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Accumulation of Uric Acid in Plasma after Repeated Bouts of Exercise in the Horse

Leena A. Räsänen, Pauliina A. S. Wiitanen,**
*Esa-Matti Lilius,† Seppo Hyyppä‡ and A. Reeta Pösö**

*Department of Basic Veterinary Sciences, Section of Biochemistry, Faculty of Veterinary Medicine, POB 57, FIN-00014 University of Helsinki, Finland; †Department of Biochemistry, University of Turku, FIN-20500 Turku, Finland and ‡ Agricultural Research Centre, Equine Research, FIN-32100 Ypäjä, Finland

ABSTRACT. Plasma concentration of uric acid, total peroxyl radical-trapping antioxidative parameter (TRAP), blood lactate concentration and plasma activity of xanthine oxidase (XO) were measured in six Standardbreed trotters after six bouts of exercise with increasing intensity on two separate days three days apart. Blood samples were taken immediately, 5, 10, 15, 30 and 60 min after each heat and 2, 4, and 6 hr after the last heat. Exercise caused an increase in TRAP and in the concentrations of lactate and uric acid. Plasma uric acid concentration increased exponentially with respect to time after the last heat performed at maximal speed, indicating a rapid increase in the rate of purine degradation. Plasma XO activity increased during exercise, but the intensity of exercise had only a minor effect on the level of XO activity. In conclusion, these data suggest that a threshold for the plasma accumulation of uric acid in terms of the intensity of exercise may exist and that XO may play a role in the formation of uric acid in horse plasma. Intense exercise causes an increase in the plasma antioxidant capacity that in the horse is mainly caused by the increase in the plasma uric acid concentration. COMP BIOCHEM PHYSIOL 114B, 139–144, 1996.

KEY WORDS. Exercise, horse, uric acid, xanthine oxidase, reactive oxygen, species, antioxidants, TRAP, lactate

INTRODUCTION

During intense exercise, muscle phosphocreatine (PCr) and glycogen are used for the rephosphorylation of ADP. When muscle PCr stores are failing ADP accumulation starts. Increased ADP level triggers the myokinase reaction, where two ADP molecules form one molecule of ATP and one of AMP. The latter is further deaminated to IMP and metabolized via inosine, hypoxanthine, xanthine to uric acid in humans and finally to allantoin in horses. High-intensity exercise has been shown to cause a significant decrease in the ATP content and a corresponding increase in the IMP content in the muscle of the Thoroughbred horse (8,22). The decline in muscle ATP is mirrored by the postexercise appearance of the end products of the pathway, uric acid and allantoin into plasma of the horse (8,19).

During the degradation of purine nucleotides, oxidation of hypoxanthine takes place in the capillary endothelial cells of muscle, liver and other tissues where xanthine dehydrogenase/oxidase (XDH/XO) oxidizes it to xanthine and to uric acid (10,18). Uric acid is transported to kidneys or to hepatocytes where uricase converts it to allantoin in nonprimate mammals (16). Uric acid can also be formed in plasma that contains XO even after moderate exercise (20). The XO form of the enzyme produces reactive oxygen species that have been claimed to initiate exercise-induced muscle injury by attracting neutrophils and by modifying their adhesion to endothelium (6,26). Antioxidants (vitamin E, ascorbic acid, uric acid, etc.) act as scavengers of free radicals and may thus have a role in preventing the tissue injury. Regular training in rats is known to increase the amount of endogenous antioxidants in plasma (17) and antioxidant enzymes in liver and in skeletal muscle (13).

It has been shown that there is a threshold for both adenine nucleotide degradation, as indicated by muscle IMP and plasma NH_3 accumulation, in equine muscle (22) and in human plasma (21), and for the plasma accumulation of hypoxanthine but not for uric acid (11) in human athletes. Studies on the possible threshold in the plasma accumulation of uric acid in trotters have not been performed, and the aim of this study was to investigate whether such a threshold exists. Furthermore, it was investigated whether

Correspondence to: Dr. L. A. Räsänen, Department of Basic Veterinary Sciences, Section of Biochemistry, Faculty of Veterinary Medicine, POB 57, FIN-00014 University of Helsinki, Finland. Tel. +358-0-708-49536 or 708-49523; Fax + 358-0-708-49799; e-mail Leena.Az.Rasanen@helsinki.fi.

Abbreviations—PCr, phosphocreatine; TRAP, total peroxyl radical-trapping antioxidive parameter; XO, xanthine oxidase; XDH, xanthine dehydrogenase.

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the plasma activity of XO plays a role in the accumulation of uric acid and whether the exercise-induced production of reactive oxygen species is accompanied by a change in the plasma antioxidant capacity.

MATERIALS AND METHODS

Horses and Experimental Design

The experimental design was approved by the Ethical Committee for Animal Experiments of Agricultural Research Centre.

Six Standardbred trotters, aged 3–10 years, were used in an experiment lasting for 4 days. Three of the horses were geldings, two stallions and one was a mare. On days 1 and 4, the horses performed at 60-min intervals three exercise bouts with increasing intensity. On day 1, horses twice trotted 3000 m and once 2000 m in that order and on day 4 twice 2100 m and once 1600 m on a 1000-m racecourse. The speed of the horses was adjusted according to their individual condition so that they ran faster heat by heat, and the third heat on day 4 was at maximal speed. The average speeds ± SD were 9.6 ± 0.3, 10.1 ± 0.3 and 10.6 ± 0.3 $m \cdot s^{-1}$ on day 1 and 10.4 ± 0.8, 10.8 ± 0.3 and 11.7 ± 0.6 $m \cdot s^{-1}$ on day 4, respectively. A pacecar was used to keep the speeds correct and steady. After each heat, horses were walked for 15 min and they then stood in a shelter. On day 2, horses did a light 30-min workout and spent 2 hr in a paddock where they were allowed to move freely. On day 3, horses spent 4 hr in the paddock.

Blood samples from the jugular vein were taken before the first heat, immediately, 5, 10, 15, 30 and 60 min after each heat and 2, 4, 6 hr after the third heat. Samples (20 ml) were drawn to a syringe either through a catheter (Intraflon 12G, Vycon, Belgium) placed into the jugular vein before the test or by venepuncture. Blood was transferred to lithium heparin tubes and for the lactate analysis to tubes containing heparin, fluoride, nitrite and a hemolyzing agent (Analzo GMRD-047, Analox Instruments Ltd., London, U.K.). Li-heparin tubes were kept on ice until the plasma was separated by centrifugation and stored frozen (−70°C) until analysed.

Analyses

Lactate concentrations were analysed with an enzymatic lactate analyzer (Analox LM5, Analox Instruments Ltd.).

The activity of XO in plasma was measured as described by Beckman *et al.* (3). The measurements were made at ambient temperature.

For the measurement of uric acid, 0.1 ml heparinised plasma was mixed with 0.5 ml methanol, and the precipitated proteins were removed by centrifugation at 15,000 **g** for 10 min. The supernatant was removed and evaporated to dryness in a water bath, 40°C, with a flow of nitrogen and dissolved in 0.5 ml 0.2 M potassium phosphate buffer, pH 5.0. The samples were filtered through a 0.22-μm filter (Millex®-GV, Millipore S. A., Molsheim, France). Uric acid concentration was analysed by high-performance liquid chromatography (24). A 10-μl aliquot was injected onto a LiChrospher® C_{18} reversed phase column (5 μm, 250 × 4 mm; Merck, Darmstadt, Germany), and uric acid was detected with an UV-detector at 256 nm. Mobile phase consisted of 0.2 M potassium phosphate buffer, pH 5.0, and a 50:25:25, v/v/v, mixture of water–acetonitrile–and methanol. Flow rate was 2 $ml \cdot min^{-1}$, and the gradient conditions were those described by Teerlink *et al.* (21). External standards that were treated similarly to the samples were used to calculate the concentrations.

Total peroxyl radical-trapping ability of plasma was measured by the modified method of Alanko *et al.* (2) and Uotila *et al.* (25). According to preliminary experiments, 2,2-azo-bis(2-amidinopropane)hydrochloride (ABAP) concentration was adjusted to 200 $mmol \cdot l^{-1}$, because of the low amount of antioxidants in equine plasma compared with human plasma.

Statistics

The results are expressed as means ± SE. Temporal patterns of parameters during the two exercise days and the recovery periods were analysed by repeated measurements analysis of variance with two within factors. Each parameter was analysed separately. The significance of *F*-ratios were evaluated using the Greenhouse-Geisser adjusted *P* values in testing the overall within subject effects and their interactions. Specific comparisons after a significant overall effect were undertaken by paired *t*-tests contrasting predetermined time points to the rest level. The differences between two successive values of each parameter were used in counting the correlations between parameters.

RESULTS

Blood lactate concentration, which was used as an indicator of the intensity of exercise (Fig. 1A), stayed significantly higher than at rest from 0 to 10 min after the third heat on the first day of exercise and from 0 min to 4 hr on the second day of exercise. The difference in the lactate responses to the heats was significant between each successive heat on the first day of exercise and between the second and third heat on the second day of exercise. The second day was significantly more strenuous ($P < 0.05$) than the first when the rest values were compared with the values obtained immediately after the third heat (Fig. 1A).

Uric acid concentration (Fig. 1B) stayed significantly higher than at rest from 0 to 60 min after the third heat on the first day of exercise and 5 min to 2 hr on the second day of exercise. The peak concentration was reached 15–30 min after the last heat performed at maximal speed. The difference in the responses to the heats was significant be-

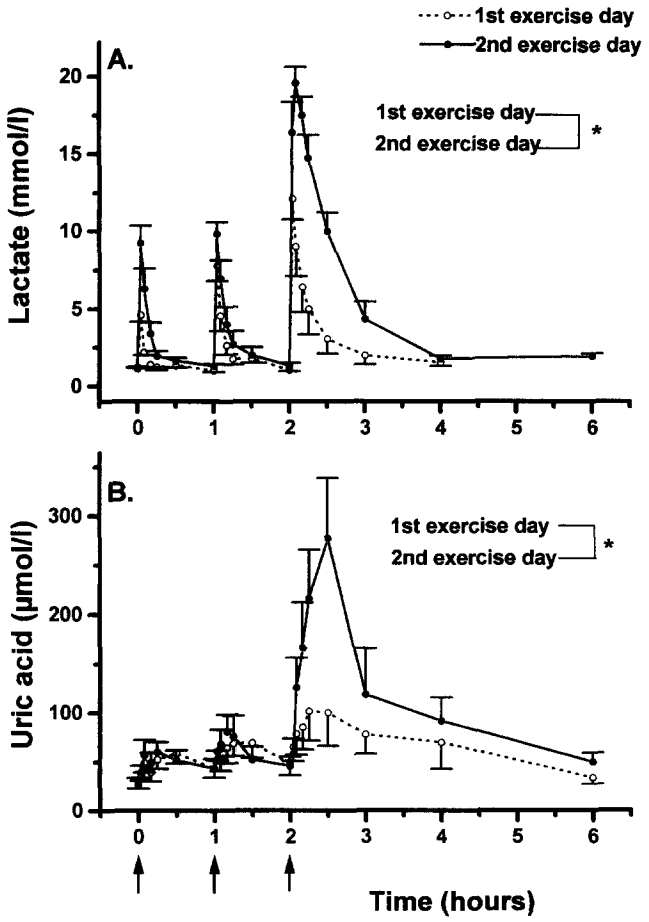

FIG. 1. **Blood lactate and plasma uric acid concentration after three bouts of exercise (pointed with arrow) on two separate days. The difference of the responses between the rest and the peak value of the third heat between the days is shown in figure: *$P < 0.05$; ns, not significant.**

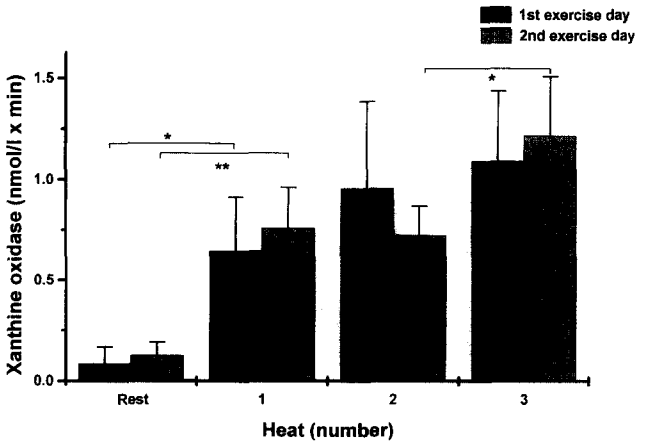

FIG. 2. **Plasma xanthine oxidase activity at rest and the individual peak activities after each heat. Differences in the figure: *$P < 0.05$, **$P < 0.01$.**

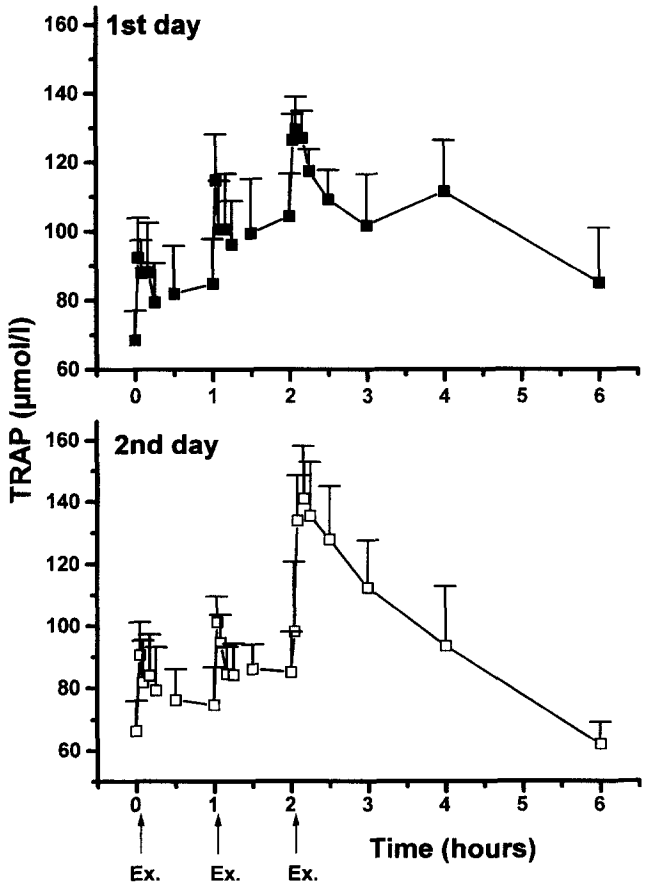

FIG. 3. **Plasma TRAP after the three heats (pointed with arrow) on the first and second day of exercise.**

tween the first and the second heat ($P < 0.05$) on the first day of exercise and between the second and the third heat ($P < 0.05$) on the second day of exercise. The second day of exercise caused a greater ($P < 0.05$) response than the first day when the rest level was compared with the value obtained 30 min after the third heat (Fig. 1B).

XO activity was significantly elevated from 5 min to 2 hr and from 5 to 60 min after the third heat on the two days of exercise, respectively. The individual peak activities after each heat showed no clear temporal pattern. For that reason the differences between the heats were calculated from the individual peak values. The first heat on both days (Fig. 2) caused a significant increase in the activity of XO. The maximal exercise, the last heat on the second exercise day, further increased the activity, whereas the other heats did not increase the activity significantly from the previous heat.

Total peroxyl radical-trapping ability (TRAP; Fig. 3) was significantly increased from the rest level from 0 min to 2

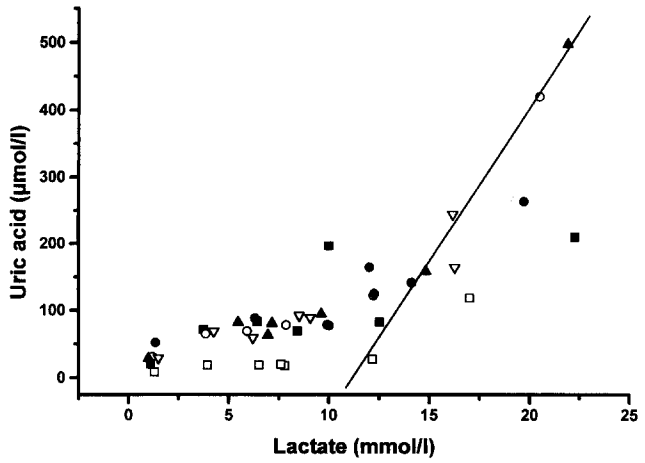

FIG. 4. Comparison of the rest and the peak concentrations of lactate with respective values of uric acid after each heat. Each symbol represents one horse. For data with a blood lactate concentration >12 mmol/l (n = 14); [Uric acid] = −226.7 + 26.4 × [blood lactate]; $P < 0.001$, $r = 0.782$.

hr and from 0 to 60 min after the third heat on the two exercise days, respectively. The difference in the magnitude of the responses between the heats was significant between the first and the second heat ($P < 0.01$) on the first day and between the second and the third heat ($P < 0.01$) on the second day. The magnitude of the responses between the two days did not differ from each other. Trolox used as a standard in TRAP determination traps two radicals per molecule (2,25), which means that the TRAP concentrations should be doubled to be comparable with uric acid concentrations.

Correlations

Changes in the XO activity correlated with the changes in uric acid concentration ($P < 0.001$; $r = 0.465$), in lactate concentration ($P < 0.001$; $r = 0.540$) and in TRAP ($P < 0.001$; $r = 0.546$). Changes in TRAP correlated to changes in uric acid concentration ($p < 0.01$; $r = 0.397$).

The rest and the peak concentrations of uric acid and lactate (Fig. 4) after each heat were compared with each other. Regression analysis of values where blood lactate concentration exceeded 12 mmol · l^{-1} (n = 14) showed an increase in the rate of uric acid accumulation above this concentration ($r = 0.782$; $P < 0.001$; Fig. 4).

DISCUSSION

Blood lactate concentration was used as an indicator of the intensity of exercise in the current study. The peak values increased heat by heat except from the first to the second heat on the second day of exercise, because two of the horses ran the first heat faster than they were supposed to. Otherwise, it fulfilled the requirements better than the heart rate, which does not increase linearly at maximal speeds, or the average speeds, which were misleading as the running distances varied.

Uric acid concentration peaked 15–30 min after the exercise, which is in agreement with earlier studies (8,19). Yamanaka *et al.* (27) have shown that the lactate threshold is also the threshold for the purine nucleotide degradation. Our results do not support this as the blood lactate concentration exceeded 4 mmol · l^{-1} even after the first heat but there was only a small increase in the concentration of uric acid (Fig. 1). There was, however, a progressive increase in the uric acid concentration after the last heat performed at maximal speed. Comparison of the concentration of uric acid with the concentration of blood lactate in Fig. 4 shows an increase in the rate of the uric acid accumulation when the blood lactate exceeds 12 mmol · l^{-1}. This agrees with the results of Sewell and Harris (22), who have shown that an intracellular pH 6.8, which corresponds to the muscle lactate concentration of 80 mmol · kg^{-1} dry muscle (9) and a blood lactate concentration of approximately 15 mmol · l^{-1} (22), triggers adenine nucleotide degradation as indicated by an increase in muscle IMP and plasma NH_3 concentration. However, more samples where lactate concentration exceeds 12 mmol · l^{-1} are required to confirm the existence of an actual threshold.

Ammonia together with IMP are formed in the deamination of AMP, catalysed by AMP deaminase. Most of the IMP is, however, reaminated to AMP in purine nucleotide cycle at the onset of recovery and just a small part is further degradated to inosine, hypoxanthine, uric acid and finally to allantoin in horses (14). The present results together with those of Sewell *et al.* (23) and Harris *et al.* (8) suggest that when the intensity of exercise increases, there is a certain point after which the reamination of IMP can no longer decelerate the loss of adenine nucleotides during the exercise. The level of muscle acidosis seems to be the key as an induced metabolic alkalosis has been shown to decrease the extent of adenine nucleotide loss thereby delaying the onset of fatigue (7). These facts suggest that detection of uric acid rather than ammonia might give more accurate knowledge of the total adenine nucleotide loss.

Increased oxidative stress caused by intense exercise has been claimed to be associated with muscle damage. Exercise-induced ischaemia causes a conversion of xanthine dehydrogenase to its reactive oxygen species producing form—XO. XO, which catalyses the conversion of hypoxanthine to xanthine and xanthine to uric acid, has been proposed to be a major contributor to the production of reactive oxygen species (5,28). The reactive oxygen species play an important role in attracting, activating and promoting the adherence of neutrophils to microvascular endothelium (6). Neutrophils migrate between the endothelial cells and into the extravascular space releasing proteolytic enzymes (e.g. collagenase and elastase) and further reactive oxygen species, thereby causing the tissue injury (4). In the

present study, plasma XO activity increased significantly due to the exercise, which is in keeping with our previous results (20).

Intensity of exercise did not seem to have an effect on the level of activity except after the maximal effort. That and the positive correlation between XO activity and the products of anaerobic metabolism, lactate and uric acid indicate that there is a rapid increase in the XO activity when the loss of ATP increases. The source of circulating XO may be the capillary endothelial cells of various tissues (10,12,18) or their outside surface from where it can be rapidly released (1). The results of the current study suggest that physical exercise may be one factor causing the release of XO from the surface of endothelial cells and that the release is probably not related to the intensity of exercise. All the plasma activity has been shown to be in the oxidase form after exercise (20), which may indicate a possible role of XO in contrast to XDH in the formation of uric acid in plasma.

Antioxidants can interrupt the chain reaction caused by the reactive oxygen species by scavenging the free radicals. The TRAP gives information on the quantitative chain-breaking antioxidant capacity (25). Plasma TRAP is composed of vitamin E, ascorbic acid, protein sulfhydryl groups and the unidentified antioxidant proportion (25). In the current study, plasma TRAP was increased as a consequence of the exercise, which suggests that in horse plasma the main component of TRAP is uric acid as indicated by the positive correlation between TRAP and uric acid in this study. This agrees with the findings in human athletes (15).

In conclusion, these data suggest that a rapid increase in the rate of adenine nucleotide degradation and for the plasma accumulation of uric acid may exist at ~12 mmol · l^{-1} blood lactate concentration after repeated bouts of intense exercise. Plasma XO activity increases rapidly after the onset of exercise, but the intensity of exercise seems to have a minor effect on the level of activity. Plasma XO in contrast to XDH activity may in part contribute to the formation of uric acid in horse plasma.

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References

1. Adachi, T.; Fukushima, T.; Usami, Y.; Hirano, K. Binding of human xanthine oxidase to sulphated glycosaminoglycans on the endothelial-cell surface. Biochem. J. 289:523–527;1993.
2. Alanko, J.; Riutta, A.; Mucha, I.; Vapaatalo, H.; Metsä-Ketelä, T. Modulation of arachidonic acid metabolism by phenols: relation to positions of hydroxyl groups and peroxyl radical scavenging properties. Free Rad. Biol. Med. 14:19–25; 1993.
3. Beckman, J.S.; Parks, D.A.; Pearson, J.D.; Marshall, P.A.; Freeman B.A. A sensitive fluorometric assay for measuring xanthine dehydrogenase and oxidase in tissues. Free Rad. Biol. Med. 6:607–615;1989.
4. Cronstein, B.N. Adenosine, an endogenous anti-inflammatory agent. J. Appl. Physiol. 76:5–13;1994.
5. Duarte, J.A.R.; Appell, H.-J., Carvalho, F.; Bastos, M.L.; Soares, J.M.C. Endothelium-derived oxidative stress may contribute to exercise-induced muscle damage. Int. J. Sports Med. 14:440–443;1993.
6. Granger, D.N. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am. J. Physiol. 255;H1269–H1275;1988.
7. Greenhaff, P.L.; Harris, R.C.; Snow, D.H.; Sewell, D.A.; Dunnett, M. The influence of metabolic alkalosis upon exercise metabolism in the thoroughbred horse. Eur. J. Appl. Physiol. Occup. Physiol. 63:129–134;1991.
8. Harris, R.C.; Marlin, D.J.; Snow, D.H.; Harkness, R.A. Muscle ATP loss and lactate accumulation at different work intensities in the exercising Thoroughbred horse. Eur. J. Appl. Physiol. Occup. Physiol. 62:235–244;1991.
9. Harris, R.C.; Snow, D.H.; Katz, A.; Sahlin, K. Effect of freeze-drying on measurements of pH in biopsy samples of the middle gluteal muscle of the horse: comparison of muscle pH to the pyruvate and lactate content. Equine Vet. J. 21:45–47;1989.
10. Hellsten-Westing, Y. Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle. Histochemistry 100:215–222;1993.
11. Hellsten-Westing, Y.; Sollevi, A.; Sjödin, B. Plasma accumulation of hypoxanthine, uric acid and creatine kinase following exhausting runs of differing durations in man. Eur. J. Appl. Physiol. Occup. Physiol. 62:380–384;1991.
12. Jarasch, E.-D.; Bruder, G.; Heid, H.W. Significance of xanthine oxidase in capillary endothelial cells. Acta Physiol. Scand. Suppl. 548:39–46;1986.
13. Lew, H.; Quintanilha, A. Effects of endurance training and exercise on tissue antioxidive capacity and acetaminophen detoxification. Eur. J. Drug. Metab. Pharmacokinet. 16:59–68; 1991.
14. Lowenstein, J.; Tornheim, K. Ammonia production in muscle: the purine nucleotide cycle. Science 171:397–400;1971.
15. Maxwell, S.R.J.; Jakeman, P.; Thomason, H.; Leguen, C.; Thorpe, G.H.G. Changes in plasma antioxidant status during eccentric exercise and the effect of vitamin supplementation. Free Rad. Res. Comms. 19:191–202;1993.
16. Motojima, K.; Goto, S. Characterization of liver-specific expression of rat uricase using monoclonal antibodies and cloned cDNAs. Biochim. Biophys. Acta. 1087:316–322;1990.
17. Rajguru, S.U.; Yeargans, G.S.; Seidler, N.W. Exercise causes oxidative damage to rat skeletal muscle microsomes while increasing cellular sulfhydryls. Life Sci. 54:149–157;1994.
18. Räsänen, L.A.; Karvonen, U.; Pösö, A.R. Localization of xanthine dehydrogenase mRNA in horse skeletal muscle by in situ hybridization with digoxigenin-labelled probe. Biochem. J. 292:639–641;1993a.
19. Räsänen, L.A.; Myllymäki, T.; Hyyppä, S.; Maisi, P.; Pösö, A.R. Accumulation of allantoin and uric acid in plasma of exercising trotters. Am. J. Vet. Res. 54:1923–1928;1993b.
20. Räsänen, L.A. Exercise induced purine nucleotide degradation and changes in myocellular protein release. Equine Vet. J. Suppl. 18:235–238;1995.
21. Sewell, D.A.; Gleeson, M.; Blannin, A.K. Hyperammonaemia in relation to high-intensity exercise duration in man. Eur. J. Appl. Physiol. Occup. Physiol. 69:350–354;1994.
22. Sewell, D.A.; Harris, R.C. Adenine nucleotide degradation in the thoroughbred horse with increasing exercise duration. Eur. J. Appl. Physiol. Occup. Physiol. 65:271–277;1992.

23. Sewell, D.A.; Harris, R.C.; Hanak, J.; Jahn, P. Muscle adenine nucleotide degradation in the thoroughbred horse as a consequence of racing. Comp. Biochem. Physiol. 101B:375–381; 1992.
24. Teerlink, T.; Mennekes, M.; Bussemaker, J.; Groenveld, J. Simultaneous determination of creatine compounds and adenine nucleotines in myocardial tissue by high performance liquid chromatography. Anal. Biochem. 214:278–283;1993.
25. Uotila, J.T.; Kirkkola, A.-L.; Rorarius, M.; Tuimala, R.J.; Metsä-Ketelä, T. The total peroxyl radical-trapping ability of plasma and cerebrospinal fluid in normal and preclamptic parturients. Free. Rad. Biol. Med. 16:581–590;1994.
26. Welbourn, C.R.B., Goldman, G.; Paterson, I.S.; Valeri, C.R.; Shepro, D.; Hechtman, H.B. Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil. Br. J. Surg. 78: 651–655;1991.
27. Yamanaka, H.; Kawagoe, Y.; Taniguchi, A.; Kaneko, N.; Kimata, S.; Hosoda S.; Kamatani, N.; Kashiwazaki, S. Accelerated purine nucleotide degradation by anaerobic but not by aerobic ergometer muscle exercise. Metabolism 41:364–369;1992.
28. Yokoyama, Y.; Beckman, J.S.; Beckman, T.K.; Wheat, J.K.; Cash, T.G.; Freeman, B.; Parks, D.A. Circulating xanthine oxidase: potential mediator of ischemic injury. Am. J.Physiol. 258:G564–G570;1990.