whole blood was centrifuged at 600 g for 10 min in order to separate plasma from red blood cells. Plasma was removed and erythrocytes were washed twice with physiological saline (0.9% sodium chloride). The obtained erythrocyte suspension (as a 5% suspension diluted according to Hb concentration in 0.15 M NaCl – 10 mM sodium phosphate buffer, pH7.2) and plasma were used for measurement of the level of lipid peroxidation. Each sample was analyzed in triplicate and the mean value was shown as a result.

Analytical procedures

Hemoglobin (Hb) amount in erythrocyte suspension was determined spectrophotometrically, using Drabkin's reagent. Ten μL 5% erythrocyte suspension was added into 995 μL Drabkin's reagent and after 20 min incubation at room temperature the absorbance at 540 nm was read against Drabkin reagent. The total hemoglobin concentration (g/L) was determined using a calibration curve.

Lipid peroxidation (LPO) was estimated by the amount of thiobarbituric acid reactive substances (TBARS) according to Gilbert et al. (1984). Into one mL of 5% erythrocyte suspension was added 0.5 mL 28% trichloroacetic acid (w/v) in 0.1 M $NaAsO_2$ and the samples were centrifuged for 10 min at 3000 rpm. One milliliter of the resulting supernatant was added to 0.5 mL TBA (1% in 0.05N NaOH) and the samples were heated at 100°C for 15 min. After cooling the absorbance was read at 532 and 453 nm against appropriate blanks. The amount of TBARS was expressed in ng malondialdehyde (MDA) per mg Hb, using a molar extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$.

Statistics

The statistical analysis was performed using MS Excel 2013. The results were expressed as mean ± SD. The statistical significance of the differences in the mean values was checked using the Student t-test for paired data.

3. RESULTS

The anthropometric data of the tested athletes are presented on Table 1. The wrestlers were representatives of various weight categories so large variations in height and weight were observed. The values of BMI were in normal limits (except № 9) taken in consideration that athletes dealing with strength sports have generally higher BMI due to well-developed musculature. All of the tested subjects were well hydrated. The percentage of body fat was in norm (except № 9), according to the American Council on Exercise.

Table 1. Anthropometric data of the tested athletes.

N	Gender	Age years	Weight kg	Height cm	BMI kg/m2	Fat kg	Fat %	Water %
1	M	21	68,2	166,0	24,75	8,0	11,6	65
2	M	21	69,9	173,2	23,3	6,0	8,6	67
3	M	20	77,3	171,0	26,44	6,6	8,6	67
4	M	22	76,6	171,0	26,2	10,0	13,0	59
5	M	20	85,1	174,5	27,95	9,2	10,7	65
6	M	22	87,0,	176,5	27,93	9,4	10,8	65
7	M	21	80,0	181,0	24,42	6,7	8,4	67
8	M	22	80,0	172,6	26,85	9,7	12,0	64
9	M	20	88,9	170,0	30,76	15,8	17,7	60
10	F	22	55,0	153,5	23,34	10,0	18,2	60
11	F	21	59,3	164,5	21,91	9,1	15,3	62
12	F	20	60,9	160,7	23,58	11,6	18,9	59

On table 2 are presented the results of the maximal oxygen uptake test and the results from the measurement of the lipid peroxidation in erythrocytes and plasma. Most of the male wrestlers (1-6) showed good aerobic efficiency (VO_2max over than 50 mL/min/kg). Three male wrestlers had average aerobic efficiency (VO_2max less than 50 mL/min/kg). All the female wrestlers demonstrated good and very good (VO_2max over 46 mL/min/kg) aerobic efficiency.

Table 2. Results of the maximal oxygen uptake test and lipid peroxidation measurement in erythrocytes and plasma.

N	Gender	Time	Maximal speed	VO_2 max	LPO in Er suspension after exercise	LPO in plasma after exercise
		min:sec	km/h	mL/kg/min	µmol MDA/gHb	µmol MDA/L
1	M	14,55	16,8	58,9	3,01	14,44
2	M	13:29	15,6	55,3	3,59	16,28
3	M	11:59	14,4	55,2	3,22	18,49
4	M	12:59	15,6	55,0	3,53	18,97
5	M	11:54	14,4	54,3	4,18	20,34
6	M	12:50	15,6	52,0	4,42	21,01
7	M	13:23	15,6	50,1	4,50	23,87
8	M	11:52	14,4	**48,9**	**5,22**	**25,67**
9	M	10:59	14,4	**46,8**	**5,12**	**25,52**
10	F	10:20	13,2	47,7	2,92	22,42
11	F	10:59	14,4	46,8	3,64	23,41
12	F	09:55	13,2	45,8	4,39	25,03

In regards to LPO it was observed that in men with maximum oxygen consumption over 50 ml/min/kg the mean LPO level in erythrocytes was 3.78 ± 0.58 µmol/gHb and in plasma was 19.06 ± 3.11 µmol/L in comparison to men with maximum oxygen consumption less than 50 ml/min/kg where the LPO in erythrocytes was 5.17 ± 0.07

μmol/gHb and in plasma 25.60 ± 0.11 μmol/L. In women it was also found that higher LPO level corresponded to lower result of the treadmill test performance.

4. DISCUSSION

The tested wrestlers were in general well trained as followed from relatively high values of oxygen uptake. According to Heyward and Gibson (2014) VO_2max from 51 to 56 mL/min/kg is classified as a good fitness level for men at age of 20 to 24 years and for women at the same age the good fitness level is referred to VO_2max from 42 to 46 mL/min/kg. The VO_2max depends on age and gender. The highest VO_2max values are measured around the age of 20, after which the aerobic fitness starts to decline with about 10% per decade for both genders. In general, men have about 20% higher values than women due to differences in body size, body composition, and blood volume (Loe et al., 2013). In this study was found that level of blood lipid peroxidation after single treadmill test conduction depends on the training level of the respondents. In athletes with better training level, a lower LPO level was observed in both erythrocyte suspension and plasma. In men with maximum oxygen consumption over 50 ml/min/kg the LPO in erythrocytes was 3.78 ± 0.58 μmol/gHb and in plasma was 19.06 ± 3.11 μmol/L in comparison to men with maximum oxygen consumption less than 50 ml/min/kg where the LPO in erythrocytes was 5.17 ± 0.07 μmol/gHb and in plasma 25.60 ± 0.11 μmol/L. Regarding the data of blood LPO in response to acute exercise in the literature exist a great variety and contradictions. An increase of MDA concentration (a marker of lipid peroxidation) in erythrocytes after an exercise performance was established by several researchers, especially in people with sedentary lifestyle (Miyazaki et al., 2001, Dane et al., 2008). However others (Dane et al., 2008) found no changes in erythrocyte MDA levels, measured before and after acute treadmill run in people involved in active sports. A possible explanation for these results is that in blood of active athletes predominate erythrocytes that are more resistant to oxidative impact, likely because of higher antioxidant defense system and/or enhanced degradation of old and oxidative modified erythrocytes. Similar controversial data have also been found for plasma LPO. A significant increase of TBARS was measured immediately post maximal multistage 20-m shuttle run test in which the tested physical education students reached VO_2max of 42.12 ± 2.74 ml/min/kg (Atashak & Sharafi 2013). However, it was registered a decrease of LPO in marathon runners with long experience and regular training, who reached VO_2max of 60.1 ± 4.3 ml/min/kg in aerobic test to exhaustion (Rokitzki et al. 1994). In a study where wrestlers conducted a single treadmill test reaching VO_2max of 51.4 ± 4.3 ml/min/kg a negative correlation between training level of the athletes evaluated by the VO_2max and TBARS concentration in plasma after exercise ($r = -0.637$, $p = 0.026$) was observed (Alexandrova et al., 2016). Therefore it could be assumed that the training level of the respondents influences the induction and development of LPO in erythrocytes and plasma.

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