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Within day changes in blood glucose and plasma insulin concentrations and erythrocytes enzyme activities in race horses at rest

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Summary

Within day changes in concentrations of blood glucose and plasma immunoreactive insulin (IRI) and erythrocytes activities of D-glucose transport (D-GT) and the glycolytic enzyme, hexokinase (HK) and pyruvate kinase (PK), were measured in resting race horses. Blood glucose and plasma IRI concentrations and activities of D-GT, HK and PK of erythrocytes were constant throughout the day in race horses. The erythrocytes activities of D-GT, HK and PK in race horses were 2 to 3.5 times greater than for untrained horses. The increases in these erythrocytes enzyme activities are considered to reflect an increased metabolic activity in the race horses resulting from the training exercises.

Keywords: blood glucose, D-glucose transport, hexokinase, pyruvate kinase, race horse

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Tägliche Veränderungen des Blutglukosespiegels, der Plasmainsulinkonzentration und der Erythrozytenenzym-Aktivitäten bei Rennpferden in Ruhe

Um den Energiestoffwechsel von Rennpferden in Ruhe näher zu erforschen wurde bei 39 Vollblütern die Glukosekonzentration, die immunoreaktive Insulinmenge im Plasma und die Aktivitäten des für den Transport von Glucose verantwortlichen Enzyms D-Glukose-GT sowie der glykolytischen Enzyme Hexokinase (HK) und Pyruvatkinase (PK) in Erythrozyten gemessen.

Ein Teil der Pferde war untrainiert, die anderen Vollblüter wurden in Rennen eingesetzt. Die entnommenen Blutproben wurden aufbereitet, das Plasma getrennt, die Erythrozyten gewaschen und homogenisiert und die Enzymaktivitäten gemessen. Der Blutzuckerspiegel wurde mit einer Glukose-Oxidase-Methode im Vollblut bestimmt. Ein Mikro-ELISa ermittelte die Insulinkonzentration im Plasma anhand einer Sandwich-Methode.

Die beiden Pferdegruppen (untrainierte und Rennpferde) unterschieden sich nicht hinsichtlich ihrer Blutglukosespiegel in Ruhe. Auch die Menge an immunoreaktivem Insulin im Plasma wies keine Differenzen auf.

Die durchschnittliche D-GT-Aktivität erreichte in den Erythrozyten der Rennpferde mehr als doppelt so hohe Werte als sie bei untrainierten Pferden gemessen wurden. Auch die HK- und PK-Level lagen bei den Pferden im Leistungssport deutlich höher als bei untrainierten Tieren.

Die Autoren ermittelten bei den Rennpferden die Veränderungen der gemessenen Blutparameter innerhalb eines Tages und zeichneten sie in Kurven auf. Die Glukose- und Insulinspiegel der Pferde besaßen in der Tendenz zyklische Peaks und Tiefpunkte, wobei ihre höchsten Werte um 7.30 Uhr und ihre geringsten Werte gegen 15.30 Uhr erreicht wurden. Die Unterschiede waren jedoch so gering, daß die durchschnittlichen Glukose- und Insulinkonzentrationen aller Testpferde während des gesamten Tages relativ konstant blieben.

Die mittlere D-GT-Aktivität der Vollblüter lag zwischen 4,4 und 6,2 nmol/min/mg. Die Hexokinase erreichte Werte von 1,3 bis 1,7 mU/mg und die Pyruvatkinasekonzentration der Erythrozyten lag bei 3,4 bis 4,2 mU/mg.

Die Studie zeigte, daß die glykolytischen Enzymaktivitäten in Erythrozyten untrainierter Pferde deutlich geringer waren als bei Pferden im Leistungssport. Auch der Glukosetransport war bei Rennpferden höher als bei Pferden mit einer anderen Nutzung. Dies deutete darauf hin, daß Rennpferde eine schnellere Bereitstellung von Energie besitzen als untrainierte Pferde.

Schlüsselwörter: Blutglukose, D-Glukosetransport, Hexokinase, Pyruvatkinase, Rennpferd

Introduction

Changes of activities of plasma enzymes such as creatine kinase, lactate dehydrogenase and aspartate aminotransferase have been monitored to indicate fitness for training *(Milne* et al. 1976). These values can be good indicators of exercise stress, but are less effective in assessing the metabolic status. Glucose is the main energy source for animals and glycolysis is a major pathway to produce ATP in red blood cells without mitochondria. Activities of glycolytic enzyme may be good indicators to assess the metabolic status in race horses. These enzyme activities are controlled by insulin, glucose uptake or other factors. In the present study, we measured changes of blood glucose and plasma immunoreactive insulin (IRI) concentrations and D-glucose transport and glycolytic enzyme activities in erythrocytes of race horses within a day, and discussed the possibility to use these parameters as indicators for assessing the metabolic status of race horses.

Materials and methods

All horses studied were Thoroughbreds. Untrained horses in breeding farms in Hokkaido, Japan. Ten sires (10 to 15 years old), 8 mares (8 to 14 years old) and 8 untrained 2-year-old Thoroughbreds (4 female, 4 male) were examined. The race horses (2 year old; 3 female, 3 male) were kept for research and trained at the Horse Riding School, Japan Racing Association. Race horses were exercised for 6 days each week, resting on Sunday. All the horses were maintained on grass supplemented with good-quality hay and concentrate. Blood was withdrawn from the jugular veins of the untrained horses into tubes containing an appropriate amount of EDTA between 1100h and 1130h. In the race horses, blood was taken 6 times at 4 hour intervals from 0730h on a Sunday. Plasma was separated by centrifugation within 5 min after sampling at 4°C and stored at -80°C. The erythrocytes were washed twice with cold PBS. The washed erythrocytes were homogenized in 4 volumes of 10mmol/l Tris-HCl, pH 7.5, containing 1mmol/l EDTA and 0.25mol/l sucrose with ultrasonic processor. The plasma membrane fractions were isolated by the method of *Belsham* and colleagues (1980). The D-glucose transport (D-GT) activities in the plasma membrane fractions was measured by a modification of the *Robinson* and colleagues (1982). The D-GT activities were expressed as nmoles per min mg protein at 37°C. Part of the original homogenate of the erythrocytes was centrifuged at 100,000g for 30 min at 4°C. The resulting supernatant was used as the extract in which enzyme activities were measured using the following methods: hexokinase (HK, EC 2.7.1.1) *(Vinuela* et al. 1963), pyruvate kinase (PK, EC 2.7.1.40) *(Hess* and *Wieker* 1974) . All enzyme assays were conducted at 25°C. All enzyme activities were expressed as mU/mg (nmoles of NADPH or NADH produced per min per mg of protein). The concentration of glucose in whole blood was measured by a glucose oxidase method *(Huggett* and *Nixon* 1957). The concentration of plasma IRI was determined by a micro ELISA sandwich method *(Arai* et al. 1989). The concentration of protein in the plasma membrane fractions and enzyme extracts were determined by the method of *Bradford* (1976), using crystalline bovine serum albumin as the standard.

Each value was expressed as the mean±SD and the difference between group means were analyzed using Student's t-test.

Tab. 1: Concentrations of blood glucose and plasma immunoreactive insulin (IRI) and activities of D-glucose transport(D-GT) and glycolytic enzymes in erythrocytes of horses

	Sire(10)	Mare(8)	Untrained 2-year-old(8)	Race horse(6)
Glucose (mmol/l)	4.2±0.7	4.4±0.9	4.0±0.6	3.4±0.7
IRI (pmol/l)	162±48	120±24	138±36	120±48
D-GT (nmol/min/mg)	2.6±0.7*	2.5±0.9*	2.8±0.9*	6.2±2.1
HK (mU/mg)	0.5±0.1*	0.4±0.1*	0.5±0.1*	1.4±0.3
PK (mU/mg)	1.0±0.2*	1.0±0.2*	0.9±0.3*	3.8±0.5

Values are presented as mean±SD.
The number in parentheses indicate the number of horses examined.
*Significantly lower (p<0. 01) than the values of race horses.

Results

Table 1 shows the blood glucose and plasma IRI concentrations and activities of D-GT, HK and PK of erythrocytes of horses. The mean blood glucose and plasma IRI concentrations did not differ between the groups examined. The mean D-GT activities in the erythrocytes of the race horses were more than twice as high as those in the other groups of horses. The HK and PK activities in the erythrocytes from the race horses were significantly elevated compared to the other groups. Table 2 shows changes within a day in blood glucose and plasma IRI concentrations and D-GT, HK and PK activities of race horses. Blood glucose and plasma IRI concentrations suggested cyclic changes peaking at 0730h and a nadir at 1530h. However, the difference was not significant. The mean concentrations of glucose and IRI were constant throughout the day. The mean D-GT activities were maintained between 4.4 and 6.2 nmol/min/mg. HK and PK activities were 1.3 to 1.7 and 3.4 to 4.2 mU/mg, respectively.

Discussion

Glucose transport and glycolytic enzyme activities in erythrocytes or hepatocytes vary with species, and herbivorous animals have lower activities than omnivorous such as the dog *(Arai* et al. 1992). D-GT and HK activities in the

Tab. 2: Changes within a day in blood glucose and plasma IRI concentrations and D-GT and glycolytic enzyme activities in erythrocytes of 6 race horses

	0730	1130	1530	1930	2330	0330	0730
Glucose	4.7±0.3	3.4±0.7	3.1±0.4	3.2±0.4	3.2±0.3	3.4±0.5	4.3±1.1
IRI	180±66	120±48	102±30	144±60	102±30	156±24	192±66
D-GT	5.9±1.6	6.2±2.1	5.7±2.3	6.2±2.3	4.7±1.5	4.4±1.0	4.4±0.9
HK	1.3±0.2	1.4±0.3	1.7±0.4	1.7±0.3	1.4±0.3	1.3±0.4	1.5±0.3
PK	3.4±0.6	3.8±0.5	3.7±0.5	3.5±0.5	3.4±0.4	3.9±1.0	4.2±0.7

Values are presented as mean±SD.
Unit: glucose, mmol/l; IRI, pmol/l; D-GT, nmol/min/mg; HK and PK, mU/mg.

erythrocytes of the untrained horses were almost the same as that in cattle, whereas the activities in the race horses increased to the level found in dogs (*Arai* et al. 1995). The higher glucose transport and glycolytic enzyme activities in the erythrocytes from race horses compared to those from untrained horses under even resting conditions suggest that glucose utilization is accelerated significantly. The glucose transporter found in erythrocytes, GLUT1, is associated with the basal uptake of glucose (*Bell* 1991) and the GT activity in erythrocytes appears to reflect the basal metabolic status of glucose in the whole body. All the horses examined in the present study were Thoroughbreds and received approximately the same diet. There were no significant differences in blood glucose or plasma IRI concentrations between the race horses and the untrained horses. The primary cause of the significant increase in D-GT and glycolytic enzyme activities in the erythrocytes of the race horses was therefore considered to be an increased basal metabolic energy accelerated with continuous exercise training.

On the other hand, since glucose is an important energy source for all mammalian cells, blood glucose concentrations should be controlled strictly. Transport of glucose into the cells is the first step in its utilization (*Ciaraldi* et al. 1986), and its transport is catalyzed by an integral membrane protein called glucose transporter (*Kasahara* and *Hinkle* 1976). D-GT and glycolytic enzyme activities of the erythrocytes in race horses were maintained throughout the day, even at rest at higher levels than in untrained horses. However, changes in glucose or IRI concentrations did not parallel changes in D-GT and glycolytic enzyme activities. D-GT and glycolytic enzyme activities are maintained constantly in a day and are considered to be useful as one indicator to assess glucose metabolism in race horses.

Conclusion

The erythrocyte activities of D-GT, HK and PK in race horses were 2 to 3.5 times greater than for untrained horses. Blood glucose and plasma IRI concentrations and activities of D-GT and glycolytic enzyme of erythrocytes were constant throughout the day in race horses.

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