The Effect of Deep Water Aqua Treadmill Training on the Plasma Biochemical Parameters of Show Jumpers

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Summary

Aqua treadmill is mainly used for rehabilitation purposes, but research indicates that this equipment could be used for training as well. The few studies performed with aqua treadmill mainly followed lactate and heart rate changes. Therefore, the aim of this study was to test the effect of high water treadmill training on several blood parameters and on the correlations between them. Eight similarly trained Standardbred show jumper horse competing at the same level were selected with age between 6 to 11 years. The horses were subjected to a one week standardized exercise test which included normal training, training with show jumping and aqua treadmill training. The aqua treadmill training consisted of a 10 min walk (filling up, 4.5 km/h), 30 min trot (13 km/h) and 4 min walk (emptying, 4.5 km/h). Blood samples were taken from the jugular vein before aqua training, at the completion of each work bout, after drying and after one and two hour rest. Blood plasma were separated and lactate, LDH, CK, AST, glucose, cholesterol, triglyceride, total-bilirubin and cortisol level were determined. In conclusion plasma lactate response itself does not reflect correctly the intensity of workload in high water level aqua training, therefore measurement of several blood parameters is advisable. Further studies needed to understand the relationship of metabolic processes altered due to the effect of partial water submersion.

Key words

horse, blood parameters, aqua treadmill, training

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Aim

Horse athletes need training to achieve good performance in a similar way than Humans. Hinchcliff et al., 2002 showed that the anaerobic capacity of horses could be increased by an appropriate conditioning program including regular and high intensity training. However, the continuous high intensity conventional training may result in a high percentage of retirement from the training program due to injuries (Eto et al., 2004). The training in water was first applied in the rehabilitation of human athletes. The buoyancy of water decreases the pressure on the joints, but makes possible to develop the muscles anaerobic capacity. The training of horses in water is not new, but recently there has been a development in the possible use of aqua treadmill for horses, following the idea that it could be used both for rehabilitation and for training purposes. Several studies had been performed with aqua treadmill (Lindner et al., 2012, 2010, Hevesi et al., 2009, Nankervis et al., 2009, Voss et al., 2002) to test its effect on metabolism using mainly heart rate and lactate as indicative variables. Therefore, the aim of this study was to test the effect of high water treadmill training on several blood parameters analyzing their change before, during and after exercise and their correlations at specific time points.

Material and methods

Experimental animals: Eight similarly trained Standardbred show jumper horse competing at the same level were selected with age between 6 to 11 years. Gender was not considered in the selection of animals tested. Horses were housed in individual boxes with the size of 3 * 3 m. The daily feed allowance consisted of 12 kg meadow hay and 2.6 kg concentrate which provided 134.5 MJ DE and 1,042 g crude protein.

Training program: The horses were trained according to the schedule presented in Table 1. Normal training was one hour training with rider, while jumping training was half an hour warming up and half an hour jumping training with rider. The protocol of the aqua training and blood sampling is reported on table 2. During the aqua treadmill training the temperature of the water was 22Cº, while the level of the water was above the shoulder joint with 15 cm. This program lasted 45 minutes (Table 2) and after the training horses were dried under infra-red lamps for 20 minutes. Horses were then taken back to the stable.

Blood sampling: 4 ml blood samples were taken before, during and after aqua treadmill training program on Thursday (Table 2). These samples were taken from the jugular vein into the sampling tubes containing NaF-oxalate or Na-heparin. 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 ºC until the analysis.

Laboratory analysis: From the blood plasma samples lactate, lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), 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.

Table 1. Training program

	Fri	Sat	Sun	Mon	Tue	Wed	Thu
Normal training	X	X	X	X	X		
Jumping						X	
Aqua treadmill				X	X		X

Table 2. Schedule of the aqua training and blood sampling

Phase	Time (min)	Speed of aquatrainer (km/h)	Blood sampling, min (code)	Activity
0	0	-	0 (T0)	Standing, preparation
1	0-10	4.5	10 (T1)	Walking, filling up the aquatrainer
2	10-40	13.0	40 (T2)	Trot in water
3	40-44	4.5	44 (T3)	Walking, emptying the aquatrainer
4	44-60	-	60 (T4)	Standing under infrared lamps
5	60-120	-	120 (T5)	Resting in the box
6	120-180	-	180 (T6)	Resting in the box

Statistical analysis: The experimental data were evaluated by the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) statistical software package using GLM (using one way ANOVA for the time of blood sampling effect) and the CORR procedures. Between means differences for time of sampling were tested by the Duncan test. The strength of the relationship between variables was determined by the Pearson correlation coefficients.

Results and discussion

Similarly to other studies (Vincze et al., 2012, Hevesi et al., 2009), plasma lactate levels decreased (Table 3) during treadmill training and elevated during the resting period. The study of Nankervis et al. (2009), have shown that the temperature of the water plays an important role in the development of cardiac rhythm. Because of the temperature of water used (i.e., 22-25°C) is usually lower than body temperature, a probable reduction of lactic acid due to the reduction of muscles' temperature could have occurred. The measured lactate values indicates that the high water level aqua training did not induce significant anaerobic energy supply (Eaton, 1994). Lindner et al. (2012) using higher maximum speed (19.8 km/h), shorter exercise period (20 min) and similar water height (80% of the withers height) did not observed markedly greater lactate level (1.5-2.0 mmol/L). Vincze et al. (2010) demonstrated that show jumpers competing at 100, 110 and 120 cm high obstacles develops 1.5, 3.5 and 3.5 mmol/L lactate level. Art et al. (1990a,b) measured 6–9 mmol/L value for show jumpers competing on 130-150 cm height. These results indicate that, that higher level competition induces significant energy demand, which deplete the aerobic capacity. Since aqua training did not result in anaerobic energy supply, it is questionable that whether this training method could be used for the training of anaerobic capacity. The aqua training had no effect on lactate dehydrogenase level; however its value

Table 3. Evolution of selected blood parameters as a result of high level aqua training

Blood parameter	Time of sampling. min							RMSE	P
	0	10	40	44	60	120	180		
Lactate (mmol/l)	0.62^{b}	0.41^{c}	0.37^{c}	0.39^{c}	0.62^{b}	1.01^{a}	1.03^{a}	0.15	<0.001
LDH (U/l)	684	676	667	672	648	673	675	83.6	0.990
Glucose (mmol/l)	4.7^{b}	3.9^{cd}	3.8^{d}	4.1^{cd}	4.4^{bc}	5.4^{a}	5.8^{a}	0.43	<0.001
Trigliceride (mmol/l)	0.35^{ab}	0.36^{ab}	0.42^{a}	0.42^{a}	0.35^{a}	0.28^{b}	0.27b	0.11	0.047
CK (U/l)	223	223	219	218	207	220	223	41.4	0.989
AST (U/l)	290	291	289	286	251	290	293	51.8	0.691
Bilirubin (mmol/l)	16.7	17.3	17.9	18.2	16.4	18.9	18.3	3.94	0.859
Cortisol (nmol/l)	186^{abc}	212^{ab}	246^{a}	246^{a}	234^{ab}	174^{bc}	129^{c}	60.3	0.002
Cholesterol (mmol/l)	2.21	2.18	2.18	2.10	2.14	2.15	2.23	0.23	0.942

was quite above the reference values (162-412 U/l, Harvey et al., 2008). Plasma concentration of LDH and other enzymes (i.e., AST, CK) are increased typically in horses during jumping or endurance exercise (Balogh et al., 2001; Krywanek et al., 1996). Lactate dehydrogenase converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased. The level of competition (age of horses) and the period of the competition season had no effect on the LDH level (Vincze et al., 2010). Most probably genetic (Gaffey and Cunningham, 1988) and/or other factors like nutrition is responsible for the level of LDH, while exercise has little influence on it. This could explain why we could not detect significant correlation between plasma lactate and LDH values (Table 4 and 5).

The glucose content of blood serum decreased during aqua training, while after it developed elevated level, even above the reference value (2.8-5.2 mmol/L, Harvey et al., 2008). This might be due to the need of glycogen replenishment in the muscles. Long workload (more than 3 hours) decreases the plasma glucose level, but a shorter exercise can decrease in increase as well depending on the intensity of workload and dietary energy source (Pösö and Hyyppa, 1999; Snow and MacKenzie, 1977). The level

Table 4. Correlation between blood parameters before aqua training (T0) and at the end of aqua training (T3)

		Lact	LDH	Gluc	Trig	CK	AST	Bil	Cort	Chol	
	Lact	-	-0.39	0.57	0.10	-0.10	-0.44	-0.24	0.49	-0.58	
	LDH	0.39	-	-0.88**	-0.45	-0.51	0.24	-0.51	-0.19	0.69+	
	Gluc	0.54	0.19	-	0.38	0.14	-0.52	0.29	0.23	-0.60	
	Trig	-0.05	-0.43	-0.19	-	0.10	-0.21	0.20	0.34	-0.03	
T0	CK	-0.25	-0.60	-0.40	0.16	-	0.69+	0.44	0.49	-0.58	T3
	AST	-0.25	0.03	-0.09	-0.38	0.55	-	-0.06	0.42	-0.12	
	Bil	-0.28	-0.17	-0.78*	-0.28	0.47	0.13	-	-0.17	-0.07	
	Cort	-0.48	-0.75*	-0.07	0.57	0.21	-0.41	-0.11	-	-0.70+	
	Chol	0.20	0.70+	0.02	-0.22	-0.60	-0.53	0.05	-0.18	-	

+ P<0.1, * P<0.05, ** P<0.01; Lact: Lactate, LDH: lactate dehidrogenase, Gluc: glucose, Trig: Triglicerides, CK: creatine kinase, AST: aspartate-aminotransferase, Bil: bilirubin, Cort: cortisol, Chol: cholesterol

Table 5. Correlation between blood parameters drying (T4) and two hours after aqua training (T6)

		Lact	LDH	Gluc	Trig	CK	AST	Bil	Cort	Chol	
	Lact	-	-0.03	0.18	0.39	-0.02	-0.72	-0.20	0.57	-0.01	
	LDH	0.03	-	-0.37	-0.08	-0.55	-0.10	-0.71*	-0.42	0.47	
	Gluc	0.12	0.49	-	-0.04	0.54	0.05	0.32	0.77*	-0.34	
	Trig	0.22	0.66+	-0.65+	-	0.31	-0.14	-0.17	0.09	-0.61	
T4	CK	0.24	-0.75*	-0.03	0.34	-	0.34	0.06	0.32	-0.93**	T6
	AST	-0.73*	-0.11	-0.15	0.12	-0.28	-	0.10	-0.16	-0.33	
	Bil	-0.46	-0.38	-0.24	0.34	-0.17	0.84**	-	0.43	0.03	
	Cort	0.08	-0.19	0.13	-0.38	0.60	-0.57	-0.65+	-	-0.18	
	Chol	0.16	0.72*	0.45	0.62+	-0.65+	-0.27	-0.21	-0.13	-	

+ P<0.1, * P<0.05, ** P<0.01; Lact: Lactate, LDH: lactate dehidrogenase, Gluc: glucose, Trig: Triglicerides, CK: creatine kinase, AST: aspartate-aminotransferase, Bil: bilirubin, Cort: cortisol, Chol: cholesterol of triglycerides decreased after the aqua training. At low intensity work the main energy source of the muscles is the metabolites of triglycerides. The glyceride component of triglycerides can be converted to glucose, explaining of the opposite change in glucose and triglycerides level in the plasma. Aqua training had no influence on the plasma CK, AST, bilirubin and cholesterol level. However, the quite elevated level of creatine kinase (reference value 2.4-23.4 U/l, Harvey et al., 2008) reflects the high intensity of aqua training. It has been found that CK level can be easily doubled in endurance horses. However, Art et al. (1990a,b) measured much lower post exercise CK levels in horses competing in higher show jumping classes. Nevertheless Pritchard et al. (2009) determined a 210 U/l reference value for Lahore working horses (Pakistan). They stated that the high levels of CK in working horses may indicate low-grade, chronic muscle damage rather than a short-term and reversible effect of overwork. This assumption was based on the fact that the reference population was working daily for short periods. Values of AST, bilirubin and cholesterol were within the reference values (Harvey et al., 2008). Contrary to that, Lopez et al. (1974) demonstrated that cholesterol level decreases as the effect of exercise. The level of cortisol increased during aqua training and decreased after that. However, Marc et al. (2000) measured cortisol peak after the dry treadmill exercise. Release of cortisol allows an individual to tolerate and adapt to challenges to homeostasis that that usually occur in life (Willmore and Costhill, 1994; Thornton, 1985). Cortisol is increased in the horse during a wide variety of exercise activity (Hyppa 2001; Horohov et al, 1999). The elevated cortisol level during aqua training indicates that this type of training does pose a stress situation to the horses, even if they had past experience. The cortisol results of Vincze et al. (2010) shows that even competition environment causing less stress to show jumpers.

The correlation between the blood parameters did not add any useful information on the measured variables. In the literature mainly the lactate level is used to test the effect of training or workload. LDH had a moderate negative correlation with cortisol and there was a good correlation between bilirubin and glucose at T0 sampling (Table 4). At the end of aqua training (T3 sampling) only level of LDH and glucose showed strong correlation. Interestingly after infra red lamp drying (T4 sampling) we had more significant correlation between blood parameters (Table 5). LDH had moderate correlation to CK and cholesterol, while between bilirubin and AST level we found a close relationship. At T4 sampling time was the only case where lactate level expressed correlation to other variable, in this case to AST. However, after two hour rest (T6 sampling) we found correlations between different variables (cholesterol-CK, LDH – bilirubin and glucose – cortisol). If we look at the overall pattern of correlations between variables, we do not see any similarities between sampling times, even not between T0 and T6. This indicates that the blood parameters we measured changing independently before, during and after aqua training. Lactate level is certainly a good indicator of aerob or anaerob energy supply. But exercises induce much complex changes in the body, which could affect the fitness of horses. Therefore in order to understand the complexity of physiological changes induced by different workloads measurement of several blood parameters is advisable.

Conclusions

Plasma lactate response itself does not reflect correctly the level of workload of high water level aqua training, therefore measurement of several blood parameters is advisable. Further studies needed to understand the metabolic processes altered due to the effect of water submersion.

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