Equine Exercise Physiology 3: 239–244, 1991

Capillary Supply in Relation to Muscle Metabolic Profile and Cardiocirculatory Parameters

K. KARLSTRÖM, B. ESSÉN-GUSTAVSSON, A. LINDHOLM and S. G. B. PERSSON
Department of Medicine and Surgery, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

ABSTRACT. Twenty-eight Swedish Standardbred trotters (age 2–9 years) performed a standardized exercise tolerance test on a treadmill. The total blood volume per kg body weight (TBV kg^{-1}), speed producing a blood lactate concentration of 4 mmol l^{-1} (V_{La4}) and a heart rate of 200 bpm (V_{200}) were measured. Biopsies from m. gluteus medius were taken and analysed for capillary supply, fibre type composition and activities of citrate synthase (CS), 3-OH-acyl-CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH). Among the horses, capillary density varied 2-fold, the percentage of type I fibres 4-fold, enzyme activities 3-fold and cardiocirculatory parameters 1.6-fold. The mean area of type IIB fibres was 2 times larger and of type IIA fibres 1.3 times larger than that of type I fibres. Interrelationships were found between capillarization, muscle characteristics and cardiocirculatory parameters. Capillary density and capillarization of type IIB fibres correlated positively with TBV kg^{-1} ($p<0.01$), V_{200} ($p<0.01$), percentage of type I fibres ($p<0.001$) and negatively with LDH ($p<0.01$). No correlations were found with capillarization of type I and IIA fibres. It is concluded that the capillary supply of muscles, especially of type IIB fibres, is of importance for aerobic capacity and exercise tolerance in horses.

Key words Capillaries; fibre types; enzyme activities; muscle; exercise tolerance test; horses.

INTRODUCTION

During muscular work oxygen has to be delivered continuously to the mitochondria for aerobic energy production. The dimensions of the cardiocirculatory system and its capillary bed as well as the structure and metabolic profile of muscle will therefore limit the capacity for aerobic metabolism and work performance. Horses have a high potential for aerobic energy production as indicated by a large blood volume and a high maximal oxygen uptake as well as an abundance of mitochondria, high activities of oxidative enzymes and a large capillary supply in the muscles.[7,12,15] The aerobic capacity and cardiocirculatory dimensions of horses can be evaluated by means of exercise tolerance tests on a treadmill.[15,16] The use of the muscle biopsy technique and histochemical and biochemical analyses has made it possible to evaluate the structure and function of the muscle.[14,18]

Skeletal muscles are composed of fibres with different contractile and metabolic characteristics (type I, IIA and IIB). In horses, the oxidative enzyme activities and mitochondrial content have been shown to be highest in type I and IIA fibres, while the glycolytic enzyme activities are highest in type IIB fibres.[20]

Interrelationships between cardiocirculatory parameters and muscle characteristics have been demonstrated in horses, but capillary supply has not been taken into consideration.[19] The purpose of this study was to determine the relationships between capillarization, cardiocirculatory parameters and muscle characteristics in Standardbred trotters.

MATERIALS AND METHODS

Horses. Twenty-eight clinically healthy Swedish Standardbred trotters were included in this study: 6 mares, 5 geldings and 17 stallions, ranging in age from 2 to 9 years. All horses were in regular training programmes. Some of the 2 year olds were used for a study of training effects on young horses, others were brought by their trainers for a routine check on work performance. Total blood volume was within ±2 SD of the predicted normal value with regard to age and sex variation.[15]

A standardized incremental exercise tolerance test (SET) was performed on a high speed treadmill (Sikob, Sweden) with a 6.25% slope. The horses trotted for 2 min at each speed of 5, 6, 7 and 8 or 6, 7, 8 and 9 m s^{-1}, aiming at a final heart rate of about 200 bpm. The young, relatively untrained horses trotted at the lower speeds. The heart rate was recorded during the last 15 seconds at each speed (Mingograph 804, Siemens-Elema). Blood samples for lactate analysis were drawn from a jugular catheter at rest and during the last 15 seconds at each speed. Blood lactate analyses were performed using an enzymatic procedure (Boehringer, test combination No 124842). A dye dilution method (Evans Blue) was used to measure total blood volume.[15] The exact speed of the treadmill was determined by timing 10 revolutions, and V_{200} and V_{La4} were extrapolated.[16]

Muscle biopsies. Within a week of SET, a sample from m. gluteus medius was taken using the needle biopsy technique.[14] The sample was divided into two parts which were prepared for histochemistry and biochemistry. All samples were stored at −80°C until analysed.

Histochemical analyses. Serial sections were cut in a cryostat at −20°C. Myofibrillar ATPase staining after pre-incubation at pH 4.6 was employed to identify type I, IIA and IIB fibres, and the fibre type composition was calculated from a minimum of 200 fibres.[3] Capillaries were visualized using the amylase-PAS method[1] and values for capillaries per fibre (cap $fibre^{-1}$), capillary density (cap mm^{-2}), the mean number of capillaries in contact with fibres of each type (CC) and CC relative to fibre type area (CC μm^{-2} 10^{-3}) were calculated.[2] Areas were measured on photographs of the sections stained for capillaries with a MOP digiplan analyser (Kontron). The mean area framed for capillary counts was 0.5 mm^2 and contained an average of 180 fibres.

Enzyme analyses. The muscle was freeze-dried for 24 hours and dissected free from blood, fat and connective tissue. 1–2 mg of tissue was homogenized with an ultrasound disintegrator in ice cold potassium phosphate buffer (0.1 M, pH 7.3) at a dilution of 1:400. Activities of citrate synthase (CS), 3-OH-acyl-CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH) were analysed using fluorometric methods.[4,5]

Statistical analyses. Conventional statistical methods were employed to calculate means, standard deviations and correlation coefficients using MacIntosh Statview 512+.

RESULTS

Means, standard deviations and ranges for capillarization, muscle characteristics and cardiocirculatory parameters are presented in Table 1. Among the 28 horses capillary density varied 2-fold, the percentage of type I fibres 4-fold, enzyme activities 3-fold and cardiocirculatory parameters 1.6-fold (Table 1, Fig. 1).

Only small differences were seen in the CC of the fibre types. The mean area of type IIB fibres was 2 times larger and of type IIA fibres 1.3 times larger than that of type I fibres. Type IIB fibres had a 1.6-fold larger mean area than type IIA fibres. CC relative to fibre area was higher for type I (70%) and type IIA fibres (50%) than for type IIB fibres. Correlations between capillary supply and muscle fibre type composition, enzyme activities and cardiocirculatory parameters are shown in Table 2. Capillary supply corre-

Table 1. *Mean, standard deviation and range of capillarization, mean fibre type area, fibre type distribution, activities of citrate synthase (CS), 3-OH-acyl-CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH), total blood volume per kg body weight (TBV kg^{-1}), speed producing a lactate concentration of 4 mmol l^{-1} (V_{La4}) and speed at a heart rate of 200 bpm (V_{200}) for Standardbred trotters (n=number of horses)*

	Mean	± SD	Range	*n*
Cap mm^{-2}	725	138	486–1031	28
Cap fibre^{-1}	2.00	0.33	1.00–2.49	28
Contact capillaries per fibre of each type (CC)				
I	5.0	0.7	3.6–6.2	27
IIA	5.6[a]	0.7	4.0–7.0	27
IIB	5.9[ab]	0.9	4.1–7.4	27
Fibre type area, μm^2				
I	1947	394	1136–3026	27
IIA	2457[a]	352	1777–3262	27
IIB	3925[ab]	744	2872–5980	27
CC relative to fibre type area, μm^{-2} 10^{-3}				
I	2.60	0.36	1.80–3.30	27
IIA	2.30[a]	0.30	1.69–2.88	27
IIB	1.54[ab]	0.35	1.07–2.33	27
Fibre type distribution, %				
I	20	8	9–38	28
IIA	46	7	34–58	28
IIB	35	10	10–50	28
Enzyme activities, mmol kg^{-1} min^{-1}				
CS	55	19	27–96	23
HAD	28	10	18–51	23
LDH	1404	278	649–1743	22
TBV kg^{-1} ml kg^{-1}	120	16	97–154	28
V_{La4}, m s^{-1}	8.96	1.22	7.42–12.12	27
V_{200}, m s^{-1}	8.16	1.19	5.84–10.11	28

[a] Significantly different from type I fibres.
[b] Significantly different from type IIA fibres.

lated positively with TBV kg^{-1}, V_{La4}, V_{200}, the percentage of type I fibres, CS and HAD and negatively with LDH and the percentage of type IIB fibres. There were significant correlations ($p<0.001$) with age for cap mm^{-2} ($r=0.77$) and CC relative to type IIB fibre area ($r=0.71$). When the correlations of the capillarization expressed in relation to fibre size (CC μm^{-2} 10^{-3}) were considered, the most significant correlations were seen with the type IIB fibres.

DISCUSSION

The relationships between capillarization and muscle oxidative capacity in this study are in accord with earlier findings in man and various animals.[7,10,11,13,17] There is also good agreement with other reported values for horse muscle regarding cap mm^{-2} and CC.[8,9]

The capillarization of muscle can be expressed in different ways, such as cap fibre^{-1}, cap mm^{-2}, CC and CC in relation to

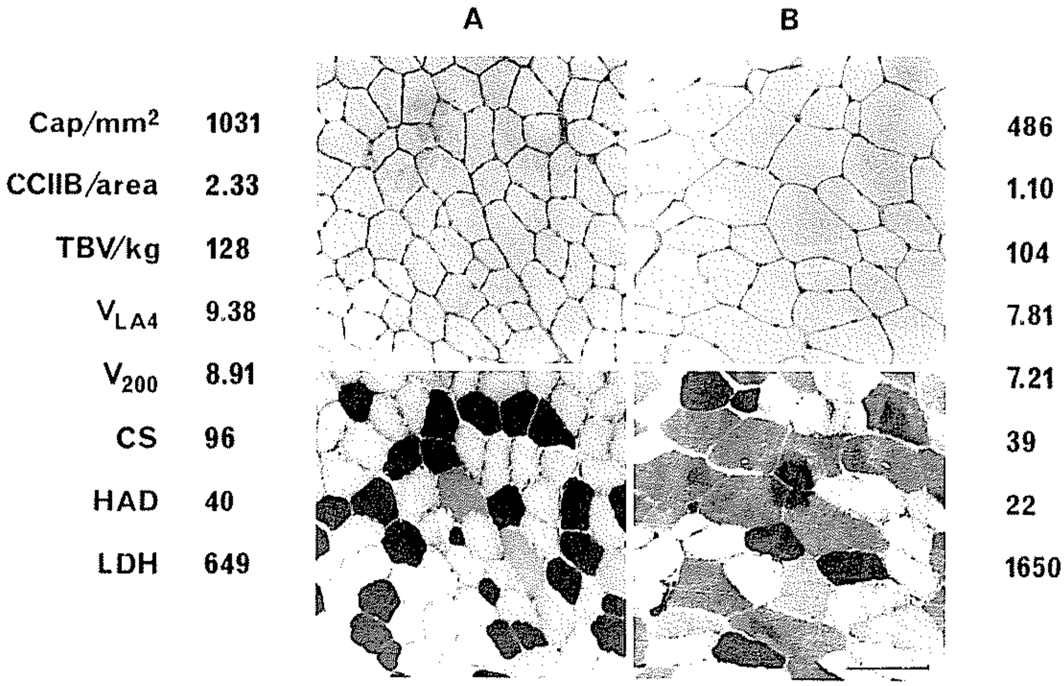

Fig 1 Capillary (top) and ATPase pH 4.6 (bottom) stainings from the horses with the highest (A) and lowest (B) capillary supply. On each side are shown individual values for capillarization, cardiocirculatory parameters and muscle enzyme activities. Abbreviations as in Table 1. Scale = 100 µm.

fibre type area. Cap fibre^{-1} does not take fibre size into account and is therefore of limited value as no information can be obtained about diffusion distances. A high cap mm^{-2} indicates small diffusion distances and an ample supply of oxygen and substrates to, as well as elimination of waste products such as lactate and heat from the muscle. One limitation of cap mm^{-2}, however, is that it gives no information about the

Table 2. *Correlations between capillary supply and muscle fibre type composition, enzyme activities and cardiocirculatory parameters*

Abbreviations as in Table 1

	% I	% IIA	% IIB	CS	HAD	LDH	TBV kg^{-1}	V_{La4}	V_{200}
Cap mm^{-2}	0.70***	0.15	−0.63***	0.63**	0.56**	−0.59**	0.52**	0.39*	0.59***
Cap fibre^{-1}	0.53**	0.17	−0.51**	0.42*	0.36	−0.43*	0.48**	0.41*	0.48**
CC relative to fibre type area									
I	0.45	−0.02	−0.30	0.28	0.31	−0.37	0.09	0.29	0.19
IIA	0.20	−0.17	−0.03	0.05	0.04	−0.54*	0.05	0.25	0.12
IIB	0.62***	0.03	−0.44*	0.42	0.46*	−0.73***	0.51**	0.46*	0.52**

$p<0.05$*, $p<0.01$**, $p<0.001$***.

capillarization of the different fibre types. More detailed information about capillarization can be derived from CC relative to fibre type area, as the supply to each fibre type is studied. From the results in this study it can be concluded that fibre type and fibre size are important for capillarization because CC of the different fibre types are quite similar, but when fibre size is taken into account a marked difference in capillarization is established.

It is noteworthy that the area of type IIB fibres is twice as large as the area of type I fibres. Even though the number of capillaries surrounding type IIB fibres is somewhat higher than for the other fibre types, the supply of oxygen and substrates to these fibres will be smaller because of their larger area. Accordingly, capillary supply will be poor in muscles having many large type IIB fibres while muscles with many small type I fibres will have an abundant capillary supply, as can be seen from Fig. 1. Therefore it is not surprising to find that cap mm^{-2} and cap $fibre^{-1}$ correlate positively with the percentage of type I fibres and negatively with that of type IIB fibres. A high percentage of type I fibres in a muscle will result in a higher frequency of type I and IIB fibres being in contact. The capillaries surrounding type I fibres are less spread apart because of the smaller area of this fibre type (Table 1). Consequently, type I fibres lying next to IIB fibres result in increased capillarization of the IIB fibres. This could explain the strong correlation between CC relative to IIB fibre area and the percentage of type I fibres. Type I fibres are more oxidative than type IIB fibres, which, in turn, have a greater glycolytic capacity than type I fibres. Therefore, the correlations of CC relative to IIB fibre area with the percentage of type I fibres and enzyme activities are interrelated.

There were almost no correlations with capillarization of type I and IIA fibres, whereas the capillary supply of IIB fibres correlated with most parameters (Table 2). The correlation found between age and CC relative to IIB fibre area, which also includes an element of training status, could hold an explanation for this. Due to the differences in the age of the horses, their time in training will obviously be different. It has been shown in a number of animals with differing life patterns that a higher level of activity causes higher capillary supplies in muscles.[7] On comparing active and inactive horses, it was found that the active horses had smaller type IIB areas than the inactive ones.[6] In this study it is not possible to distinguish between the effect of age and that of training, but it seems as though the primary activity-related adaptation in the muscle is that the type IIB fibres become smaller and thereby more capillarized. The characteristics of this fibre type will therefore have a greater influence on the other parameters than types I and IIA. V_{200} primarily reflects the oxygen conveying capacity while TBV kg^{-1} is a marker for both the dimensional capacity of the cardiovascular system and the oxygen transport capacity.[16] Further, both TBV kg^{-1} and capillarization are dependent on training, which induces an increase in aerobic potential.[1,2,8,11,15] Consequently, the relationship with the capillary density of the skeletal muscles is to be expected. The influence of CC relative to IIB area on these parameters might not only be caused by the higher delivery of oxygen and substrates to the IIB fibres, but also by the facilitated efflux of lactate, ammonia and heat from them. Type IIB fibres have a high glycolytic capacity and may, therefore, have a high production of lactate during work. With an easier removal of waste products, the detrimental effects caused by a lowered pH might be postponed, thereby improving the work performance of the horse.

It can be concluded that relationships ($p<0.01$) exist between the capillary supply of skeletal muscle and the functional and dimensional capacities of the cardiocirculatory system as well as structural and biochemical properties of the muscle. It was found that the capillarization of type IIB fibres seems to be of importance for the aerobic capacity and exercise tolerance in horses.

This was shown by the significant relationships ($p<0.01$) to the cardiocirculatory parameters.

ACKNOWLEDGEMENTS

The present study was supported by grants from the Swedish Council for Forestry and Agricultural Research and the Swedish Racing Board (ATG).

REFERENCES

1. Andersen, P (1975). Capillary density in skeletal muscle of man Acta Physiol Scand 95, 203–205.
2. Andersen, P and Henriksson, J. (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise J Physiol., Lond 270, 677–690.
3. Brooke, M. H and Kaiser, K. K (1970) Muscle fibre types: How many and what kind? Arch Neurol 23, 369–379.
4. Essén, B., Lindholm, A and Thornton, J (1980). Histochemical properties of muscle fibre types and enzyme activities in skeletal muscles of Standardbred trotters of different ages. Equine Vet J 12, 175–180.
5. Essén-Gustavsson, B, Karlström, K and Lindholm, A (1984). Fibre types, enzyme activities and substrate utilisation in skeletal muscles of horses competing in endurance rides. Equine Vet J. 16, 197–202
6. Essén-Gustavsson, B and Lindholm, A (1985) Muscle fibre characteristics of active and inactive Standardbred horses. Equine Vet J. 17, 434–438
7. Essén-Gustavsson, B (1986) Activity- and inactivity-related muscle adaptation in the animal kingdom In Saltin, B. (ed.): *Biochemistry of Exercise VI*, Human Kinetic Publishers, Champaigne, IL, pp 435–444
8. Henckel, P (1983). Training and growth induced changes in the middle gluteal muscle of young Standardbred trotters. Equine Vet. J. 15, 134–140
9. Hodgson, D R., Rose, R J, Dimauro, J. and Allen, J. R (1985) Effects of a submaximal treadmill training programme on histochemical properties, enzyme activities and glycogen utilisation of skeletal muscle in the horse. Equine Vet J 17, 300–305.
10. Hoppeler, H, Mathieu, O, Weibel, E R., Krauer, R., Lindstedt, S. L and Taylor, C R (1981). Design of the mammalian respiratory system VIII. Capillaries in skeletal muscles Respiration Physiology 44, 129–150
11. Hoppeler, H., Howald, H, Conley, K., Lindstedt, S L, Claassen, H., Vock, P and Weibel, E R (1985). Endurance training in humans: aerobic capacity and structure of skeletal muscle J Appl Phys 59, 320–327
12. Hoppeler, H, Jones, J H, Lindstedt, S L., Claassen, H, Longworth, K. E, Taylor, C R, Straub, R and Lindholm, A (1987) Relating maximal oxygen consumption to skeletal muscle mitochondria in horses *In* Gillespie, J R and Robinson, N E (eds.): *Equine Exercise Physiology 2*, ICEEP Publications, Davis, CA, pp. 278–289.
13. Hudlicka, O., Hoppeler, H and Uhlmann, E (1987). Relationships between the size of the capillary bed and oxidative capacity in various cat skeletal muscles Eur. J Physiol. 410, 369–375
14. Lindholm, A and Piehl, K (1974) Fibre composition, enzyme activity and concentrations of metabolites and electrolytes in muscles of Standardbred horses Acta Vet Scand. 15, 287–309.
15. Persson, S G. B. (1967) On blood volume and working capacity in horses Acta Vet Scand Suppl 19, 1–189.
16. Persson, S. G B (1983). Evaluation of exercise tolerance and fitness in the performance horse. *In* Snow, D H, Persson, S G B and Rose, R J (eds.): *Equine Exercise Physiology*, Granta Editions, Cambridge, pp 441–457
17. Romanul, F. C A (1965) Capillary supply and metabolism of muscle fibres. Arch Neurol 12, 497–509.
18. Snow, D H (1983) Skeletal muscle adaptions: A review *In* Snow, D. H., Persson, S. G B. and Rose, R J (eds): *Equine Exercise Physiology*, Granta Editions, Cambridge, pp 160–183.
19. Valberg, S., Essén-Gustavsson, B., Lindholm, A and Persson, S G B (1985). Energy metabolism in relation to skeletal muscle fibre properties during treadmill exercise Equine Vet J. 17, 439–444.
20. Valberg, S and Essén-Gustavsson, B (1987) Metabolic response to racing determined in pools of type I, IIA and IIB fibres *In* Gillespie, J R and Robinson, N. E (eds.): *Equine Exercise Physiology 2*, ICEEP Publications, Davis, CA, pp 290–301