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Influence of Training Exercise on Clinical Plasma Chemistry Parameters and Cardiac Markers in Race Horses

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ABSTRACT

Physiological variations of clinical chemistry parameters as a function of exercise reflecting general health and performance are largely unknown in race horses. To assess changes in blood biochemical variables, with special emphasis on cardiac markers, blood samples were drawn from twelve healthy race horses (6 males and 6 females; age range from 2 to 6 years) before and after the exercise training. Biochemical parameters in the renal, liver and lipid profile as well as the muscle enzymes creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were measured with standard methods using an automated analyzer Hitachi 917 (Roche Diagnostics). Cardiac troponin I (cTnI) plasma concentrations were analyzed with immunometric luminescence technology utilizing an automated immunoassay analyzer Vitros ECi (Ortho-Clinical Dianostic). With the exception of triglycerides, all other biochemical variables showed increased levels after the exercise, with values for alanine aminotransferase (ALT), albumin and total protein reaching statistical significance. The activities of CK and LDH, but not of AST, also displayed a significant increase after the training. Comparing with pre-exercise mean value of 0.015 ng/ml, a more than 10 fold increase in cTnI concentration (0.177 ng/ml) was observed, with a maximum value as high as 0.498 ng/ml. The majority of animals (58%) showed levels exceeding lower cut-off concentration of cTnI at 0.12 ng/ml indicative of a small myocyte injury. In conclusion, small changes in levels of the routine blood chemistry parameters due to training exercise are common in race horses. There was biochemical evidence of minor myocardial cell damage as assessed by cardiac troponin I plasma concentrations.

Key words: training exercise, biochemical parameters, cardiac markers, troponin I, race horses

INTRODUCTION

The effect of physical exercise on metabolic parameters which indicates general health and performance in race horses has rarely been reported (Sommer *et al*., 1986). Little is also known, whether and to what extent exercise training results in cardiac damage, with release of cardiac marker proteins into the circulation (Phillips *et al*., 2003). The question is relevant in

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terms of the possibility of early identification of animals with occult cardiac disease as well as of those that are proned to later development of cardiomyopathy, and thus are not suitable for horse racing.

Previous studies have used striated muscle enzymes creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) to indicate fitness for training (Sommer *et al*., 1986). Due to their presence in significant amount both in cardiac and skeletal muscle, these enzymes lack specificity for myocardial injury. The recent development of sophisticated immunoassay systems for determining levels of the contractile proteins of the troponin complex, cTnI and troponin T (cTnT), has allowed for cardiac marker testing with high clinical sensitivity and specificity (Collinson *et al*., 2001). Because of species conservation of the epitopes on troponin molecules, the monoclonal antibodies in immunoassay that have initially been developed for human application can also recognized cardiac troponins from other mammals (O' Brien *et al*., 1997).

The purpose of the present study was to determine alterations in routine blood chemistry parameters and cardiac marker protein levels in race horses after training exercise, with special emphasis on changes in cTnI concentrations. The results obtained could be served as a baseline information for future studies on cardiac disease in horses.

MATERIALS AND METHODS

Animals

Twelve clinically normal thoroughbred race horses (6 females and 6 males; age range 2-6 years) were examined. Each race horse was in exercise training for 6 days per week, consisting of fast galloping and slow work. Blood samples were taken around 2 hours before (22 hours after the last training period) and between 3-4 hours after the exercise. Blood was withdrawn from the jugular vein into heparinized tubes and the plasma obtained after centrifugation was kept at –20°C until analysis of biochemical parameters.

Laboratory analysis

Plasma glucose concentration was determined by glucose oxidase method. Levels of blood urea nitrogen (BUN) and creatinine as well as activities of the enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP), gammaglutamyl transferase (GGT) and the striated muscle enzymes CK, AST and LDH were measured with standard enzymatic methods. The enzymatic colorimetric tests were used for measurements of total cholesterol and triglyceride concentrations, as well as the cholesterol content in high density lipoprotein (HDL) after precipitation of apolipoprotein B-containing lipoprotein with phosphotunstic acid and magnesium chloride. Total protein and albumin were determined by biuret method and bromocresol green method, respectively. Measurements of all these biochemical variables were performed using the fully-automated analyzer Hitachi 917 (Roche Diagnostics).

Concentrations of cTnI were measured with immunometric luminescence assay on an Vitros ECi analyzer (Ortho-Clinical Diagnostic). The lower limit of detection of this assay is 0.02 ng/ml. Based on studies on humans, the 99th percentile reference limit, the lowest concentration to provide a 10% coefficient of variation (CV), and the receiver operator characteristic curve (ROC) optimized cut-off were 0.08, 0.12 and 0.40 ng/ml, respectively (Apple and Jaffe, 2006).

Statistical analysis

Data were analyzed with the Statistical Package for the Social Sciences (SPSS), version 11.0 and presented as mean ± standard deviation. Intergroup variations were assessed by the Mann-Whitney U-test. A p value of < 0.05 was

considered to indicate significant difference.

RESULTS

The short-term effects of exercise on plasma glucose and biochemical parameters in liver, renal and lipid profile are demonstrated in Table 1. With the exception of triglycerides, all other metabolic variables showed an increase in their levels after exercise training. Nevertheless, a statistical significant difference was seen only for ALT, albumin and total protein.

With regard to parameters in cardiac profile, there was a mild to moderate degree of elevation of striated muscle enzyme activities after exercise, but the difference reached statistical significance only for CK and LDH (Table 2). A significant increase in cTnI concentration of more than 10 fold the baseline value was also observed. By using different cut-off concentrations of cTnI of 0.08 (99th percentile), 0.12 (10% CV) and 0.40 (ROC) ng/ml, the percentage of elevation was calculated to be 67%, 58% and 8%, respectively.

In Figure 1, individual cTnI concentrations before and after exercise training are demonstrated. At baseline, all horses displayed very low cTnI levels, with values ranging from 0.00 to 0.036 ng/ml. After exercise, 2 of the animals showed an unchanged or a decreased level. The other 10 exhibited a distinct increase in cTnI concentrations, with a maximum value as high as 0.498 ng/ml.

Table 1 Mean (± SD) values and P values of biochemical parameters before and after training exercise in 12 race horses.

	Before	After	P Value
Glucose (mg/dl)	104.2 ± 7.9	105.8 ± 10.5	0.563
ALT (U/L)*	11.5 ± 3.5	16.7 ± 6.0	0.014**
ALP (U/L)*	174.9 ± 32.5	191.1 ± 34.5	0.204
GGT (U/L)*	27.8 ± 8.3	29.8 ± 8.0	0.385
Albumin (g/dl)	3.18 ± 0.20	3.48 ± 0.21	0.003**
Total protein (g/dl)	6.13 ± 0.44	6.62 ± 0.49	0.028**
Total cholesterol (mg/dl)	80.6 ± 9.9	83.6 ± 9.7	0.435
Triglycerides (mg/dl)	36.8 ± 8.2	31.5 ± 9.2	0.119
HDL cholesterol (mg/dl)*	41.2 ± 5.7	43.0 ± 5.2	0.339
BUN (mg/dl)*	14.8 ± 2.3	16.1 ± 2.1	0.159
Creatinine (mg/dl)	1.38 ± 0.23	1.41 ± 0.18	0.929

* Abbreviation: see text

** P value < 0.05

Table 2 Mean (± SD) values and P values of cardiac enzymes and cardiac troponin I before and after training exercise in 12 race horses.

	Before	After	P Value
CK (U/L) *	168. 9 ± 41.7	228.5 ± 76.0	0.007**
AST (U/L) *	296.2 ± 64.2	346.1 ± 123.6	0.273
LDH (U/L) *	470.7 ± 87.4	698.4 ± 125.8	0.001**
cTnI (ng/ml) *	0.015 ± 0.013	0.177 ± 0.149	0.002**

* Abbreviation: see text

** P value < 0.01

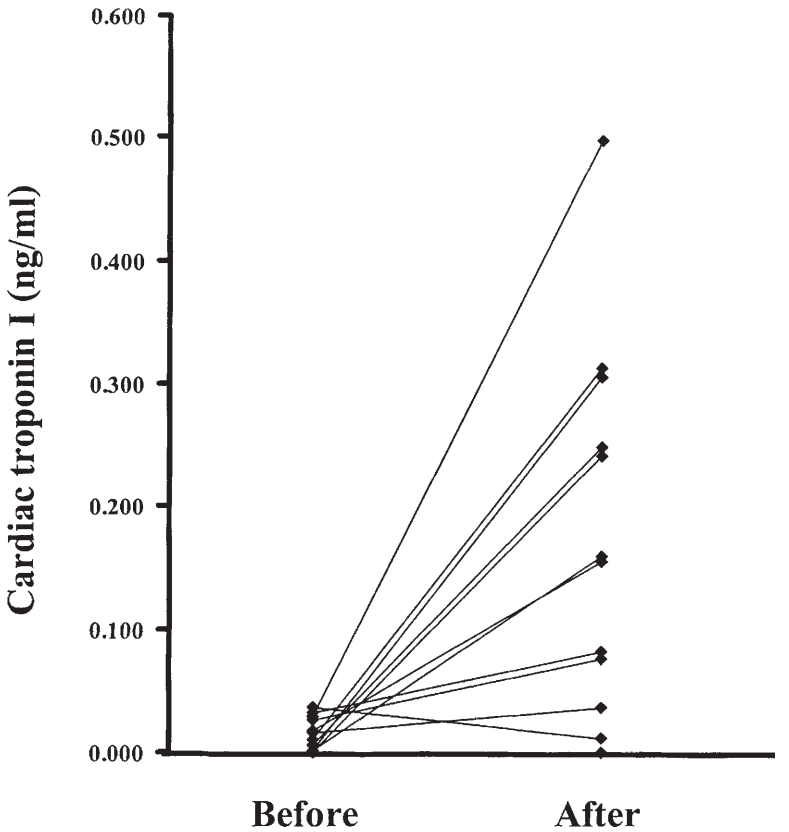

Figure 1 Individual cardiac troponin I concentration before and after training exercise in 12 race horses.

DISCUSSION

Results from the present study clearly demonstrated that biochemical alterations after training exercise are common in race horses. Nonetheless, most of the parameters in liver, renal and lipid profile showed minor increases in their levels, with a statistically significant difference observed only for ALT, total protein and albumin. As in our study, a mild increase in plasma glucose concentration and a decrease in triglyceride level were seen in race horses after the races (Hosoya *et al*., 2004). Similar to our observation, a significant increase in total protein and albumin concentrations has been reported in humans after a marathon running, and is probably secondary to the effect of dehydration after the races (Kratz *et al*., 2002; Foran *et al*., 2003).

With regard to the striated muscle enzymes, there was a distinct elevation in the post-exercise activities of CK, AST and LDH compared with baseline values. In human, there was a marked increase in levels of CK and, to a lesser extent, AST after a marathon running (Kratz *et al*., 2002). However, since these enzymes are abundantly present both in cardiac and skeletal muscle, their diagnostic values for myocardial cell damage are substantially limited.

Troponin I belongs to the troponin complex system of the myofibril that, together with troponin T and troponin C, regulates the calcium-dependent interaction of actin and myosin. Cardiac troponin I (cTnI) has a unique structure distinguishable from that of the skeletal muscle isoform. Initially, sophisticated immunoassay for determining cTnI has been developed for human

application. Nonetheless, the homology of cTnI among mammalian species allows for the cross-reaction of human anti-cTnI antibodies with equine cTnI (O'Brien *et al*., 1997). In recent years, several studies have used cTnI as biochemical marker for the assessment of cardiac damage in horses (Phillips *et al*., 2003; Schwarzwald *et al*., 2003; Peek *et al*., 2004). Phillips *et al*. (2003) reported a reference range for cTnI (Dimension® heterogenous immunoassay module, Dade Behring) in race-training thoroughbred horses of 0.00 - 0.11 ng/ml. By using a different cTnI immunoassay (Vitros ECi®, Ortho-Clinical Diagnostic), we found pre-exercise values ranging from 0.00 to 0.036 ng/ml (mean value 0.015 ng/ml). Three to four hours after the training, the values rose to a mean concentration of 0.177 ng/ml (range 0.00-0.498 ng/ml). Seven of the 12 animals exhibited cTnI concentration of more than 0.12 ng/ml indicative of minor myocardial damage. Only one horse showed cTnI value exceeding the myocardial infarction cut-off concentration of 0.40 ng/ml.

To our knowledge, studies on the effect of exercise on the release of cardiac troponins have been conducted only in human (Rifai *et al*., 1999; Neumayr *et al*., 2001; Herrmann *et al*., 2003; Urhausen *et al*., 2004). Results from these trials have shown that a significant number of persons showed increased cTnI concentrations after strenous exercise (triathlon, marathon running, alpine bicycling). Depending on the cut-off values used and the types of exercise events, the percentages of elevated cTnI were between 9 and 86 which is similar to our observation in horses (8 – 67%). It should be noted, however, that our data are derived from animals on a relatively lower exercise level. Nevertheless, results from all the studies mentioned above clearly pointed to the existence of an exertion-related myocardial cell damage. Since the extent of cardiac injury observed is usually minimal, it can be assumed that the release of cTnI after exercise training in our horses is from the cytosolic pool (3% of the total cTnI content) of the myocytes which may indicate reversible cell damage.

The limitations of the present study need to be considered. We have not shown a result of subgroup analysis that revealed a non-significant trend for sex-related difference in the effect of exercise on cTnI release, with male animals exhibited higher post-exercise values than females. This may be due to the small sample size. The significance of this finding remains to be determined in trials with a larger number of animals. Similarly, the limited number of study animals has not allowed for determination of a possible age-related difference in cTnI concentration. Another limitation was that we have not measured CK-MB isoenzyme mass, another specific marker of myocardial cell damage. However, in contrast to the troponins, there is poor species conservation of CK-MB molecule and the immunoassays developed for human application are too selective for epitopes on the human form of CK-MB from higher primates (Holt, 1998). In addition, the electrophoretic method for CK-MB determination is not available for use in our laboratory. Furthermore, the activity of CK-MB in plasma of horses has been reported to be very low or undetectable (Holt, 1998).

CONCLUSION

Physiological alterations in several blood clinical chemistry parameters following exercise training are common in race horses. In particular, a small but significant increase in cTnI plasma concentrations post-training was observed in the majority of animals indicative of minor myocardial cell damage. The clinical significance of these changes is at present still not obviously known and remains to be determined in future investigations.

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