Research article

Effect of single bout of maximal excercise on plasma antioxidant status and paraoxonase activity in young sportsmen

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The purpose of this study was to elucidate the participation of plasma PON1 (paraoxonase activity [PON] and arylesterase activity [ARE]) in antioxidant defense in response to a single bout of maximal exercise. PON, ARE, lipid profile, lipid peroxidation (thiobarbituric acid reactive substances [TBARS]), total antioxidant status (ferric reducing ability of plasma [FRAP]), concentration of uric acid [UA], and total bilirubin (TBil) were determined in the plasma before, at the bout and 2 h after maximal exercise on a treadmill in young sportsmen. Chosen physiological parameters also were controlled during maximal exercise. Following maximal exercise, the unaltered level of TBARS and increased FRAP were registered. ARE increment was the highest (37.6%) of all measured variables but lasted for a short time. UA increment was lower than ARE but long-lasting and correlated with FRAP. PON activity increment was associated with the combined effect of body weight, lean, body mass index (BMI) and basal metabolic rate (BMR). We conclude that PON1 is a co-factor of the first line of antioxidant defense during maximal exercise. Its activity is associated with body composition and not the physical fitness of the subjects.

Keywords: PON1 activity, antioxidant defense, exercise, fitness, lipid profile

Introduction

A single bout of exercise generates reactive oxygen species (ROS) leading to oxidative stress, which can subsequently lead to molecular, cellular, and vascular damage.[1] However, repeated exposure to exercise, especially regular exercise, improves physical health and develops favorable adaptations as a result of increased activity of the protective systems. Among other things, it stimulates antioxidant defense[2] and has a beneficial effect on the lipid profile.[3]

HDL particles are capable of protecting LDL from oxidation.[4] This protective ability of HDL varies among the HDL particles as a result of differences in HDL composition, antioxidants and enzymes associated with HDL.

Many studies indicate that paraoxonase 1 (PON1), an HDL-associated enzyme, takes part in the antioxidant function of HDL.[5] It has hydrolytic activity towards paraoxon (paraoxonase activity [PON]) and towards phenyl acetate (arylesterase activity [ARE]). ARE is more stable than PON,[6] which is sensitive to different modulating factors. Furthermore, the PON1 enzyme is able to prevent oxidation of LDL, cell

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membranes, and HDL particles.[7,8] It also hydrolyzes lipid peroxides and hydroperoxides in oxidized HDL and LDL, as well as hydrogen peroxide (H_2O_2) produced under oxidative stress.[8] Moreover, human PON1 was found to decrease accumulation of cholesterol in macrophages.[9] Therefore, it seems that PON1 plays an important role in prevention of atherosclerosis, which was also demonstrated using PON1-knockout mice[10] as well as mice overexpressing PON1.[11] Some research suggests that PON1, along with other antioxidants, can participate in the defence of cells from oxidative stress evoked by single exercise. However, the results are conflicting as discussed in our previous review.[12]

The aim of our study was to investigate the effect of a single bout of maximal exercise on the level of total antioxidant capacity and chosen markers of the antioxidant status in plasma, particularly PON and ARE. Moreover, associations between antioxidant status and chosen physiological parameters were explored.

Subjects and methods

Subjects

A group of 32 healthy, male, amateur, young sportsmen volunteered for the investigation. They were engaged in a 3-year regular aerobic training program, which consisted of an average of four times weekly 2-h physical and technical practice sessions. None of the subjects was taking any drug known to affect lipid and lipoprotein metabolism or antioxidant capacity of plasma. All participants were non-smokers. A personal interview was carried out to assess the exact intensity of their training program. A written informed consent was signed by all participants and, in cases of minority, also by their parents before admission to the study. The study protocol was approved on 26 September 2006 by the Bioethics Committee of Medical University of Lodz nr RNN/163/06/KE.

Study design

All participants underwent testing between 8–11 a.m. after a 12-h overnight fast. Baseline measurements were done after a 20-min rest, directly before the maximal exercise test as listed in Table 1. Blood samples were then obtained for future analyses (marked as 'before'). The subjects had then completed maximal exercise on the treadmill with continuous measurement of cardiorespiratory parameters (Table 2). The participants were instructed to run until they reached exhaustion. The test was terminated at volitional fatigue. Blood samples were obtained at the bout of maximal exercise (marked as 'at the bout'). The cardiorespiratory parameters were monitored 10 min before, throughout the maximal exercise, and up to their return to normal values, *i.e.* 5–10 min after maximal exercise. Two hours after completion of maximal exercise, blood samples were taken for the third time (marked as 'after maximal exercise'). Plasma from all blood samples was analyzed for biochemical variables listed in Table 3.

Baseline measurements

Body composition and basal metabolic rate (BMR) were assessed with Bodystat 1500 (Bodystat Ltd, UK). Heart rate (HR) and blood pressure (BP) were taken with a sphygmomanometer OMRON M4-1. Maximal minute ventilation (MVV) was performed with Lungtest 1000 spirometer (MES S.c., Krakow, Poland). Master Screen Body, Jaeger (Viasys Healthcare GmbH, Germany) was used to perform body plethysmography: FVC, TLCOc/VA and TLC-He. Both Lungtest 1000 spirometer and Master Screen Body software are compatible with the American Thoracic Society standards.[13]

Characteristics of maximal exercise

The maximal exercise was performed on the Trackmaster treadmill according to our modification of the Bruce protocol.[14] All subjects reached their VO_{2max}.

Cardiorespiratory parameters were monitored continuously with the VO2000 MedGraphics Cardiorespiratory Diagnostic Systems, which worked compatibly with Breeze.suite 6.2A MedGraphics software.

Human plasma

Venous blood samples were drawn from antecubital vein into lithium heparin containing Vacutainer tubes. Samples were centrifuged (3000 *g*, 4ºC, 15 min) and stored at –80ºC for further analyses.

Plasma volume shifts influenced by maximal exercise were determined in randomly chosen subjects. The volume decreases did not exceed 5% before versus at the bout of maximal exercise and 1.5% before versus 2 h after maximal exercise. Since the volume decreases were low, we assumed that they will not essentially influence our results. Therefore, we decided not to correct the obtained results for plasma volume changes.

Chemicals

The majority of chemicals was purchased from Sigma-Aldrich Chemical (St Louis, MO, USA). Trizma base

Table 1 Descriptive characteristics of subjects

Parameter	*n*	Mean	± SD
Age (years)	32	18.34	2.62
Height (cm)	32	177.34	6.89
Weight (kg)	32	69.28	9.11
Body fat (%)	32	12.74	5.38
Lean (kg)	32	60.38	8.13
BMI (kg/m^2)	32	22.02	2.55
BMR (kcal)	32	1872.31	206.92
HR at rest (beats/min)	32	68.50	12.86
Systolic pressure (mmHg)	32	138.38	15.26
Diastolic pressure (mmHg)	32	73.94	9.75
MVV (l/min)	28	172.59	36.81
FVC (l)	28	5.36	0.86
TLCOc/VA (mmol/min/kPa/l)	27	1.82	0.21
TLC/He (l)	27	6.70	0.96

FVC, forced vital capacity; TLCOc/VA, hemoglobin standardized lung diffusing capacity for CO corrected for the alveolar volume; TLC-He, total lung capacity measured by helium dilution; *n*, number of subjects.

Table 2 Markers of physiological status measured at the bout of maximal exercise

Parameter	*n*	Mean	± SD
RR (br/min)	29	52.41	9.73
VE (l/min)	29	123.01	24.72
VO_{2max} (ml/min)	29	4149.31	861.20
VO_{2max} (ml/kg/min)	29	59.80	11.42
VCO_{2max} (ml/min)	29	4955.93	887.14
HR_{max} (beats/min)	27	185.89	24.85
VO_{2max}/HR (ml/beat)	27	23.33	6.70
RER	29	1.21	0.11
METS	29	17.09	3.26

RR, respiratory rate; VE, ventilation; VO_{2max}, maximal oxygen consumption; VCO_{2max}, maximal carbon dioxide production; HR_{max}, maximal heart rate; METS, metabolic equivalents; RER, respiratory exchange ratio; *n*, number of subjects.

was purchased from Fluka, Buchs, Switzerland and Triton X-100 from Serva Feinbiochemica, Heidelberg, Germany.

Measurement of biochemical variables in plasma

Lipid profile as well as UA and TBil were determined by automated standard methods in an Olympus AU 640 autoanalyzer in the laboratory of Military Teaching Hospital No. 2 in Lodz, Poland.

The enzyme PON1 was evaluated employing paraoxon (PON) and phenyl acetate (ARE) as substrates. Both activities were measured with the procedure described by Nakanishi *et al.*[15] The rate of generation of products was monitored in an Ultrospec III spectrophotometer (Pharmacia LKB) using the Spectro-Kinetics software.

FRAP was measured according to the procedure described by Benzie and Strain.[16]

Products of lipid peroxidation in plasma were assayed as TBARS levels and determined similarly to the procedure described by Kasielski and Nowak.[17]

Statistical analysis

All measurements were expressed as mean ± SD. The biochemical changes among variables obtained before maximal exercise, at the bout of maximal exercise, and 2 h after maximal exercise were compared by using Friedman ANOVA followed by Wilcoxon matched pairs tests post-hoc. Pair-wise correlations were calculated using the Pearson product moment correlation coefficient and coefficients of multiple correlation (R) were calculated using multiple linear regression analysis to estimate the influence of multiple variables. P-values with $P < 0.05$ were considered statistically significant. All performed tests were two-tailed. All statistical tests were performed using Statistica Software, v8.

Results

The descriptive characteristics of the subjects are shown in Table 1. The markers of physiological status of the subjects at the bout of maximal exercise are presented in Table 2. Our results, particularly the mean level of VO_{2max} (59.80 ml/kg/min), show that the subjects are well trained as they claimed. According to Astrand,[18] this value indicates high fitness of young men. With the applied protocol, our subjects reached VO_{2max} in the mean time of 12 min 29 s with mean speed of 9.5 km/h and treadmill slope of 9º. All mentioned parameters together with maximal oxygen pulse (VO_{2max}/HR) were used as fitness indicators in our study.

Arterial pressure observed in our subjects is slightly higher than expected in young men. However, it was measured only once, before the experiment, and can be explained by the effect of white coat hypertension. The young age of the subjects and strong motivation to obtain high results during exercise made them particularly susceptible to this effect.

The effect of acute bout of maximal exercise on PON/ARE and other biochemical values

All biochemical variables are presented in Table 3. The basal values are within normal range. The concentrations and activities of all biochemical variables determined in plasma significantly increased

Table 3 Biochemical characteristics of plasma during maximal exercise

Parameter	*n*	Before maximal exercise		Bout of maximal exercise		After maximal exercise	
		Mean	± SD	Mean	± SD	Mean	± SD
TChol (mM/l	26	4.45	1.06	4.78	1.20*	4.45	1.13^
HDL-C (mM/l)	26	1.34	0.25	1.44	0.26*	1.33	0.25^
LDL-C (mM/l)	26	2.61	0.81	2.75	0.95*	2.53	0.88^
TG (mM/l)	26	1.10	0.80	1.30	0.89*	1.30	1.00*
PON (U/l)	28	371.43	244.88	436.04	275.17*	388.89	256.43^
ARE (U/ml)	29	132.36	57.91	182.13	97.56*	143.43	64.03^
TBil (μM/l)	26	16.43	7.39	18.73	8.43*	16.88	8.19^
UA (μM/l)	26	322.54	70.40	352.58	73.80*	387.04	86.00*^
FRAP#	30	1.24	0.21	1.44	0.33*	1.61	0.37*^
TBARS (mM/l)	29	3.14	1.39	3.57	2.03	3.04	1.24

TChol, total cholesterol; HDL-C, high density lipoprotein cholesterol; HDL-C, low density lipoprotein cholesterol; TG, triglycerides; #FRAP is expressed in mM/l Fe^{2+}.
Statistical significance ($P < 0,05$) *versus value before maximal exercise, ^versus value at acute bout of maximal exercise.
n, number of subjects.

at the bout of maximal exercise (Fig. 1) with the exception of the value of TBARS. Maximal exercise proved to have the highest influence on ARE of PON1, as 37.6% increment in ARE was observed in comparison to the value before maximal exercise. Its increase was over twice the value of PON (17.4%) and FRAP (16.1%) increment. A much lower increment was detected for TBil concentration (14%), with the lowest increment for UA (9.3%). In the lipid profile, the highest percentage increment was observed for TG concentration (18%). HDL-C concentration increased by 9.7%, TChol by 7.4% and LDL-C concentration showed the smallest increment as it increased only by 5.36%. The observed increments of concentrations and activities were mostly short lasting, as the return to baseline values was observed after only 2 h of rest. Only TG concentration remained at the increased level 2 h after the completion of maximal exercise. However, both UA concentration and FRAP were still significantly increasing 2 h after completion of the treadmill run. We conclude that PON1 antioxidant defense during maximal exercise is short lasting in comparison to the function of UA and other compounds contributing to total antioxidant capacity.

Correlations between PON/ARE and other variables

Moderate positive correlation between PON and HDL-C concentration was found in all three time points during maximal exercise with $P < 0.05$ (before $r = 0.53$, at the bout $r = 0.43$ and after $r = 0.43$). Likewise moderate positive correlation was found between UA concentration and FRAP (before $r =$

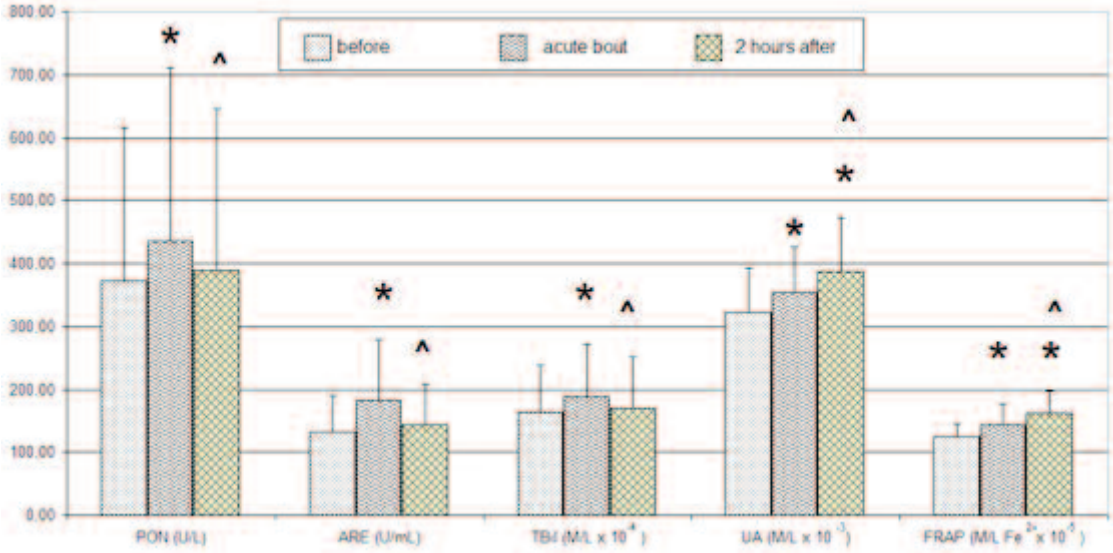

Figure 1 Changes in antioxidant protection indicators in plasma induced by acute bout of maximal exercise (mean ± SD). PON, paraoxonase activity; ARE, arylesterase activity; TBil, total bilirubin concentration; UA, uric acid concentration; FRAP, ferric reducing ability of plasma

0.66, at the bout $r = 0.73$ and after $r = 0.68$) with $P < 0.001$. The correlation between UA and FRAP was the strongest correlation obtained in our study.

Multiple linear regression analysis showed that ARE after maximal exercise was associated with the combination of maximal HR and VO_{2max}/HR ($R^2 = 0.30$; $F = 4.62$; $P < 0.02$). Neither of these parameters separately was associated with ARE after maximal exercise.

The percentage PON increment was influenced by a combination of factors, namely weight, lean, BMI, and BMR ($R^2 = 0.36$; $F = 3.24$; $P < 0.03$). However, none of these factors separately were significantly associated with the PON increment.

Discussion

In the present study, the total antioxidant capacity of plasma, measured as FRAP, increased at the acute bout of maximal exercise, which is consistent with other studies.[19] FRAP consists mainly of the aqueous antioxidants in serum, which include UA and TBil. UA accounts for approximately 60% of all free radical-scavenging activity in human serum.[16] We found that plasma UA level increased at the bout of maximal exercise and still increased 2 h after maximal exercise, as observed in other studies.[19] In our study, a high correlation between FRAP and UA concentration was found, which implies its significance in total antioxidant capacity of plasma. During strenuous exercise, energy is delivered from purine nucleotides. Their increased catabolism results in accumulation of hypoxanthine, xanthine and UA. UA is then secreted or leaks from the cells to plasma acting as a very potent free radical scavenger and a chelator of transitional metal ions.[20] However, it is accepted that UA has a dual function: pro-oxidant in lower doses and antioxidant in higher concentrations.[21] The UA concentration observed in our study was high enough to act as an antioxidant.

Similarly, TBil plasma concentration increase at maximal exercise in our investigation is in agreement with other observations.[22]

We detected that TBARS levels were not altered by maximal exercise. In many studies, increase in TBARS was observed during maximal exercise. Yet, in some studies, TBARS levels did not change (for review see Vollaard *et al.*[23]). We conclude that the elevation of plasma antioxidants compensated the oxidative stress caused by physical activity.

Maximal exercise caused an increase in PON1 activities with a higher increase in ARE than PON, which can be understood as a higher increase in the enzyme's bioavailability than its hydrolytic activity in plasma.[6] An increase in both PON1 activities at the acute bout of maximal exercise was followed by a return to basal levels within 2 h. Similarly, in a study performed by Tomas *et al.*,[24] PON increased immediately after the bout of exercise; however, it decreased in the following 2 h. On the contrary, a single bout of exercise inhibited serum PON in rats.[25] Tsakiris *et al.*[26] described a decrease in both PON and ARE after exercise in basketball players. However, in other studies, no change of PON1 activity following intense exercise was observed.[27,28]

The increase in PON1 activity at the bout of maximal exercise, observed in our study, is an element of intrinsic antioxidant mechanism evoked during maximal exercise in order to counteract the deleterious effects of free radicals on lipoproteins. The observed return of PON1 activity to basal level 2 h after maximal exercise is consistent with the results of an *in vitro* study of Cao *et al.*,[29] who described a reduction of PON1 activity after oxidative incubation and Cu^{2+}-induced peroxidation of LDL. Oxidized LDL appeared to inactivate PON1 through interactions between the enzyme free sulfhydryl group and oxidized lipids formed during LDL oxidation.[30] In this reaction, the enzyme is consumed, which is expressed by a decrease in ARE and PON. It should be noted that the temporary activity of the enzyme is a product of its synthesis in the liver, the level of its utilization, and the actual configuration of its catalytic center.

In our study, maximal exercise caused an increase in TChol and lipoprotein fractions. In other studies, increases,[31] decreases,[32] and no change[19,33]) in TChol, LDL-C and TG have been observed. However, the increase in HDL-C and decrease in LDL-C and TG have been more consistent.[3,34]

In order to determine if changes in PON1 activities were not a result of HDL-C increments, correlations between PON1 activities and HDL-C level were performed. A moderate association was found between PON, but not ARE, and HDL-C levels throughout the study. Therefore, our study indicates that the increase of HDL-C level contributes to PON1 activity increase at maximal exercise but the correlation is not strong. This implies that additional mechanisms responsible for PON1 activity changes during and after maximal exercise are employed. However, some authors did not observe correlation between PON1 and HDL-C levels during exercise.[24] Besides concentration of HDL-C, qualitative alterations in HDL molecule may be responsible for PON1 activity increment during exercise. The

qualitative properties of HDL particles depend upon the susceptibility to oxidation of unsaturated fatty acids transported in phospholipids and triglycerides.[7] Moreover, qualitative properties of HDL particles are strongly associated with apolipoprotein A-I (ApoA-I) located on HDL. ApoA-I and phospholipids are necessary for PON1 stability and optimal activity.[35] To explain further the association between PON1 and HDL in response to exercise, the concentration of ApoA-I should be determined alongside PON1 activity.

A novel finding of our investigation is that PON increment (%) at the acute bout of maximal exercise is higher in subjects who have higher weight, BMI, lean, and BMR. This phenomenon may result from an adaptation to increased ROS production caused by an augmentation of cellular respiratory processes in well-trained sportsmen with high muscle mass and BMR. Adaptive 25% improvement of plasma antioxidant status in sportsmen in comparison to sedentary controls was shown by Brites *et al*.[36] On the contrary, in other research a negative correlation was found between PON1 activity and body weight as well as BMI. PON1 activity was reduced in obese subjects at rest.[37,38] and in patients with metabolic syndrome.[39] Moreover, PON1 activity increased after weight reduction by gastric banding procedure in morbidly obese individuals.[40] Only Rector *et al*.[41] described lower serum PON1 activity associated with body weight reduction. It should be emphasized that we looked for these associations in completely different circumstances, *i.e*. in healthy subjects during maximal exercise. The negative correlations between PON1 activity and BMI in research mentioned above may be associated with the obesity of examined subjects while all sportsmen included in our investigation had BMI within normal range.

We have performed a search for associations between PON1 activities and physical fitness indices such as VO_{2max}, speed at VO_{2max}, treadmill slope at VO_{2max}, time of reaching VO_{2max}, and VO_{2max}/HR. We found that ARE after maximal exercise was exceptionally influenced by the combined effect of maximal HR and VO_{2max}/HR. Until now, it was shown that physically active subjects had higher basal PON1 activity than sedentary controls.[42,43] Our results bring very weak evidence for the association between physical fitness and PON1 activity.

Conclusions

Maximal exercise in young sportsmen leads to an increase of total antioxidant activity of plasma, which is mainly due to UA, and to a lesser degree TBil and PON1 activity. The effect of UA on antioxidant defense is long-lasting, and TBil and PON1 is short-lasting. The increment of PON1 activity during maximal exercise does not depend on physical fitness parameters of subjects, but on the combined effect of weight, BMI, lean, and BMR.

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References

1. Ji LL. Exercise and oxidative stress: role of the cellular antioxidant systems. *Exerc Sport Sci Rev* 1995; **23**: 135–166.
2. Lesgards JF, Durand P, Lassarre M *et al*. Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. *Environ Health Perspect* 2002;**110**: 479–486.
3. Durstine JL, Haskell WL. Effects of exercise training on plasma lipids and lipoproteins. *Exerc Sport Sci Rev* 1994; **22**: 477–521.
4. Parthasarathy S, Barnett J, Fong LG. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta* 1990; **1044**: 275–283.
5. Ji LL Antioxidants and oxidative stress in exercise. *Proc Soc Exp Biol Med* 1999; **222**: 283–292.
6. Beltowski J, Wojcicka G, Mydlarczyk M, Jamroz A. Cervistatin modulates plasma paraoxonase/arylesterase activity and oxidant-antioxidant balance in the rat. *Pol J Pharmacol* 2002; **54**: 143–150.
7. Brites F, Zago V, Luz Muzzio M, Wikinski R, Schreier L. HDL capacity to inhibit LDL oxidation in well-trained triathletes. *Life Sci* 2006; **78**: 3074–3081.
8. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parma SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest* 1998; **101**: 1581–1590.
9. Rozenberg O, Shih DM, Aviram M. ,Human serum paraoxonase 1 decreases macrophage cholesterol biosynthesis: possible role for its phospholipase-A_2-like activity and lysophosphatidylcholine formation. *Arterioscler Thromb Vasc Biol*. 2003; **23**: 461–467.
10. Shih DM, Gu YR, Xia M *et al*. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature* 1998; **394**: 284–287.
11. Tward A, Xia YR, Wang XP *et al*. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. *Circulation* 2002; **106**: 484–490.
12. Otocka-Kmiecik A, Orlowska-Majdak M. The role of genetic (PON1 polymorphism) and environmental factors, especially physical activity in antioxidant function of paraoxonase. *Postepy Hig Med Dosw* 2009; **63**: 668–-677
13. American Thoracic Society, Standarization of spirometry 1987 update. *Am Rev Respir Dis* 1987; **136**: 1285–1296.
14. Bruce RA, Kusumi F, Hosmer D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am Heart J* 1973; **85**: 546–562.
15. Nakanishi M, Takanami Y, Maruyama T *et al*. The ratio of serum paraoxonase/arylesterase activity using an improved assay for arylesterase activity to discriminate PON1(R192) from PON1(Q192). *J Atheroscler Thromb* 2003; **10**: 337–342.
16. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal Biochem* 1996; **239**: 70–6.
17. Kasielski M, Nowak D. Long-term administration of *N*-acetylcysteine decreases hydrogen peroxide exhalation in subjects with chronic obstructive pulmonary disease. *Respir Med* 2001; **95**:

448–456.
18. Astrand PO. *Experimental studies on physical working capacity in relation to sex and age*. Copenhagen: Mundsgaard, 1952.
19. Liu ML, Bergholm R, Makitmattila S *et al*. A marathon run increase the susceptibility of low density lipoprotein (LDL) to oxidation *in vitro* and modifies plasma antioxidants. *Am J Physiol* 1999; **276**: E1083–E1091.
20. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. *Curr Pharm Des* 2005; **11**: 4145–4151.
21. Sanguinetti S, Batthy C, Trostchansky A *et al*. Nitric oxide inhibits prooxidant actions of uric acid during copper-mediated LDL oxidation. *Arch Biochem Biophys* 2004; **423**: 302–308.
22. Benitez S, Sanchez-Quesada JL, Lucero L *et al*. Changes in low-density lipoprotein electronegativity and oxidizability after aerobic exercise are related to the increase in associated non-esterified fatty acids. *Atherosclerosis* 2002; **160**: 223–232.
23. Vollaard NB, Shearman JP, Cooper CE. Exercise-induced oxidative stress: myths, realities and physiological relevance. *Sports Med* 2005; **35**: 1045–1062.
24. Tomas M, Elosua R, Senti M *et al*. Paraoxonase1–192 polymorphism modulates the effects of regular and acute exercise on paraoxonase1 activity. *J Lipid Res* 2002; **43**: 713–720.
25. Pawlowska D, Moniuszko-Jakoniuk J, Soltys M. Parathion-methyl effect on the activity of hydrolytic enzymes after single physical exercise in rats. *Pol J Pharmacol Pharm* 1985; **37**: 629–638.
26. Tsakiris S, Karikas GA, Parthimos T, Tsakiris T, Bakogiannis C, Schulpis KH Alphatocopherol supplementation prevents the exercise induced reduction of serum paraoxonase 1/arylesterase activities in healthy individuals. *Eur J Clin Nutr* 2009; **63**: 215–221.
27. Romani R, De Medio GE, di Tullio S *et al*. Modulation of paraoxonase 1 and 3 expression after moderate exercise training in the rat. *J Lipid Res* 2009; **50**: 2036–2045.
28. Briviba K, Watzl B, Nickel K *et al*. A half-marathon and a marathon run induce oxidative DNA damage, reduce antioxidant capacity to protect DNA against damage and modify immune function in hobby runners. *Redox Rep* 2005; **10**: 325–331.
29. Cao H, Girard-Globba A, Berthezene F, Moulin P. Paraoxonase protection of LDL against peroxidation is independent of its esterase activity towards paraoxon and is unaffected by the QoR genetic polymorphism. *J Lipid Res* 1999; **40**: 133–139.
30. Brites FD, Evelson PA, Christiansen MG *et al*. Soccer players under regular training show oxidative stress but an improved plasma antioxidant status. *Clin Sci* 1999; **9**: 381–385.
31. Dufaux B, Order U, Muller R, Hollmann W. Delayed effects of prolonged exercise on serum lipoproteins. *Metabolism* 1986; **35**: 105–109.
32. Thompson PD, Cullinanane EM, Henderson O, Herbert PN. Acute effects of prolonged exercise on serum lipids. *Metabolism* 1980; **29**: 662–665.
33. Fallon KE, Sivyer G, Sivyer K, Dare A. The biochemistry of runners in a 1600 km ultramarathon. *Br J Sports Med* 1999; **33**: 264–269.
34. Pronk NP. Short term effects of exercise on plasma lipids and lipoproteins in humans. *Sports Med* 1993; **16**: 431–448.
35. Sorenson RC, Bisgaier CL, Aviram M, Hsu C, Billecke S, La Du BN. Human serum paraoxonase/arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: apolipoprotein A-I stabilizes activity. *Arterioscler Thromb Vasc Biol* 1999; **19**: 2214–2225.
36. Brites FD, Evelson PA, Christiansen MG *et al*. Soccer players under regular training show oxidative stress but an improved plasma antioxidant status. *Clin Sci (Lond)* 1999; **96**: 381–385.
37. Ferretti G, Bacchetti T, Moroni C *et al*. Paraoxonase activity in high-density lipoproteins: a comparison between healthy and obese females. *J Clin Endocrinol Metab* 2005; **90**: 1728–-1733.
38. Ferretti G, Bacchetti T, Masciangelo S, Bicchiega V. HDL-paraoxonase and membrane lipid peroxidation: a comparison between healthy and obese subjects. *Obesity (Silver Spring)* 2010; **18**: 1079–1084.
39. Senti M, Tomas M, Fito M *et al*. Antioxidant paraoxonase 1 activity in the metabolic syndrome. *J Clin Endocrinol Metab* 2003; **88**: 5422–5426.
40. Uzun H, Zengin K, Taskin M, Aydin S, Simsek G, Dariyerli N. Changes in leptin, plasminogen activator factor and oxidative stress in morbidly obese patients following open and laparoscopic Swedish adjustable gastric banding. *Obes Surg* 2004; **14**: 659–665.
41. Rector RS, Warner SO, Liu Y *et al*. Exercise and diet induced weight loss improves measures of oxidative stress and insulin sensitivity in adults with characteristics of the metabolic syndrome. *Am J Physiol* 2007; **293**: E500–E506.
42. Senti M, Tomas M, Anglada R *et al*. Interrelationship of smoking, paraoxonase activity, and leisure time physical activity: a population-based study. *Eur J Intern Med* 2003; **14**: 178–184.
43. Cabrera de Leon A, Rodriguez-Perez M del C, Rodríguez-Benjumeda LM *et al*. Sedentary lifestyle: physical activity duration versus percentage of energy expenditure. *Rev Esp Cardiol* 2007; **60**: 244–250.