COBISS: 1.08
Agris category code: L50

THE EFFECT OF WORKLOAD TYPE AND BASELINE COVARIATE ON THE RESPONSE OF PLASMA BIOCHEMICAL PARAMETERS IN SHOW JUMPERS

Anikó VINCZE [1], Csaba SZABÓ [1, 2], Ákos HEVESI [3, 4], Sándor VERES [3], Dániel ÜTŐ [3]

ABSTRACT

Studies with horses indicated that the responses of blood biochemical parameters to different exercises vary. Some study also indicated that significant individual variance exist in blood parameters, which makes difficult to detect treatment effect. Therefore, the aim of this study was to examine the correlation between plasma biochemical parameters of show jumpers before and after of high level aqua training and jumping course completion, and the effect of using baseline level as a covariate on the significance of horse effect. Four normally trained show jumpers ageing from 6 to 11 years were trained with high-intensity aqua treadmill in three periods during three days and after they did compete in the Indoor Show Jumping Championship. Blood samples were taken before and immediately after aqua treadmill training program and both days of the competition before and immediately after the course. From the blood plasma samples lactate, lactate dehidrogenase (LDH), creatine kinase (CK), aspartate amino transferase (AST/GOT), glucose, cholesterol, triglyceride, total-bilirubin and cortisol level were determined. Aqua training did result significant changes only in glucose, trygliceride and cortisol level. In contrast show jumping resulted significantly higher levels in all parameters measured except GOT. We found positive correlation between same blood parameters before and after exercise and competition in bilirubin, cholesterol, LDH, GOT, CK and cortisol. Our result clearly demonstrate that using baseline variables as covariate eliminates the significant individual effect. In conclusion when evaluating fitness of horses the type of exercise should be considered and biochemical values measured at rest should be used as covariate factor.

Key words: horses / sport / jumpers / animal physiology / blood parameters / aqua treadmill

1 INTRODUCTION

The idea of blood-based assessment of training, conditioning and performance is certainly not new. The training induce adaptive processes which results in changes in haematological and biochemical indices. The extent of changes depends on several factors: type of exercise, intensity of work (strength, duration and frequency) and individual variation (Krumprych, 2006). Several studies have shown that physiological responses to treadmill exercise do not replicate responses to field exercise. Plasma lactate concentrations in Standardbred horses pulling a 10 kilopond draught load were lower on the treadmill than on the racetrack (Gottlieb-Vedi and Lindholm, 1997), blood lactate in trotters were lower during exercise on a level treadmill than during exercise on a racetrack (Couroucé *et al.*, 1999). In sport horses it had been also found that blood lactate concentrations were lower on the level treadmill compared with exercise over ground (Sloet van Oldruitenborgh-Osterbaan and Barneveld, 1995).

Large variance, even 20–30 CV% can exist in horse blood biochemical variables among individuals at rest (Lumsden *et al.*, 1980; Krumprych, 2006). Exercise can even elevate the level of variance (Krumprych, 2006). In humans it is demonstrated that there is a correlation

1 Kaposvár Univ., Guba Sándor 40, 7400 Kaposvár, Hungary
2 **Corresponding author**: szabo.csaba@ke.hu
3 Szent István Univ., Budapest, István 2, 1078, Budapest, Hungary
4 Hungarian Equine Rehabilitation and Health Service Ltd., Guba Sándor 40, 7400 Kaposvár, Hungary

between blood biochemical parameters before and after physical challenge (Grigoriev *et al.*, 1995). This means that the response given to a physical activity can be affected by the baseline level. For similar reason the baseline level of plasma urea N concentrations have been used as a covariate factor to reduce source of unidentified variance (error) in treatment effect in studies with pigs (Coma *et al.*, 1995). This could be even more important in studies where the number of subjects often count four to six, like trials with horses. Therefore, the aim of this study was to examine the correlation between plasma biochemical parameters of show jumpers before and after of high level aqua training and jumping course completion, and the effect of using baseline level as a covariate on the significance of horse effect.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS

Four normally trained Standardbred show jumpers aged from 6 to 11 years were used in the test at the Pannon Equestrian Academy, Kaposvár University. Gender was not considered in the selection of animals tested.

2.2 TRAINING METHOD

The four jumping horses were trained with high-intensity aqua treadmill in three periods during three days (Table 1). These horses did compete in the Indoor Show Jumping Championship in Hungary at the Equestrian Academy of Kaposvár. The horses finished one class each day (Saturday and Sunday). One week rest was between the three test periods.

The normal training and jumping training were one hour per day with rider. During the aqua treadmill training the temperature of the water was 22 °C, the level of the water was above the shoulder joint with 15 cm. This program lasted 45 minutes: 10 minutes walking, 30 minutes trotting and 5 minutes walking. After the training they were dried under infra-red lamps for about 20 minutes.

2.3 BLOOD SAMPLING

4 ml blood samples were taken before and immediately after aqua treadmill training program on Thursday and both days of the event before and immediately after the first course. These samples were taken from the jugular vein into the sampling tubes containing NaF-oxalate and Na-heparine. The blood samples were stored on ice until spinning. The samples were spanned at 3000 rpm for 3 minutes. Plasma were pipetted to an eppendorf tube and stored at a temperature −18 °C until the analysis.

2.4 LABORATORY ANALYSIS

From the blood plasma samples lactate, lactate dehidrogenase (LDH), creatine kinase (CK), aspartate amino transferase (AST/GOT), glucose, cholesterol, triglyceride, total-bilirubin and cortisol level were determined in the laboratory of the Kaposi Mór Teaching Hospital (Kaposvár, Hungary) using Roche Modular SWA (Hoffmann-La Roche Ltd.) measuring system.

2.5 STATISTICAL ANALYSIS

The experimental data were evaluated by the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) statistical software package using GLM procedure and type III SS. In case of significant treatment effect mean differences were tested by Tukey test. The strength of the relationship between variables determined with Pearson correlation coefficients.

3 RESULTS AND DISCUSSION

Aqua training did result significant changes only in glucose, trygliceride and cortisol level (Table 2). In contrast show jumping resulted significantly higher levels in all parameters measured except GOT. These differences in agreement with other scientific results confirm that various excercises result in different responses of blood parameters (Gottlieb-Vedi and Lindholm, 1997, Cour-

Table 1: *Weekly training schedule*

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Normal training		X			X		
Jumping			X				
Aqua treadmill	X	X		X			
Competition						X	X

***Table 2:** The effect of training method and individual variance on some plasma biochemical parameters in show jumpers*

	Sampling				Horse				Effect		
Parameter	BA	AA	BC	AC	A	B	C	D	**S**ampling	**H**orse	S*H
Bilirubin	15.0^{ab}	15.9^{ab}	14.0^{b}	17.0^{a}	15.2^{ab}	16.5^{a}	17.4^{a}	13.0^{b}	***	***	NS
Glucose	4.8^{ab}	4.0^{c}	5.0^{a}	4.5^{b}	4.6	4.7	4.6	4.7	***	NS	NS
Lactate	0.63^{b}	0.40^{b}	0.87^{b}	2.41^{a}	1.0^{b}	1.0^{b}	2.0^{a}	1.0^{b}	***	***	***
Trygliceride	0.36^{b}	0.44^{a}	0.33^{b}	0.45^{a}	0.34^{b}	0.39^{ab}	0.41^{a}	0.42^{a}	***	***	NS
Cholesterol	2.1^{b}	2.0^{b}	2.1^{b}	2.2^{a}	2.3^{a}	2.3^{a}	1.8^{c}	2.1^{b}	***	***	NS
LDH	668^{ab}	611^{b}	623^{b}	735^{a}	751^{a}	618^{b}	615^{b}	680^{ab}	**	*	NS
GOT	308^{b}	294^{b}	324^{ab}	356^{a}	294^{b}	286^{b}	415^{a}	313^{b}	***	***	NS
CK	232^{b}	205^{b}	237^{b}	280^{a}	186^{c}	225^{b}	316^{a}	253^{b}	***	***	NS
Cortisol	155^{b}	216^{a}	120^{c}	176^{b}	137^{b}	152^{b}	199^{a}	154^{b}	***	***	***

BA – before aqua training, AA – after aqua training, BC – before competition, AC – after competition
$^{a, b, c}$ Means in a row of an effect lacking a common superscript differ ($P < 0.05$)

oucé *et al.*, 1999; Sloet van Oldruitenborgh-Osterbaan and Barneveld, 1995). Hevesi *et al.* (2009) and Voss *et al.* (2001) demonstrated similar effect compared to dry treadmill exercise. The lack of clear metabolic response after aqua training most probably is a result of the cooling effect of the water, which limits the extent of chemical processes. The appropriate temperature, the continous more intensive flexor-extensor exercise, the massage effect of water and increased capillary activity must be important factors to explain the lower lactate-level during aquatrainig (Hevesi *et al.*, 2010). Valette *et al.* (1993) estimated the anaerobic treshold about 2.25 mmol/l. In this respect the competition resulted anaerobic muscle work, despite the short intensive work. In our previous study (Vincze *et al.*, 2010) we measured higher post competition level of lactate (3.5 mmmol/l) for conventionally trained show jumpers competing on 110 and 120 cm class. Art *et al.* (1990) found about 9 mmmol/l post competition lactate level for horses competing in 150 cm classes. These results suggests that there must be a close correlation between the effort required to pass the obstacle (height of the obstacles) and the lactate response. Release of cortisol allows an individual to tolerate and adapt to challenges to homeostasis that occur in every life (Willmore and Costill, 1994; Thornton, 1985). The level of cortisol is increased in the horse during a wide variety of exercise activity (Horohov *et al.*, 1999; Hyyppa 2001; Snow and Rose, 1981), and the release appears to be affected by both intensity and duration of exercise (Thornton, 1985; Snow and MacKenzie, 1977). In our study both type of exercise significantly increased the level of cortisol. Interestingly, our data shows that the aqua training was a more stressful exercise, based on both pre- and post-exercise values. However, if we calculate the response given it is similar.

Similarly to the observation of Hevesi *et al.* (2010) we also found significant individual (horse) effect in the case of most parameters measured. The differences between horses often was higher than the response given to the competition as an excercise. This high individual variation can reduce the number of significant differences instudies whre the number of experimental units is rather limited. Grigoriev *et al.* (1995) demonstrated in humans that there is a correlation between blood biochemical parameters before and after physical challenge. We found positive correlation between billirubin, trygliceride, cholesterol, LDH, GOT, CK and cortisol before and after treadmill exercise (Table 3). Other researchers observed highest correlation with blood lactate concentration 2 and 5 mins after exercise on treadmill (Evans *et al.*, 1993), in our results the lactate level before and after training did not correlate significantly. This may be due to the lactate transport activity, Standardbred horses can be divided into two populations: one with high and

***Table 3:** Correlation coefficient (P-value) between the same blood parameters before and after exercise and competition*

	Billirubin	Glucose	Lactate	Trygliceride	Cholesterol	LDH	GOT	CK	Cortisol
Aqua treadmill	**0.87**	0.17	−0.47	**0.74**	**0.63**	**0.76**	**0.82**	**0.77**	**0.64**
	(<0.01)	(0.59)	(0.12)	(<0.01)	(0.03)	(<0.01)	(<0.01)	(<0.01)	(0.02)
Competition	**0.56**	0.25	0.47	0.37	**0.79**	**0.58**	**0.98**	**0.92**	**0.56**
	(0.01)	(0.24)	(0.02)	(0.07)	(<0.01)	(0.01)	(<0.01)	(<0.01)	(<0.01)

***Table 4:** The effect of baseline level used as a covariate on the significance of individual differences in blood parameters*

	Horse				Model 1	Model 2	
Parameter	A	B	C	D	Horse	Horse	Baseline as covariate
Bilirubin	16.1[ab]	17.7[b]	18.3[a]	14.4[b]	*	NS	**
Glucose	4.2	4.5	4.2	4.4	NS	NS	+
Lactate	1.2[b]	1.3[a]	2.9[a]	1.5[a]	*	NS	*
Trygliceride	0.37[b]	0.47[b]	0.45[b]	0.48[a]	*	+	***
Cholesterol	2.3[a]	2.3[a]	1.9[b]	2.1[b]	***	NS	+
LDH	781[a]	629[b]	622[b]	742[b]	**	*	*
GOT	302[b]	287[b]	426[a]	325[b]	***	NS	***
CK	198[b]	230[b]	323[a]	268[ab]	***	NS	***
Cortisol	158[b]	173[b]	252[a]	176[b]	***	***	***

[a, b, c] Means in a row lacking a common superscript differ ($P < 0.05$) (calculated in model 1)

the other with low lactate transport activity in their RBC (Vaihkönen and Pösö, 1998). Lactate transport capacity appears to be inherited, with the high capacity being caused by the dominant allele (Vaihkönen *et al.*, 2002).

Rumley *et al.* (1985) demonstrated total CK did not correlate with finishing time at 30 min or 30 hrs post race. However, in our study the CK level very closely correlated in both aqua treadmill and competition basis before exercise and after exercise. The most of the blood parameters has significant correlation before and after competition or exercise. This information indicates that evaluating the effect of exercise on blood biochemical parameters can not be judged without the knowledge of baseline levels. Furthermore, the response given to a workload is depends on the baseline value of the blood parameter. Therefore, when evaluating exercise induced changes in blood parameters it can be suggested that the baseline levels should be used as a covariate to reduce the effect of individual variation. We have tested that hypothesis on our dataset and the results are presented in Table 4.

Our results clearly demonstrate that in the case of horses using the baseline levels as a covariate makes individual effect non-significant in most of the cases. The exception of LDH and cortisol indicates that other factors than baseline level affects considerable the individual response. Waguespack *et al.* (2011) tested the usefulness of baseline plasma urea level as covariate on the number of detected significant differences in pigs throughout several experiments. In their case using the baseline level did not resulted in noteworthy increase in the number of significant differences. However in the case of pigs the baseline level was a value measured at the beginning of the trial and the effect of nutritional treatment was measured several weeks later. Therefore, it is obvious that there is a week correlation between the two values. However in the case of horse studies there is a relative short time difference between the baseline and test measurements. Since in our study there was a considerable difference between post exercise blood parameters of aqua training and show jumping competition, it had no meaning to test that theory. However, in evaluating the effects of treatment using the same type of workload, this approach can be useful.

4 CONCLUSIONS

Different type of exercise results in different pattern of blood biochemical parameters, therefore results of different type of exercises can not be compared directly. When evaluating the effect of exercise on blood biochemical parameters, the values measured before exercise should be used as covariate in order to get correct result.

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