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**The correlation of blood lactic acid and glycerol levels
in Purebred Arabian horses during exercise tests**

Współzależność stężenia glicerolu i kwasu mlekowego we krwi
koni czystej krwi arabskiej podczas testów wysiłkowych

Summary. Physical effort influences free glycerol concentration in the blood of horses, and also glycerol bounded in triacylglycerols. The aim of this study was to evaluate the influence of the intensity of exercise that was measured by blood lactic acid (LA) level on plasma glycerol concentration in Purebred Arabian horses. During the training sessions of 36 Arabian horses (26 stallions 3–6 years old and 10 mares aged 3–4 years), 53 exercise tests were performed. Blood samples were taken at rest, at the end of the training session composed by 1200 meters gallop at racetrack with a speed 8–13 m/s, and thirty minutes later. Blood LA and plasma glycerol concentrations were determined by enzymatic tests. The post-exercise LA value correlated positively with the concentration of glycerol that was determined at the same time ($r = 0.63$) and 30 minutes later ($r = 0.80$).

Key words: Arabian horses, exercise, glycerol, lactic acid

INTRODUCTION

Although after an effort the level of many biochemical parameters in the blood increase, only the determination of lactate acid (LA) level is used in practice to evaluate the physical condition of horses [Lindner 2000, Gondim *et al.* 2007]. It is claimed that a high, post-exercise level of LA is correlated with quite rapid runs [Desmecht *et al.* 1996, Guhl *et al.* 1996, Davie and Evans 2000]. Moreover, the blood LA concentration after strenuous exercise test is correlated with the quality of horse racing performance [Evans *et al.* 1993, Courouce *et al.* 1997, Roneus *et al.* 1999].

Changes in the rate of LA production by muscle reflect changes in the degree to which anaerobic glycolysis contributes to energy production. The second source of energy for working muscles is from processes of lipolysis and oxidizing free fatty acids (FFA). Under the influence of the raised adrenaline and glucagon, FFA are released during exercise from the fatty tissues as well as from triacylglycerols (TG) circulating in the blood. This takes place both during endurance tests and sprints [Hodgson *et al.* 1986, Watt *et al.* 2004, Helge *et al.* 2007]. Alongside the release of FFA, the degeneration of TG also leads to the release of glycerol, which does not undergo any further metabolism during exercise and it accumulates in the circulatory system. Therefore, in exercise study, the level of plasma glycerol is still used as an indicator of lipolysis intensity [Goto *et al.* 2007, Ormsbee *et al.* 2007]. Despite the consumption of FFA by the muscles at this time, TG concentration in the horses' blood increases [Warren *et al.* 1999, Hyypa 2001]. It is thought that lipolysis and burning FFA is simultaneously accompanied by the synthesis of TG in liver [Poso *et al.* 1989]. The increase of plasma TG concentration is specific for the exercising horses; it is not observed in either people or rodents. The influence of exercise on the plasma levels of lipid metabolites was investigated in trotters but it was only partially studied in galloping racehorses [Poso *et al.* 1989, Kędzierski and Podolak 2002, Podolak *et al.* 2004]. Since physical effort influences free glycerol concentration in the horse's blood, and also glycerol bounded in TG, it would seem appropriate to determine this changing parameter in accordance with exercise intensity.

The aim of this study was to evaluate the influence of the intensity of exercise measured by blood LA level on the plasma glycerol concentration during exercise tests in Purebred Arabian horses.

MATERIAL AND METHODS

A total of 36 Purebred Arabian horses (26 stallions 3–6 years old and 10 mares aged 3–4 years) were used in this study. Some of these horses were investigated two or three times, as a result, 53 exercise test were performed. The horses were prepared for racing from minimum 3 months according to training programs conducted in their state-run stables. All the subjects were clinically healthy and they had competed in official races during the study. The research was carried out from June to September during the height of the race season in Poland. The training sessions were composed of 1200 meters gallop at racetrack with a speed 8–13 m/s.

The blood was sampled from external jugular venapuncture into test tubes containing lithium heparin. Samples were taken three times during research days: during rest, marked as sample A; at the end of the training session, marked B; and thirty minutes later, marked C. Blood LA concentration was determined via enzymatic cuvette test manufactured by Dr Lange (Berlin, Germany). Plasma glycerol level was determined by a Dr Lange company's test based on enzymatic lipolysis and the final measure of glycerol concentration.

The data are expressed in mmol/l and shown as means \pm SE. Comparisons between the analysed results were made using the Student's t-test (ANOVA, Microsoft Excel NT).

Table 1. The concentration of blood lactic acid (LA) and plasma glycerol determined in Purebred Arabian horses during race training sessions (means \pm SE)
 Tabela 1. Stężenie kwasu mlekowego (LA) we krwi i glicerolu w osoczu krwi koni rasy arabskiej podczas sesji treningowych (średnia \pm błąd standardowy)

The range of blood LA levels just after exercise mmol/l Zakres wartości stężenia LA we krwi pobieranej tuż po wysiłku	Glycerol/Glicerol, mmol/l					
	A	B	C	Δ B-A	Δ C-A	Δ C-B
2.3–5.8 (3.92 \pm 1.14 ^x) n = 24	0.35 \pm 0.10 ^{ax}	0.51 \pm 0.15 ^{bx}	0.32 \pm 0.09 ^{ax}	0.16 \pm 0.10 ^x	-0.03 \pm 0.07 ^x	-0.19 \pm 0.12 ^x
6.0–12.5 (10.3 \pm 1.89 ^y) n = 18	0.36 \pm 0.12 ^{ax}	0.63 \pm 0.21 ^{by}	0.63 \pm 0.27 ^{by}	0.27 \pm 0.14 ^y	0.27 \pm 0.23 ^y	0.005 \pm 0.25 ^y
13.5–22.0 (16.3 \pm 2.84 ^z) n = 11	0.48 \pm 0.24 ^{ax}	0.90 \pm 0.31 ^{bz}	1.25 \pm 0.49 ^{cz}	0.42 \pm 0.11 ^z	0.77 \pm 0.37 ^z	0.35 \pm 0.34 ^z

A – sample taken at rest, B – just after exercise, C – 30 minutes after effort, Δ – difference between obtained data.

Data compared in horizontal line marked with different letters a, b, c are statistical different at $p \leq 0.05$; as x, y, z – compared in vertical line

A – próbki pobierane w spoczynku, B – tuż po zakończeniu wysiłku, C – po 30 min. odpoczynku, Δ – różnice między uzyskanymi wynikami.

Średnie oznaczone: a, b, c – porównywane poziomo oraz x, y, z – porównywane pionowo różnią się istotnie przy $p \leq 0,05$.

RESULTS

According to blood LA level determined just after exercise, the horses were divided into three groups (Table 1). A significant increase in plasma glycerol concentration was observed in all investigated horses after effort. The following changes of this parameter during 30 minutes of regeneration were different and they were compared with the intensity of exercise. When LA level after exercise was in the range 2.3–5.8 mmol/l the glycerol concentration decreased to the initial level during 30 minutes of regeneration. In horses having LA results between 6.0–12.5 mmol/l after gallop, the glycerol level remained elevated for another 30 minutes. The LA values above 13.5 mmol/l were compared with the increase of plasma glycerol during 30 minutes' rest.

The analysis of the obtained results through the application of the correlation coefficient indicated an essential connection between the examined parameters. The post-exercise LA value correlated positively with the concentration of glycerol after exercise (glycerol B; $r = 0.63$) and glycerol 30 minutes later (glycerol C; $r = 0.80$), as well as with differences between glycerol concentration after 30 minutes recovery and at rest (glycerol C-A; $r = 0.82$), or glycerol measured after completion of exercise and during rest (glycerol B-A; $r = 0.71$). As is shown in Fig. 1, after an exercise causing LA concentration in the blood higher than 4 mmol/l, plasma glycerol level determined after 30 minutes rest remained higher than its initial level.

DISCUSSION

Results of the present study indicate that a positive correlation between horses' blood LA and changes in plasma glycerol concentration measured after exercise occurs. The more intense exercise and LA production, the more plasma glycerol level increases.

During the physical effort, under the influence of increased levels of glucagon and catecholamines, TG are mobilized from adipose tissue and FFA and glycerol are liberated and released into the bloodstream. FFA are an important source of energy for exercise, they are transported into muscle when they undergo oxidation process. However, about one-third of circulated FFA is extracted by liver cells and reestrified. This results in increased TG synthesis in the liver, and subsequently their secretion into bloodstream. With increasing supply of FFA during exercise, there is a competitive secretion of TG. Blood TG are hydrolyzed in various tissue capillaries, especially in muscle and FFA and glycerol are released again. Poso et al. [1989] revealed that plasma glycerol and TG levels determined after effort were positively correlated with the intensity of the exercise in Standardbred trotters. The results presented here indicated, that in galloping Arabian horses a positive correlation between plasma glycerol and blood LA levels indicating exercise intensity exists.

However, a higher level of this factor was seen when blood LA concentration after exercise was compared with plasma glycerol level measured 30 minutes after the end of effort. Moreover, plasma glycerol concentration determined 30 minutes after more intense exercise was proportionally higher than just after the end of exercise. This observation suggests that in Purebred Arabian horses during the recovery period after intensive gallop the lipolysis and glycerol secretion into blood still exists. A similar reaction was not

found out in trotters [Hyypa *et al.* 1997, Hyypa 2001]. It is known from the Freeston *et al.* [1991] studies that even 30 minutes after intense exercise an elevated level of catecholamines in the blood is still possible. A symptom of the prolonged influence of catecholamines on fatty tissue are intensified lipolysis and an increased concentration of metabolites like FFA and glycerol in the blood. Also, during exercise the increase of plasma glycerol level measured in presented conditions can represent the activity of lipolysis in this period. It indicates that in horses the activity of lipolysis increased despite the high LA concentration. Boyd *et al.* [1974] demonstrated that the excess of lactate is capable of retarding the lipolysis process in men. On the other hand, training increases the tolerance of horses in relation to a high LA concentration.

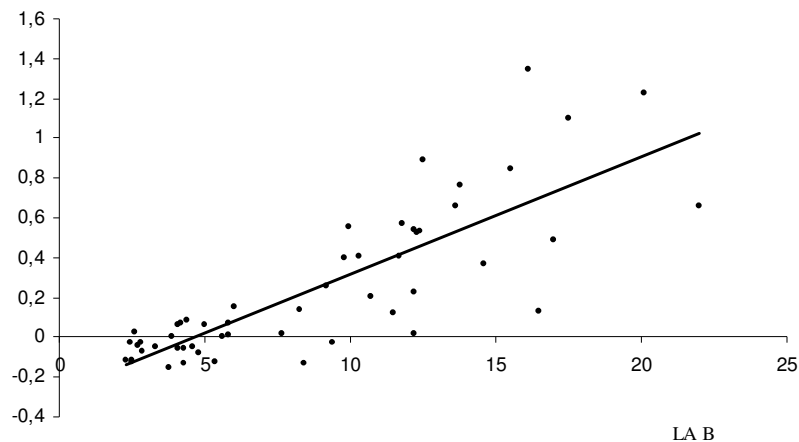


Fig. 1. Comparison of blood LA level determined just after exercise and differences in plasma glycerol levels obtained 30 minutes after the effort and at rest in Purebred Arabian horses
Ryc. 1. Współzależność stężenia LA we krwi i różnicy między wartościami glicerolu uzyskanymi 30 minut po zakończeniu wysiłku i w spoczynku w osoczu krwi koni rasy arabskiej

CONCLUSIONS

In the present work the greatest correlation was shown between LA measured just after exercise and the difference of glycerol levels determined 30 minutes after exercise and at rest. These results indicate that determining glycerol concentration in blood plasma can be applied to evaluate the intensity of exercise in trained Arabian horses.

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Streszczenie. Intensywny wysiłek fizyczny zwiększa stężenie wolnego glicerolu w osoczu krwi koni, a także glicerolu związanego w postaci triacylogliceroli. Celem badań było określenie zależności między intensywnością wysiłku mierzoną poziomem kwasu mlekowego (LA) we krwi a stężeniem glicerolu w osoczu krwi koni czystej krwi arabskiej. Badaniami objęto 26 ogierów w wieku 3–6 lat i 10 trzy- i czteroletnich klaczy. Łącznie przeprowadzono 53 próby wysiłkowe. Krew do badań pobierano w spoczynku, tuż po zakończeniu galopu na dystansie 1200 m pokonywanego z prędkością 8–13 m/s i po 30 min odpoczynku. Stężenie LA we krwi i glicerolu w uzyskanym osoczu oznaczano metodami enzymatycznymi. Poziom LA oznaczany po wysiłku był wprost proporcjonalny do stężenia glicerolu, zarówno oznaczonego tuż po zakończeniu próby ($r = 0,63$), jak i 30 min. później ($r = 0,80$).

Słowa kluczowe: konie rasy arabskiej, glicerol, kwas mlekowy, wysiłek