A Monitoring of Allantoin, Uric Acid, and Malondialdehyde Levels in Plasma and Erythrocytes After Ten Minutes of Running Activity

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Summary

Uric acid is the final product of human purine metabolism. It was pointed out that this compound acts as an antioxidant and is able to react with reactive oxygen species forming allantoin. Therefore, the measurement of allantoin levels may be used for the determination of oxidative stress in humans. The aim of the study was to clarify the antioxidant effect of uric acid during intense exercise. Whole blood samples were obtained from a group of healthy subjects. Allantoin, uric acid, and malondialdehyde levels in plasma and erythrocytes were measured using a HPLC with UV/Vis detection. Statistical significant differences in allantoin and uric acid levels during short-term intense exercise were found. Immediately after intense exercise, the plasma allantoin levels increased on the average of 200 % in comparison to baseline. Plasma uric acid levels increased slowly, at an average of 20 %. On the other hand, there were no significant changes in plasma malondialdehyde. The results suggest that uric acid, important antioxidant, is probably oxidized by reactive oxygen species to allantoin. Therefore allantoin may be suitable candidate for a marker of acute oxidative stress.

Key words

Allantoin • Uric acid • Oxidative stress • Antioxidants • Short-term intense exercise

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Introduction

Reactive oxygen species (ROS) have recently become a significant research subject. Many scientists are seeking to assess their role in physiological and mainly pathological processes. This is why ROS have become the subject of intense medical research and its results are slowly being applied in medical practice. There are currently a number of methods for evaluating oxidative stress (Giustarini *et al.* 2009, Kaneko *et al.* 2012, Chung and Benzie 2013). Monitoring radical reactions in the body is difficult and therefore usually substances produced by the effects of free radicals or compounds with antioxidant capabilities are determined instead. Indicators of oxidative stress can be measured in various body fluids or tissues (Abdul-Rasheed *et al.* 2010, Schrag *et al.* 2013).

An important antioxidant is uric acid (UA), traditionally thought to be only the end product of purine degradation in humans with no other physiological function. The formation of UA can be accompanied by the emergence of ROS (Becker 1993). Most other mammals are able to further degrade UA to allantoin. UA is converted to allantoin by uricase, but humans have several "nonsense" mutations in the gene encoding uricase, and therefore this gene is not expressed, although it is still present (Yeldandi *et al.* 1992).

Although the human body does not contain an active uricase enzyme, ROS may non-enzymatically change the UA to allantoin, parabanic acid, oxaluric acid, oxonic acid, cyanuric ucid and urea (Volk *et al.* 1989, Kaur and Halliwell 1990, Hicks *et al.* 1993). Allantoin is

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the most prevalent and its level in body fluids is thus regarded as an indicator of an increased production of ROS (Tolun *et al.* 2010, 2012, Il'yasova *et al.* 2012, Turner *et al.* 2012, Chung and Benzie 2013). The human body carefully protects its UA reserves. UA is completely filtered in kidneys, but more than 90 % is resorbed into the blood in the tubules. Such a treatment of UA is therefore not consistent with the theory that UA is only the end product of purine metabolism (Becker *et al.* 1991).

The antioxidant importance of UA lies not only in the fact that it is found in all body fluids and tissues, but also that its plasma concentration (150-450 μmol/l) is much higher than that of other antioxidants (Becker *et al.* 1991, Becker 1993). One-electron oxidation of UA with strong oxidants creates a urate anion radical that is efficiently taken up by ascorbic acid (Maples and Mason 1988, Becker 1993). More important is the ability of UA to create stable coordination complexes with iron ions. Through this mechanism, UA inhibits the Fenton reaction (giving rise to the highly reactive hydroxyl radical). Inhibition of the formation of the hydroxyl radical is considered the most important antioxidant effect of UA (Ames *et al.* 1981). UA itself also reacts with a hydroxyl radical and hypochlorous acid, and inhibits the formation of oxo-heme oxidants (Maples and Mason 1988, Becker *et al.* 1991).

The role of ROS in skeletal muscle damage after strenuous physical activity has been discussed a great deal recently. Physical load significantly increases the production of ROS; their destructive effects can then influence sports performance and contribute to the onset of muscle fatigue (Ji 1999, Castrogiovanni and Imbesi 2012, Gravier *et al.* 2013, Ramos *et al.* 2013).

The level of ROS production depends on the duration, intensity and type of physical activity, is highly variable because it depends on physical condition, diet, genetic and various other factors.

The aim of this work is to monitor the levels of allantoin, UA, and malondialdehyde (MDA) in plasma and erythrocytes of volunteers burdened with strenuous exercise, when a significant production of ROS is expected.

Materials and Methods

Reagents and chemicals

Allantoin, ortho-phosphoric acid, sodium hydroxide, sodium chloride, hydrochloric acid, phosphate buffer (8.3 mmol/l, pH 7.2), sodium dihydrogenphosphate, sodium hydrogenphosphate, metaphosphoric acid (MPA), uric acid, 1,1,3,3-tetramethoxypropane (TMP), ethylenediaminetetraacetic acid (EDTA), acetic acid, 2,4-dinitrophenylhydrazine (DNPH) and 2-thiobarbituric acid (TBA) were obtained from Sigma Chemical Company. AG 1-X8 Resin, 100-200 mesh, chloride form, was purchased from Bio-Rad Laboratories (Hercules, CA, USA), HPLC-gradient grade acetonitrile (ACN) and methanol was from Merck (Darmstadt, Germany). Lyophilized UA, creatinine and lactate mixed standard (CHEM I Calibrator, Level 3, Lot 4HD007) and liquid assayed chemistry UA, creatinine and lactate control (Dade TRU-Liquid Moni-Trol Control, Level 1, Lot TLM0503-1 and Level 2, Lot TLM0503-2) were from Dade Behring (Newark, DE, USA). All other chemicals were of analytical grade. Because UA is slightly soluble in alkaline solution, but an alkali environment leads to degradation of UA into allantoin, a commercial lyophilized standard was used.

Instrumentation

Chromatographic analyses were performed with a liquid chromatograph Shimadzu (Kyoto, Japan) equipped with a LC-10AD solvent delivery system, a SIL-10AD autosampler, a CTO-10AS column oven, a SPD-10A variable wavelength spectrophotometric detector, and a SCL-10A system controller. Data were collected digitally using the chromatography software Clarity (DataApex, Prague, Czech Republic).

Spectrophotometric analyses were carried out on a Shimadzu (Kyoto, Japan) UV-1700 PharmaSpec spectrophotometer.

Subjects

A total of 30 healthy subjects (15 women aged 22-36 years, mean age 29 years, and 15 men aged 21-40 years, mean age 31 years) were included in the study (for the group characteristics, see Table 1). None of the studied subjects exhibited any renal, hepatic, gastrointestinal, pulmonary or oncological diseases. They were considered healthy according to a physical examination and routine laboratory tests. All study participants were informed of all risks and gave written informed consent to participate in this study, which was approved by the Hospital Committee on Human Research (Regional Hospital of Pardubice, Czech Republic) according to the Helsinki Declaration.

Table 1. Group characteristics.

	Women	Men	P
Age (years)	29 ± 4	31 ± 5	-
BMI (kg/m^2)	22.7 ± 3.8	23.4 ± 4.1	-
Hemoglobin (g/l)	139 ± 7	151 ± 9	***
P_ALA (μmol/l)	1.8 ± 0.5	2.0 ± 0.9	-
RBC_ALA (nmol/g Hb)	17 ± 5	19 ± 7	-
P_UA (μmol/l)	220 ± 60	315 ± 68	***
RBC_UA (nmol/g Hb)	74 ± 16	83 ± 26	-
P_MDA (μmol/l)	0.79 ± 0.21	0.88 ± 0.29	-
RBC_MDA (nmol/g Hb)	67 ± 18	75 ± 24	-
P_Lactate (mmol/l)	1.2 ± 0.7	1.4 ± 0.9	-

P – Plasma, RBC – Red Blood Cells, ALA – Allantoin, UA – Uric Acid, MDA – Malondialdehyde, Hb – Hemoglobin, BMI – Body Mass Index. *** P<0.001.

The subjects were trained in different kinds of sports such as football, basketball, swimming, cycling, badminton and squash. All volunteers attended 10-min run at a speed of about 9.6±0.3 km per hour on flat ground (4 x 400 m oval track; 1600±50 m).

Whole blood samples collection

Peripheral venous blood samples were obtained from each volunteer immediately before starting the experiment (before intense exercise), immediately after intense exercise (10-min run) and 60 min after intense exercise. Blood (6 ml) was collected into plastic tubes with EDTA (The Vacuette Detection Tube, No. 456023, Greiner Labortechnik Co., Kremsmünster, Austria). Plasma was separated from red blood cells by centrifugation (1700 x g, 15 min, 8 °C) and immediately stored at –80 °C. Erythrocytes were washed four times by isotonic solution (0.85 % sodium chloride) and after their lysis by ice-cold deionized water immediately stored at –80 °C.

Determination of allantoin

Plasma and erythrocyte allantoin was measured as previously reported (Kanďár *et al.* 2006, Kanďár and Záková 2008a). Briefly, 300 μl of standard solution, plasma or red blood cells hemolysate were pipetted into a well-capped 1.5 ml polypropylene (PP) tube (Thermo Fisher Scientific, Pardubice, Czech Republic). 600 μl of cold ACN were added and the solution was vortexed for 60 s, incubated (4 °C, 10 min), and centrifuged (22 000 x g, 4 °C, 10 min). Sediment was re-extracted with 600 μl of cold ACN/0.01 mol/l H_3PO_4, pH 7.3 (1:1). The combined supernatants were immediately applied to solid phase extraction (SPE) column (AG 1-X8 anion-exchange resin). A purified sample was evaporated to dryness under nitrogen (Linde Gas, Prague, Czech Republic) at 60 °C. Dry residue was dissolved in 200 μl of 0.1 mol/l NaOH and incubated (100 °C, 20 min). After cooling, 300 μl of 1.5 mmol/l DNPH in 2.5 mol/l HCl were added and the solution was incubated (50 °C, 50 min). The reaction mixture was then filtered through a nylon filter (pore size 0.20 μm, 4 mm diameter; Supelco, Bellefonte, PA, USA) and transferred into 1.0 ml amber vial. 20 μl was injected onto a HPLC equipped with a guard column Discovery C18, 20 x 4 mm i.d., 5 μm, an analytical column Discovery C18, 150 x 4 mm i.d., 5 μm (Supelco), and a UV/Vis detector. For the gradient elution, two mobile phases were used: A – 5 % ACN in 8.3 mmol/l phosphate buffer (v/v), pH 6.1±0.1, and B – 50 % ACN in 8.3 mmol/l phosphate buffer (v/v), pH 6.1±0.1. The flow rate was kept constant at 0.5 ml/min, and the separation ran at 37 °C. Optimum response of glyoxylate-2,4-dinitrophenylhydrazone derivative, corresponding to allantoin, was observed when the wavelength was set to 360 nm. The analytical parameters of plasma and erythrocyte allantoin analysis were as follows: intra-assay with coefficient of variation (CV) 5.7 % (n=10) and CV 3.8 % (n=10), inter-assay with CV 8.3 % (n=12) and CV 6.6 % (n=12), and recovery 94.1 % (n=5) and 97.2 % (n=5). The calibration curve was linear in the whole range tested (0.5-50.0 μmol/l). The lowest concentration that could be quantified with acceptable accuracy and precision was 0.5 μmol/l (6.0 pmol/inject). The limit of detection, defined as a signal-to noise (S/N) ratio of 3:1, was 0.15 μmol/l (1.8 pmol/inject).

Determination of uric acid

Plasma and erythrocyte UA was measured as previously reported (Kanďár and Záková 2008b). Briefly, 200 μl of standard solution, plasma or red blood cells hemolysate were pipetted into a well-capped 1.5 ml PP tube. 400 μl of cold 10 % MPA were added and the solution was vortexed for 60 s, incubated (4 °C, 10 min), and centrifuged (22 000 x g, 4 °C, 10 min). Supernatants were filtered through a nylon filter (pore size 0.20 μm, 4 mm diameter) and transferred into 1.0 ml amber vials. 10 μl was injected onto a HPLC system. The chromatography analysis of UA was accomplished using an isocratic elution on a Discovery C18, 250 x 4 mm i.d.,

5 µm, analytical column fitted with a Discovery C18, 20 x 4 mm i.d., 5 µm, guard column at 25 °C. The mobile phase was a mixture of methanol and 25 mmol/l sodium dihydrogenphosphate (v/v), pH 4.8±0.1. The flow rate was kept constant at 0.5 ml/min. Optimum response of UA was observed when wavelength was set to 292 nm.

The analytical parameters of plasma and erythrocyte UA analysis were as follows: intra-assay with CV 2.3 % (n=10) and CV 3.8 % (n=10), inter-assay with CV 7.7 % (n=12) and CV 9.2 % (n=12), and recovery 98.1 % (n=5) and 94.3 % (n=5). The calibration curve was linear in the whole range tested for plasma (20-1000.0 µmol/l) and erythrocyte lysate (1-100 µmol/l). The lowest concentration that could be quantified with acceptable accuracy and precision was 1.0 µmol/l (3.3 pmol/inject). The limit of detection, defined as a signal-to noise (S/N) ratio of 3:1, was 0.3 µmol/l (1.0 pmol/inject).

Determination of malondialdehyde

Plasma and erythrocyte MDA was measured as previously reported (Kandár *et al.* 2002). Briefly, 200 µl of standard solution (MDA obtained by acid hydrolysis of TMP), plasma or red blood cells hemolysate were pipetted into a well-capped 2.0 ml amber glass tube, 600 µl of 0.1 % EDTA and 200 µl of 28 mmol/l TBA in 8.75 mol/l acetic acid were added and the solution was vortexed for 60 s and incubated (100 °C, 60 min). After cooling, 500 µl of cold n-butanol were then added, the solution was vortexed for 30 min, and centrifuged (2910 x g, 4 °C, 20 min). The upper n-butanol layer was filtered through a nylon filter (pore size 0.20 µm, 4 mm diameter) and transferred into 1.0 ml amber vial. 10 µl was injected onto a HPLC equipped with a guard column LiChroCart 4 x 4 mm, Purospher Star RP-18e, 5 µm, an analytical column LiChroCart 125 x 4, Purospher Star RP-18e, 5 µm (Merck, Darmstadt, Germany), and a UV/Vis detector. For the isocratic elution of $MDA(TBA)_2$ derivative, a mixture of 35 % methanol in 8.3 mmol/l phosphate buffer (v/v), pH 7.2, was used as a mobile phase. The flow rate was kept constant at 0.5 ml/min, and the separation ran at 37 °C. Optimum response of $MDA(TBA)_2$ derivative was observed when the wavelength was set to 532 nm. The analytical parameters of plasma and erythrocyte MDA analysis were as follows: intra-assay with CV 5.2 % (n=10) and CV 5.7 % (n=10), inter-assay with CV 8.4 % (n=12) and CV 9.1 % (n=12) and recovery 96.6 % (n=5) and 95.4 % (n=5). The calibration curve was linear in the whole range tested (0.2-10.0 µmol/l). The lowest concentration that could be quantified with acceptable accuracy and precision was 0.2 µmol/l (0.80 pmol/inject). The limit of detection, defined as a signal-to noise (S/N) ratio of 3:1, was 0.06 µmol/l (0.24 pmol/inject).

Determination of lactate

Lactate in the plasma was measured with the set Lactate Flex® by standard procedure using an automatic biochemistry analyzer Dimension® RxL Max® (Siemens Healthcare Diagnostic Ltd., Deerfield, IL, USA).

Determination of hemoglobin

Hemoglobin in the red blood cells hemolysate was measured with a HEMOGLOBIN set (Lachema, Brno, Czech Republic). Briefly, 5.00 ml of working solution (0.8 mmol/l potassium cyanide and 0.5 mmol/l potassium ferricyanide in 1.1 mmol/l N-methyl-D-glucamine buffer, pH 8.3) were mixed with 0.02 ml of the red blood cells hemolysate sample or standard solution in a test tube. After incubation (room temperature, 10 min), the absorbance was read at 543 nm against the working solution on a UV-1700 PharmaSpec spectrophotometer.

Statistical analysis

Data were analyzed using the Sigmastat version 3.5 (Systat Software Inc., Point Richmond, CA, USA) and the STATISTICA version 12 (StatSoft CR s.r.o., Prague, Czech Republic). The data are presented as median ± IQR (interquartile range). Differences between women and men were analyzed using the Mann-Whitney Rank Sum Test. A two-factor analysis of variance (ANOVA) was performed to investigate of changes in levels of plasma and red blood cells hemolysate allantoin, UA, MDA and plasma lactate as a function of time (immediately after exercise and one hour after exercise) and age. *Post-hoc* comparisons were made using the Holm Sidak test, with alpha set at 0.05. The Holm Sidak test can be used for both pairwise comparisons and comparisons versus a control group. It is more powerful than Tukey and Bonferroni test, and, consequently, it is able to detect differences that these tests do not. It is recommended as the first-line procedure for pairwise comparison testing.

Results

In this study, we monitored the levels of allantoin and UA in the plasma and erythrocytes of

volunteers after a 10-min running activity. In addition, we determined the plasma and erythrocyte levels of MDA, a classic indicator of oxidative stress, and plasma lactate concentration, an appropriate indicator of muscle load and anaerobic glycolysis.

We found a significant increase in the plasma concentration of allantoin immediately after the 10-min running activity, on average by 200 %. After an hour of rest, allantoin levels almost returned to their initial value (Fig. 1A). Significant increase in the erythrocyte concentration of allantoin was observed too, and this trend continued during the first hour after the workout. This is a completely different process than that in plasma (Fig. 1B). Allantoin is a highly polar substance, thus is completely, just like for example creatinine, practically totally eliminated *via* the kidneys (Lagendijk *et al.* 1995, Berthemy *et al.* 1999). Therefore, after one hour, we found allantoin in plasma at virtually the same levels as before the running activity. Allantoin from erythrocytes is apparently released very slowly into plasma. It is possible that in erythrocytes the oxidative stress during physical load is more intensive and lasts longer.

Changes in the plasma levels of UA had a completely different course than the changes in the levels of allantoin. The concentration of UA reached its maximum one hour after the end of the workout (Fig. 1C). The erythrocyte level of uric acid immediately after running activity was significant increased, after an hour of rest a decline was observed (Fig. 1D). This corresponds to an increased level of erythrocyte allantoin, an oxidative product of UA. It is known that the level of UA in plasma increases during physical exertion. This is probably also partially caused by an inhibition of the renal clearance of UA with lactate (Fig. 2C), which is accumulated in plasma during exercise (Hellsten *et al.* 2001). Another possible mechanism for increasing the concentration of UA in plasma is the metabolic conversion of hypoxanthine to UA in hepatocytes. The resulting UA is then used as an antioxidant in myocytes and erythrocytes exposed to oxidative stress, resulting in increased levels of allantoin. The enrichment of muscles and erythrocytes with UA during exercise could therefore mean an increase in the availability of antioxidant substances stimulated by the increasing production of ROS.

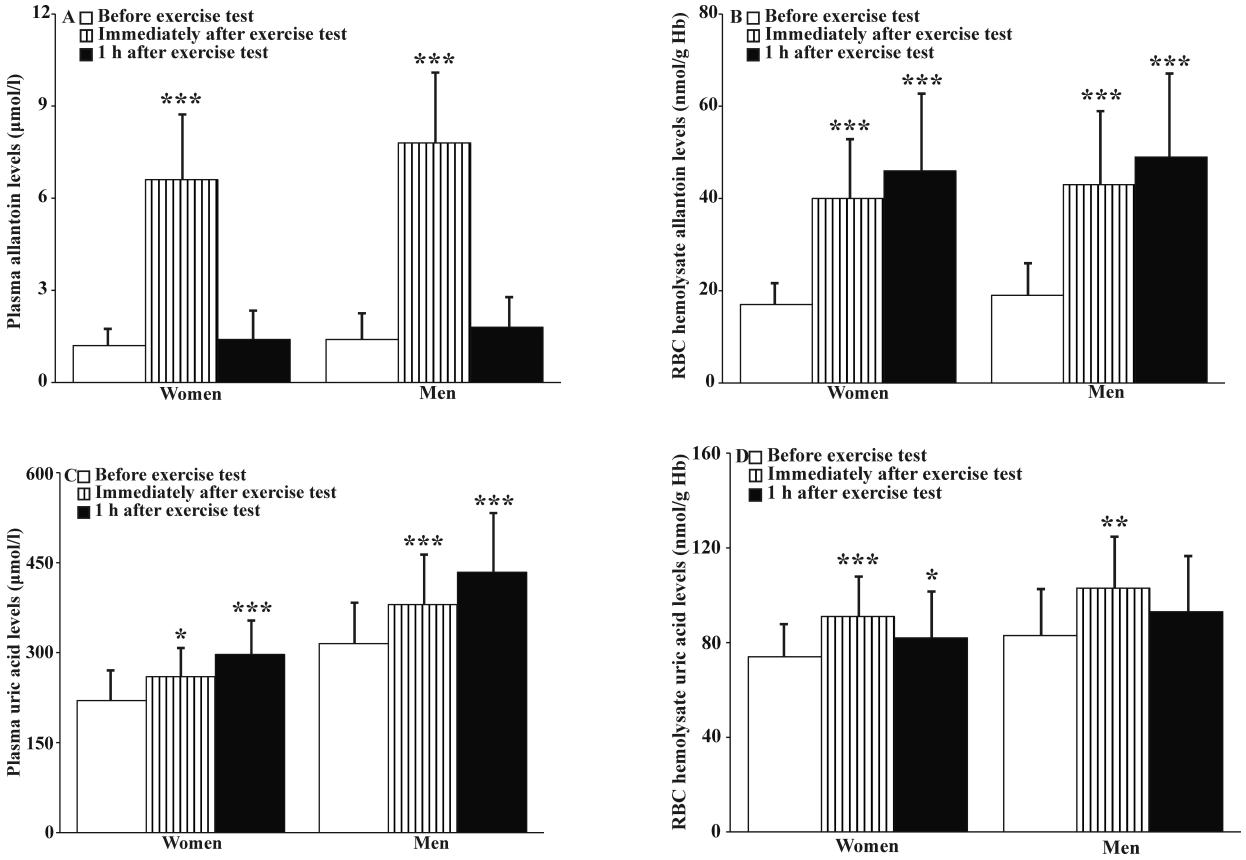

Fig. 1. Plasma allantoin **(A)**, red blood cells hemolysate allantoin **(B)**, plasma uric acid **(C)**, and red blood cells hemolysate uric acid **(D)** levels in healthy subjects (15 women and 15 men; duplicate assays) before running activity, immediately after running activity and 1 h after running activity. The results are expressed as median ± IQR (interquartile range). *p<0.05; **p<0.01; ***p<0.001 (compared to levels before running activity).

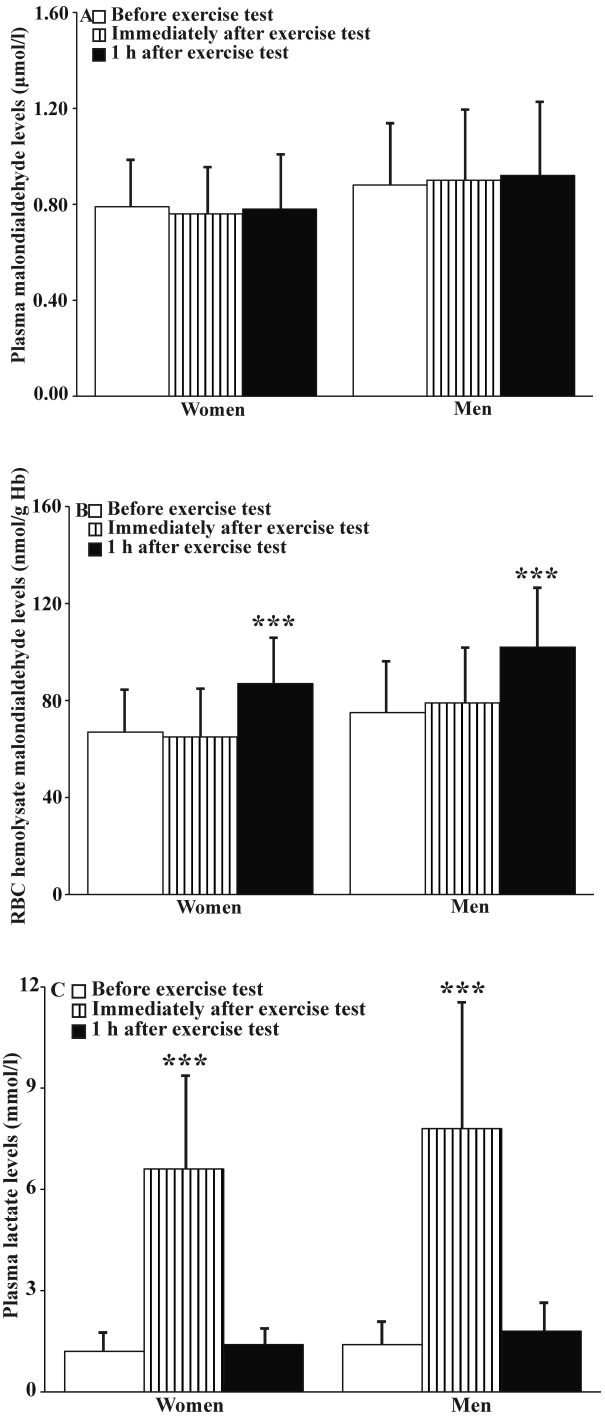

Fig. 2. Plasma malondialdehyde **(A)**, red blood cells hemolysate malondialdehyde **(B)**, and plasma lactate **(C)** levels in healthy subjects (15 women and 15 men; duplicate assays) before running activity, immediately after running activity and 1 h after running activity. The results are expressed as median ± IQR (interquartile range). *p<0.05; **p<0.01; ***p<0.001 (compared to levels before running activity).

As for changes in the plasma levels of MDA, we found no significant difference after the physical exertion and after an hour of rest (Fig. 2A). MDA is a product of lipid peroxidation, which is a relatively slow process (Yücel *et al.* 1998), and the resulting MDA immediately reacts with biomolecules within the cell, as indicated by the elevated levels of MDA in erythrocytes (Fig. 2B). It can also indicate increased oxidative stress in erythrocytes. Increased lipid peroxidation occurs in erythrocyte membranes due to an excessive production of ROS during exercise.

ANOVA was performed to examine the main effects of time (immediately and one hour after exercise), age, and interaction (time x age) on the measured variables (Table 2). There was significant main effect of time in the ANOVA of plasma allantoin (immediately after exercise), plasma UA (both immediately and one hour after exercise in men, one hour after exercise in women), plasma lactate (immediately after exercise), and red blood cells hemolysate (RBC) allantoin (both immediately and one hour after exercise), RBC UA (immediately after exercise) and RBC MDA (after one hour exercise). Effect of age in the ANOVA of plasma allantoin in women ($p=0.040$ and 0.022), RBC allantoin in men ($p=0.016$ and 0.034), plasma UA in men (0.031), RBC UA in women (0.049 and 0.045), plasma MDA (0.039) and RBC MDA (0.031 and 0.013) in women was observed, but statistically insignificant (power of test was <0.800). Traditionally, the power of the performed test should be >0.8.

Discussion

Current laboratory diagnostics of oxidative stress focuses on both the observation of the increased production of oxidants, disorders in antioxidant systems and monitoring of oxidative damage; and in recent years, the ability of the antioxidant system to adequately respond to oxidative stress (Kanďár *et al.* 2007, Ogasawara *et al.* 2009, Dorjgochoo *et al.* 2012, Mesaros *et al.* 2012). Allantoin was recently suggested as an indicator of oxidative stress (Kanďár *et al.* 2006, Kanďár and Záková 2008a, Yardim-Akaydin *et al.* 2006, Gruber *et al.* 2009).

It was shown that skeletal muscle consumes UA during exercise (McAnulty *et al.* 2007). Waring *et al.* (2001) suggested the important antioxidant role of UA at exercise. UA levels in cells are replenished by uptake from blood after exercise (Hellsten *et al.* 2001). A significant relationship between plasma UA levels and acute oxidative stress during exercise was found (Mikami *et al.* 2000).

Table 2. Summary table for the two factor analysis of variance (ANOVA) investigating the changes in levels of allantoin, uric acid, malondialdehyde and lactate in women and men as a function of time (immediately after exercise and one hour after exercise) and age.

Set	Factor	Immediately after exercise				One hour after exercise			
		F	p	Power (1-β)	Conclusion	F	p	Power (1-β)	Conclusion
Women	Time	221.460	**<0.001**	**1.000**	*Significant*	3.245	0.097	0.269	-
P_ALA	Age	3.061	**0.040**	0.558	-	3.642	**0.022**	0.686	-
(μmol/l)	Time*Age	0.974	0.498	0.050	-	0.417	0.890	0.050	-
Men	Time	92.857	**<0.001**	**1.000**	*Significant*	0.742	0.406	0.050	-
P_ALA	Age	0.925	0.529	0.0500	-	1.727	0.190	0.204	-
(μmol/l)	Time*Age	0.200	0.985	0.0500	-	0.110	0.998	0.050	-
Women	Time	99.230	**<0.001**	**1.000**	*Significant*	87.425	**<0.001**	**1.000**	*Significant*
RBC_ALA	Age	1.946	0.144	0.262	-	1.605	0.222	0.174	-
(nmol/g Hb)	Time*Age	0.379	0.912	0.050	-	0.433	0.879	0.050	-
Men	Time	141.822	**<0.001**	**1.000**	*Significant*	150.828	**<0.001**	**1.000**	*Significant*
RBC_ALA	Age	3.989	**0.016**	0.750	-	3.197	**0.034**	0.591	-
(nmol/g Hb)	Time*Age	0.739	0.658	0.050	-	0.666	0.712	0.050	-
Women	Time	5.295	**0.040**	0.471	-	17.196	**0.001**	**0.968**	*Significant*
P_UA	Age	0.779	0.629	0.050	-	0.834	0.591	0.050	-
(μmol/l)	Time*Age	0.009	1.000	0.050	-	0.026	1.000	0.050	-
Men	Time	19.004	**<0.001**	**0.981**	*Significant*	64.023	**<0.001**	**1.000**	*Significant*
P_UA	Age	2.820	0.052	0.498	-	3.289	**0.031**	0.612	-
(μmol/l)	Time*Age	0.016	1.000	0.050	-	0.098	0.999	0.050	-
Women	Time	25.551	**<0.001**	**0.997**	*Significant*	6.558	**0.025**	0.580	-
RBC_UA	Age	2.859	**0.049**	0.508	-	2.941	**0.045**	0.529	-
(nmol/g Hb)	Time*Age	0.106	0.998	0.050	-	0.095	0.999	0.050	-
Men	Time	12.672	**0.004**	**0.893**	*Significant*	4.185	0.063	0.364	-
RBC_UA	Age	1.688	0.199	0.194	-	1.285	0.335	0.102	-
(nmol/g Hb)	Time*Age	0.061	1.000	0.050	-	0.036	1.000	0.050	-
Women	Time	0.941	0.351	0.050	-	0.109	0.747	0.050	-
P_MDA	Age	3.071	**0.039**	0.561	-	2.375	0.086	0.379	-
(μmol/l)	Time*Age	0.025	1.000	0.050	-	0.050	1.000	0.050	-
Men	Time	0.173	0.685	0.050	-	0.409	0.534	0.050	-
P_MDA	Age	1.300	0.329	0.105	-	1.097	0.427	0.066	-
(μmol/l)	Time*Age	0.004	1.000	0.050	-	0.015	1.000	0.050	-
Women	Time	0.432	0.523	0.050	-	40.915	**<0.001**	**1.000**	*Significant*
RBC_MDA	Age	3.312	**0.031**	0.617	-	4.238	**0.013**	0.789	-
(nmol/g Hb)	Time*Age	0.037	1.000	0.050	-	0.030	1.000	0.050	-
Men	Time	0.612	0.449	0.050	-	34.614	**<0.001**	**1.000**	*Significant*
RBC_MDA	Age	1.730	0.189	0.205	-	2.268	0.097	0.349	-
(nmol/g Hb)	Time*Age	0.020	1.000	0.050	-	0.094	0.999	0.050	-
Women	Time	82.520	**<0.001**	**1.000**	*Significant*	0.861	0.372	0.050	-
P_Lactate	Age	0.611	0.754	0.050	-	0.823	0.598	0.050	-
(mmol/l)	Time*Age	0.236	0.967	0.050	-	0.024	1.000	0.050	-
Men	Time	104.457	**<0.001**	**1.000**	*Significant*	4.086	0.066	0.354	-
P_Lactate	Age	1.217	0.366	0.088	-	1.392	0.292	0.124	-
(mmol/l)	Time*Age	0.554	0.796	0.050	-	0.019	1.000	0.050	-

The F test statistic is provided for comparisons within each factor and between the factors. The p value is the probability of being wrong in concluding that there is a true difference between the groups. There are significant differences if $p<0.05$. The Power or sensitivity is the probability that the test will detect the observed difference among the groups if there really is a difference. The closer the power is to 1, the more sensitive the test. Traditionally, the power of the performed test should be >0.8. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously. P – Plasma, RBC – Red Blood Cells, ALA – Allantoin, UA – Uric Acid, MDA – Malondialdehyde.

Many studies showed that UA is oxidized by ROS mainly to allantoin (Matrens *et al.* 1987, Santos *et al.* 1999, Kanďár and Záková 2008a). Oxidation of UA to allantoin during exercise suggests both that UA is of significant as an antioxidant and that ROS are formed (Hellsten *et al.* 2001). Therefore UA and allantoin levels as well allantoin/UA ratio are suitable indicators in the assessment of the level of acute oxidative stress in human during exercise.

Hellsten *et al*. (1997) found a physiological increase in allantoin levels in the plasma of 7 voluntary blood donors, who performed five minutes of intense exercise. They state that these volunteers had allantoin concentrations in the range of 11.9±2.6 μmol/l before exercise, and during exercise the concentration more than doubled. The level of UA in plasma rose from 305±16 μmol/l to 426±20 μmol/l within 45 min after the exercise. We achieved similar results, but with a much higher variance in the measured levels. They also compared the levels of allantoin and UA directly in muscles and obtained the following results: the UA level in muscle tissue decreased from 0.260±0.023 μmol/g to 0.084±0.016 μmol/g during exercise and then increased sharply during the first three minutes after exercise. The allantoin concentration increased from 0.030±0.007 μmol/g to 0.100±0.014 μmol/g during exercise and it decreased to 0.079±0.002 μmol/g during the first three minutes after exercise. Muscle biopsy is an invasive intervention, so a simpler way needed to be found to monitor oxidative stress in cells during physical exertion. Erythrocytes seemed to be suitable cells.

It is generally known that oxidative stress markers are age-depended. Some indications were observed; on the other hand the power of the performed tests was below the desired power of 0.800. ANOVA power is affected by the sample size. Furthermore, a small age difference (14 years in women and 19 years in men) is in the study group.

It can be concluded that the importance of ROS in sports is not yet fully appreciated and influencing the production and effects of ROS might be a way to improve the performance of athletes. Our results suggest that during sports activities, the levels of allantoin and UA in plasma and erythrocytes are increased. UA is probably oxidized by ROS to allantoin. Our study based on the monitoring of UA and allantoin levels in plasma and erythrocytes during 10-min running activity reveal that allantoin may be suitable candidate for a marker of acute oxidative stress. Determination of allantoin levels in erythrocytes is for the monitoring of an acute oxidative stress more suitable because plasma allantoin is rapidly and completely eliminated *via* the kidneys. In addition, whole blood is more available biological material than a biopsy sample.

Conflict of Interest

There is no conflict of interest.

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