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Proteinuria, GGT Index and Fractional Clearance of Electrolytes in Exercising Athletic Horses

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Abbreviations: DTT, dithiothreitol; FE-K, potassium fractional clearance; FE-Na, sodium fractional clearance; FF, filtration fraction; GFR, glomerular filtration rate; MW, molecular weight; RBF, renal blood flow; SDS, sodium dodecyl sulphate; UP, urinary protein

INTRODUCTION

Assessment of kidney function in athletic horses is very important because electrolytic and acid-base balance is fundamental for athletic performance (Edwards *et al*., 1989; Carr, 2003; McKeever, 2004). As is well known, increases in serum urea and creatinine concentrations do not occur until approximately 75 % of the nephrons become non-functional and even urinalysis does not provide enough information due to the typical amount of crystalluria that often masks useful diagnostic findings (Marr, 1998; Reed and Bayly, 1998). Further parameters are thus necessary in order to obtain an adequate evaluation of renal function. Thus, routine screening should include:

a) accurate assessment of renal tubular function, measuring: the fractional clearance of electrolytes, specifically for sodium and potassium (FE-Na, FE-K); the urinary gamma glutamyl transferase concentration and its urinary activity expressed as percent ratio to urinary creatinine concentration (GGT index) (Rossier *et al*, 1995; Carr, 2003).
b) assessment of glomerular anatomical and functional integrity, measuring proteinuria (UP) (Reed and Bayly, 1998; Carr, 2003).

Exercise induced abnormalities of the urine are well documented in humans and have been termed “athletic pseudonephritis”, characterised by proteinuria, pigmenturia and haematuria. These changes are consistent with nephritis, but are transient and do not appear to be associated with any pathological changes in the kidney. Similar findings have also been described for dogs and horses, but there is a lack of information about the correlation between exercise intensity and amount and patterns of proteinuria (Schott *et al*, 1995; McKeever, 2004). The aim of this study was to evaluate variations in the above mentioned urinary parameters before and after controlled exercise on a treadmill in athletic horses in training.

MATERIALS AND METHODS

Twentyone healthy athletic trotter horses in training, of both sexes (15 males, 6 females), with a mean age of 4.0±1.0 years were considered for this study. Urine samples were collected by spontaneous micturition before and within 1 hour after the end of controlled exercise on a treadmill. All urine samples were centrifuged at 1500 rpm for 5 min. Creatininuria (alkaline picrate spectrophotometric method, Bioclinic), proteinuria (Blue Coomassie spectrophotometric method, Bioclinic), urinary sodium and potassium (IL 943) and gamma glutamil transferase (Carboxy-glupa spectrophotometric method, Bioclinic) were assessed in the supernatant fraction. With the same analytical methods serum sodium, potassium and creatinine were also assessed. Urinary sodium and potassium fractional clearances and the GGT index were calculated. After this, the supernatant fraction was added with sodium azide (0.1 mmol/L) and frozen at −25 °C. SDS-PAGE was performed by a flatbed electrophoresis system: “Multhipor II System” (GE Health Care, Biosciences). Polyacrylamide precasted homogeneous and gradient gels (ExelGel SDS Homogeneous 12.5 and ExelGel SDS Gradient 8–18, GE Biosciences) were used. Buffer strips were placed along the anodic and cathodic sides of the gels (Buffer Strips, GE Health Care Biosciences). Urine supernatants were diluted with a reducing buffer (Tris, SDS, DTT) added with bromophenol blue. A known molecular weight (MW) protein pool was used as a reference standard (BenchMark™ Protein ladder, Invitrogen). Samples and standard were heated at 95 °C for 3–5 minutes and then 14 μl and 9 μl, respectively were applied onto the gel. Run conditions were 50 mA, 30 W, 600 V. Blue Coomassie stain was used. Electrophoretic lanes were analyzed visually and by a 1-D software (TotalLab, GE Health Care Biosciences, Italy).

Data analysis Values of FE-Na, FE-K, the GGT index and UP measured pre and post exercise are presented as mean ± std. Analysis of variance was used to determine statistical differences between pre and post exercise results.

TABLE I
Mean and std of pre and post exercise parameters

Before Exercise		After Exercise	p
FE-Na	0.012±0.015 %	0.2±0.2 %.	0.0002
FE-K	19,8±11.5 %	49.0±25.5 %	1.05×10^{-5}
GGT index	4,6±3,5 %	14,4±4,3 %	4.6×10^{-10}
UP	11,9±12,7 mg/dl	74,5±33,5 mg/dl	8.1×10^{-10}

p = probability of significance

RESULTS

ANOVA test showed a statistically significant variance between pre and post exercise results in all parameters (Table I).

SDS-PAGE interpretation of pre-exercise samples showed thin bands in the albumin zone and a difficult to interpret higher molecular weight (MW) wide-vanished band.

Post exercise lanes showed wider and denser albumin bands. Samples characterized by higher proteinuria showed bands corresponding to high and low MW proteins.

DISCUSSION

As the large amount of crystals in most equine urine samples hinders the detection of haematuria and pigmenturia, the investigation of other chemical and biochemical parameters in urine becomes necessary (FE-Na, FE-K, quantitative and qualitative assessment of proteinuria, GGT index). While renal blood flow can be reduced during exercise, the decreases in GFR do not necessary parallel the decreases in RBF through a vasoconstriction of the efferent arteriole inducing an increase in the filtration fraction (FF). This mechanism may contribute to enhance filtration of macromolecules during exercise, because of an alteration in the permeability of the glomerular barrier due to damage of the electrical impedance (then followed by an increase in albuminuria) (Schott *et al*, 1995; McKeever H.M., 2004). Schott *et al*. (1995) observed that strenuous exercise in humans results in mixed glomerular (medium and high MW) and tubular (low MW) proteinuria. The detection of a higher amount of proteinuria and mixed patterns of proteinuria in some of the post exercise urine samples of this study confirms that both a glomerular filtration change and a decreased tubular reabsorption are also present in horses. The tubular involvement is also shown by a higher GGT index, FE-Na and FE-K. In the post exercise period, particularly, the restoration of RBF and the action of several hormones (ANP, aldosterone) could have induced higher natriuresis and kaliuresis and the consequent increase in the observed fractional clearances of electrolytes.

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