

Metabolic responses in endurance horses during racing in relation to uric acid profile, leucocytes, heart rate and plasma biochemical parameters

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ABSTRACT: Uric acid has stronger pro-oxidant than antioxidant properties during equine endurance events and thus, limits performance and has serious repercussions on health. The aim of the study was to investigate the changes in uric acid, leucocytes, plasma biochemical parameters and heart rate in metabolic endurance horses. Thirty Arabian endurance horses were physically examined and blood samples were collected pre and post-race. After physical examination, the successfully completed ($n = 10$) and metabolic disordered ($n = 20$) endurance horses were identified. Blood samples in heparinised vacutainer tubes were used for the determination of uric acid, triglyceride, creatine kinase, aspartate transaminase, packed cell volume, lactate, total protein and plasma protein. Blood sample in ethyl diaminotetra-acetic acid vacutainer tubes were used for the analysis of leucocytes. The age, body weight, heart rate, humidity and ambient temperature were also recorded. One way Analysis of variance and pairwise correlations were used for the analysis. A value of $P \leq 0.05$ was considered as significantly different. The mean values of uric acid, lactate, leucocytes, plasma protein, total protein, heart rate, creatine pinase and Packed cell volume were significantly different between the successfully completed and metabolic disordered endurance horses ($P < 0.0001$), respectively. The mean values of aspartate transaminase and triglyceride were significantly different between the successfully completed and metabolic disordered endurance horses: $P < 0.0130$ and $P < 0.0004$, respectively. There were significant positive correlations between uric acid and lactate ($r = 0.5196$; $P < 0.0271$), between uric acid and plasma protein ($r = 0.6025$; $P < 0.0175$), between uric and Packed cell volume ($r = 0.5206$; $P < 0.0268$), between uric acid and triglyceride ($r = 0.5541$; $P < 0.0170$) and between uric acid and heart rate ($r = 0.5629$; $P < 0.0150$) in the metabolic disordered endurance horses. In conclusion, heart rate, triglyceride, blood lactate and packed cell volume were significantly associated with uric acid, a biomarker of oxidative stress. Therefore, uric acid could be used to evaluate performance and health status in endurance horses during training and endurance events.

Keywords: uric acid; oxidative stress; endurance horses; leucocytes; biochemical; heart rate

List of abbreviations

MD = metabolic disordered horses; **SC** = successfully completed the race; **ROS** = reactive oxygen species; **I-R** = ischaemic and reperfused heart; **LDL** = low-density lipoprotein; **XO** = xanthine oxidase; **ATP** = adenosine triphosphate; **ADP** = adenosine diphosphate; **AMP** = adenosine monophosphate; **IMP** = inositol monophosphate; **EDTA** = ethyl diaminotetra-acetic acid; **PCV** = packed cell volume; **AST** = aspartate transaminase; **CK** = creatine kinase; **TG** = triglyceride; **UA** = uric acid; **RUGS** = Research University Grant Scheme

Circulating uric acid has both pro-oxidant and antioxidants properties (Sautin and Richard 2008). For example, uric acid can scavenge free radicals

discharged into the blood, such as reactive oxygen species (ROS) which are produced by macrophages (Nathalie et al. 2007; Sautin and Richard 2008) and

which result in a deleterious effect on muscles cells due to oxidative stress (Fazio et al. 2007; Piccione et al. 2007). Uric acid may function either as an antioxidant in plasma or as a pro-oxidant primarily within cells (Sautin and Richard 2008), and can generate free radicals in a mixture of radical-forming systems (Maple and Mason 1988). Accordingly, uric acid can become pro-oxidant by forming radicals when combined with other oxidants and oxidant-generating substances and these radicals appear to target mainly lipid membranes and low-density lipoprotein (LDL). Simultaneously, the hydrophobic milieu created by lipids is adverse for the antioxidant effects of uric acid, and oxidised lipids can even convert uric acid into an oxidant (Bagnati et al. 1999; Muraoka and Miura 2003).

The uric acid build up from adenine nucleotides is of significance when energy balance is critical. Due to the low reactivity of uricase, uric acid increases exponentially when the metabolic pathway is clogged during exercise (Tullson et al. 1995; Essen-Gustavsson et al. 1999; Moriwaki et al. 1999; Castejon et al. 2006). The main source of free radical production in the ischaemic and reperfused heart are xanthine oxidase (XO)-catalysed reactions (Kuppasamy and Zweier 1989; Downey 1990; Gandhi and Gunjan 2009). During ischaemia, due to the energy required by the contracting myocardium, ATP is broken down to ADP and AMP. AMP is constantly degraded to hypoxanthine, which is subsequently converted to xanthine and uric acid by XO. This reaction is coupled two the one-electron reduction of O_2 , giving rise to O_2^- , if O_2 supply is inadequate. High intensity exercise generates a cellular milieu which serves to promote activation of the XO pathway Hellsten (1994). After intense muscular contraction hypoxanthine accumulates and uric acid concentrations become elevated in both contracting muscle and in the plasma (Norman et al. 1987; Hellsten-Westing et al. 1993; Ji 1999). Factors contributing to the onset of fatigue include heat production which occurs during exercise, loss of electrolytes and water, lactic acid production, metabolic alkalosis, fluid and electrolyte shifting and subclinical conditions (Whiting 2009).

Metabolic responses during endurance races result from a build-up of free radicals in the muscles leading to poor performance and serious repercussions on health status. Thus, this study aims at appraising the changes in uric acid profile, leucocytes, heart rate and plasma biochemical parameters during an endurance race of 80 km.

MATERIAL AND METHODS

Thirty Arabian horses participated in an endurance competition of 80 km and were used for the study; out of this number 20 had metabolic syndrome and were subsequently eliminated from the race and 10 completed the race successfully. The race was conducted in accordance with FEI rules. The age and body weight of the horses ranged between five and 20 years and 350–450kg, respectively. Veterinary inspections were conducted after each phase of the race on all competing horses and physical parameters were recorded.

The physical parameters evaluated were heart rate, mucous membrane, skin recoil, gut motility or sound and gait. The horses were also observed for soreness or injuries on the back, withers, girth area, body or distal extremities (Khaled and Ahmad 2008; Lawan et al. 2010).

At the end of the endurance race the horses were categorised as having successfully completed (SC) the race or as metabolic disordered (MD). The criteria for evaluating a horse as SC depended on the horse's ability to maintain normal gastrointestinal, respiratory, cardiac, or musculoskeletal status. In addition, these horses had heart rates equal to or below 64 beats/min and exhibited excellent hydration status after 20–30 min of recovery period. As MD horses were classified those that could not meet the above mentioned criteria and were subsequently eliminated from the endurance race (Khaled and Ahmad 2008).

The ambient temperature and humidity were recorded at an interval of 30 min from the beginning of the race to the finish. The mean temperature ($^{\circ}C$) and humidity (%) were 29.06 ± 1.1 $^{\circ}C$ and $71.73 \pm 4.05\%$ respectively during the period of the endurance race. The geographical terrain was good and water points were also provided at specific places along the track. The ambient temperature and humidity were measured using the portable thermohygrometer H1936440N[®] (Hanna Instruments, Romania).

Blood samples were obtained from all horses via jugular venipuncture into ethyl diaminetetraacetic acid (EDTA) vacutainer tubes for haematological tests and into heparinised vacutainer tubes for biochemical analysis. Blood sample collection was performed immediately after a 20 min recovery period and samples were analysed immediately in the laboratory located within the premises of the event before and after the race.

The differential leucocyte count was analysed using Cell DYN 3700 (Abbot®), while the packed cell volume (PCV) was analysed using a Hettich-Hematocrit 210 and Hawksley microhematocrit reader®. The plasma biochemical, uric acid, aspartate transaminase (AST), creatine kinase (CK), lactate, triglyceride and total protein were determined with a chemistry analyser (Hitachi 920®) using standard diagnostic kits (Roche®). The data were analysed using ANOVA and pairwise correlation analysis using the statistical software package JMP 9 (SAS). Analyses were considered as significant at $P < 0.05$.

RESULTS

Pre and post SC and MD blood biochemical, haematological and physical parameters of endurance horses after covering a distance of 80 km are presented in Table 1. The results indicated that even at the pre-race sampling, most of the biochemical parameters were significantly elevated in the MD endurance horses except the heart rate which was within the normal range.

Table 1 presents the pre and post SC and MD blood biochemical parameters and heart rate of endurance horses, indicating significantly higher serum concentrations of uric acid, lactate, leucocytes, plasma protein, total protein, heart rate, CK and PCV in the MD endurance horses compared to the SC ($P < 0.0001$). The mean values of AST and

triglyceride were also significantly higher in MD versus SC endurance horses: $P < 0.0130$ and $P < 0.0004$, respectively.

There were significant positive correlations between uric acid and lactate, leucocytes, plasma protein, total protein, heart rate, triglyceride, CK and PCV between the pre and post SC and MD endurance horses: Uric acid and lactate ($r = 0.5196$; $P < 0.0271$); between uric acid and plasma proteins ($r = 0.6025$; $P < 0.0175$); between uric acid and PCV ($r = 0.5206$; $P < 0.0268$); between uric acid and triglyceride ($r = 0.5541$; $P < 0.0170$); and between uric acid and heart rate ($r = 0.5629$; $P < 0.0150$).

DISCUSSION

In the present study, there were significant differences in the pre and post SC and MD biochemical and physical parameters of endurance horses. The significant differences in these parameters could be due to factors contributing to the onset of fatigue which include heat production during exercise, loss of electrolytes and water, lactic acid production, metabolic alkalosis, fluid and electrolyte shifting and subclinical conditions (Whiting 2009). Uric acid levels were significantly different between the pre and post SC and MD endurance horses and there was a strong positive correlation between uric acid and triglyceride. This difference could be due to oxidative stress associated with uric acid during strenuous racing. The accumulated free radicals

Table 1. Pre and post SC and MD blood biochemical, haematological and physical parameters of endurance horses after covering a distance of 80km expressed in their conventional units

Parameters	Pre-ride SC	Post-ride SC	Pre-ride MD	Post-ride MD
Uric acid ($\mu\text{mol/l}$)	7.67 ^b \pm 6.47	10.10 ^b \pm 5.31	78.11 ^a \pm 52.94	80.96 ^a \pm 52.32
AST (IU/l)	283.37 ^b \pm 63.02	349.04 ^{ab} \pm 97.01	547.55 ^a \pm 423.35	512.49 ^a \pm 366.27
CK(IU/l)	187.33 ^b \pm 57.68	348.86 ^b \pm 111.37	1417.25 ^a \pm 790.93	1273.94 ^a \pm 1013.81
PCV (l/l)	0.31 ^b \pm 0.071948	0.33 ^b \pm 0.03	0.47 ^a \pm 0.06	0.46 ^a \pm 0.07
Lactate (mmol/l)	1.03 ^c \pm 0.21	1.00 ^c \pm 0.11	8.59 ^a \pm 2.67	6.76 ^b \pm 2.95
Total Protein (g/l)	68.94 ^b \pm 9.45	68.43 ^b \pm 4.66	79.85 ^a \pm 7.56	82.30 ^a \pm 8.17
Plasma protein (g/l)	62.11 ^b \pm 2.52	65.14 ^b \pm 5.39	79.96 ^a \pm 8.55	79.04 ^a \pm 8.83
Triglyceride (mmol/l)	0.25 ^b \pm 0.09	0.25 ^b \pm 0.09	0.69 ^a \pm 0.40	0.63 ^a \pm 0.37
Leucocytes	5.73 ^b \pm 1.51	5.53 ^b \pm 1.55	9.68 ^a \pm 3.32	9.78 ^a \pm 3.39
Heart rate (bpm)	38.80 ^c \pm 4.02	43.14 ^c \pm 7.82	57.10 ^b \pm 7.91	68.24 ^a \pm 13.68

Values are expressed as mean \pm SD ^{a,b,c}, within each row; means with different superscripts are significantly different at $P < 0.05$

AST = aspartate transaminase; CK = creatine kinase, PCV = packed cell volume

(Fazio et al. 2007; Piccione et al. 2007), could then attack lipid membranes of muscles as was indicated by the increase in triglyceride concentrations leading to an unfavourable environment for uric acid antioxidant effects (Muraoka and Miura 2003). The difference may also be down to the oxidation of lipids which subsequently convert uric acid to an oxidant (Bagnati et al. 1999); this would serve to promote the oxidative effects of oxidant-generating substances (Bagnati et al. 1999; Muraoka and Miura 2003; Fazio et al. 2007; Piccione et al. 2007). Increases in uric acid as a result of prolonged lopsided energy distribution could lead to metabolic syndromes during endurance events (Tullson et al. 1995; Essen-Gustavsson et al. 1999; Moriwaki et al. 1999; Castejon et al. 2006).

The current study indicated a strong positive association of lactate with uric acid and there were significant differences in lactate and uric acid concentrations between the SC and MD. This was probably due to oxidative stress and the common source of these substances: the contracting muscle tissues. A blood lactate concentration of ≥ 4 mmol/l denotes an unfit horse in training protocols (Dudley and Terjung 1985; Guhl et al. 1996; Hinchcliff et al. 2002; Castejon et al. 2006; Kedzierski et al. 2009; Fielding et al. 2009; Lindner et al. 2009). Lactate is a scavenger of free radicals; however, the formation of lactate cannot be divorced from metabolic acidosis which could exhibit pro-oxidant activity and lactic acidosis has been associated with the generation of free radicals and lipid peroxidation (Bralet et al. 1991; Groussard et al. 2000). Furthermore, other studies have shown an increase in both lactate and oxidative stress biomarkers during resistance exercise (Hudson et al. 2008).

All horses in the MD group had elevated heart rates compared to those from the SC group and there was a strong positive correlation between heart rate and uric acid. This could be due to the accumulation of uric acid in the contracting myocardium during ischaemia and reperfusion of the heart during races leading to metabolic problems and poor performance of endurance horses (Kuppasamy and Zweier 1989; Downey 1990; Hellsten-Westing et al. 1993; Gandhi and Gunjan 2009). This is in agreement with earlier studies that identify derangements in cardiovascular function, development of metabolic problems and exhaustion as being due to persistently elevated heart rates after an endurance race and which implicate elevated heart rate as the most important indica-

tor of reduced performance during equine endurance events (Rose et al. 1977; Carlson 1985; Rowell 1986; Hodgson and McConaghy 1994; Schott and Charlton 1996; Schott et al. 1997; McKeever 2000; Harold 2010; Lawan et al. 2012). Also, lower heart rate was used as a determinant of equine fitness in response to strenuous endurance rides (Cottin et al. 2006; Bashir and Rasedee 2009).

In the present study, there were significant differences in the values of PCV, plasma proteins, CK and AST between the SC and MD. The significant differences in PCV, plasma proteins and total proteins could be indicative of dehydration status in the MD group may be due to the action of xanthine oxidase in free radical production as a result of muscle cell membrane permeability (Mills et al. 1997; Castejon et al. 2006). The significance difference in CK between SC and MD in the working muscles could be due to high energy demand (Lowenstein 1990; Rose and Hodgson 1994), and is perhaps associated with muscular damage (Piccione et al. 2008). Horses with rhabdomyolysis have elevated levels of muscle enzymes, CK, AST and lactate (Hodgson and McConaghy 1994). The significant differences between SC and MD in leucocyte number could be due to the free radicals discharged into the circulation by macrophages and which result in harmful effects on tissues and organs (Nathalie et al. 2007; Sautin and Richard 2008; Piccione et al. 2008; Gandhi and Gunjan 2009).

CONCLUSION

In conclusion, heart rate, triglyceride, lactate and PCV were significantly associated with uric acid, a biomarker of oxidative stress. Further studies are required to determine if uric acid could be used to evaluate performance and health status in endurance horses during training and endurance events.

Acknowledgements

The researchers would like to acknowledge the Research University Grant Scheme (RUGS) for making this project a success. Appreciation goes to Mr. Mohamed Halmi Othman and Mr. Abdullah Mison for their efforts and assistance and the staff of the Veterinary Teaching Hospital, University Putra Malaysia, especially Mr. Salehuddin and Drs. Mohammad Fairuz Jamaluddin, Dr. Naguib and

Mimi Armiladiana Mohamad, for their assistance, advice and encouragement.

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Received: 2012–08–24

Accepted after corrections: 2012–11–13

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