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Takashi Abe

Charles F. Kearns

Hélio C. Manso Filho

Yoshiaki Sato

Margaret M. Sleeper
University of Pennsylvania, sleeper@vet.upenn.edu

See next page for additional authors

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Abstract
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Keywords
muscle thickness, plasma lactate, packed cell volume, kaatsu-training

Disciplines
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Author(s)
Takashi Abe, Charles F. Kearns, Hélio C. Manso Filho, Yoshiaki Sato, Margaret M. Sleeper, and Kenneth H. McKeever

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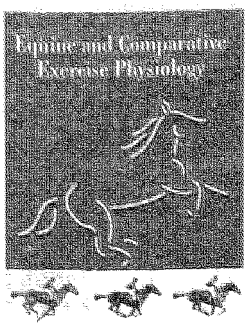

Acute vascular occlusion in horses: effects on skeletal muscle size and blood flow

T Abe[1], CF Kearns[2], HC Manso Filho[3], Y Sato[4], M Sleeper[5] and KH McKeever[3]

[1]Department of Exercise and Sport Science, Tokyo Metropolitan University, 1-1 Minami-Ohsawa, Hachioji, Tokyo 192-0397, Japan
[2]Cardiovascular/Endocrine Biology, Schering-Plough Research Institute, Kenilworth, NJ, USA
[3]Equine Science Center, Department of Animal Sciences, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA
[4]Sato Institute for Rehabilitation and Fitness, Fuchu, Tokyo, Japan
[5]Ryan Veterinary Hospital, University of Pennsylvania, Philadelphia, PA, USA
Corresponding author: abebe@comp.metro-u.ac.jp

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Abstract

The purpose of this study was to demonstrate whether acute vascular occlusion was safe and if it would result in changes to limb muscle size in horses. Six healthy, unfit Standardbred mares were used. Horses (standing at rest) wore an occlusion cuff at the most proximal position of the left forelimb. The right forelimb was used as control. An occlusion pressure of 200 mmHg was set for 5 min followed by a 2 min recovery. Three sets of occlusions were given to each horse. Muscle thickness was measured using B-mode ultrasound. The circumference of the forelimb and first phalanx was measured using a flexible tape measure. Pulsed-wave Doppler was performed on the *radialis* artery with a 5–10 MHz mechanical transducer at baseline and at each occlusion. Peak flow velocity (PFV) and the flow velocity integral (FVI) were measured each time. Mid-forelimb, but not first phalanx, girth was increased ($P < 0.05$) in the occluded but not in the control leg following occlusion. Extensor and flexor muscle thickness was increased ($P < 0.05$) in the occluded but not in the control leg. There were no changes ($P > 0.05$) in PFV or FVI at any measurement time point. Acute vascular occlusion may be a suitable and safe model for studying muscle hypertrophy in horses.

Keywords: muscle thickness; plasma lactate; packed cell volume; Kaatsu-training

Introduction

Vascular occlusion has been shown to cause muscle growth/enlargement in humans and has been suggested as being beneficial to those populations who could not otherwise tolerate high-intensity training[1,2]. Orthopaedic problems are the primary cause of poor race performance in horses and also prevent the equine athlete from training. Vascular occlusion may be beneficial to horses not only as a preventative measure, but also when they cannot tolerate high-intensity exercise such as during injury. However, occlusion reperfusion is thought to be a potential cause of founder; therefore the safety of vascular occlusion in horses needs to be determined. The purposes of the present study were to demonstrate whether vascular occlusion (1) would result in changes to limb muscle size in horses and (2) would not detrimentally affect hoof blood flow, and thereby increase the risk of founder.

Methods

Animals

All methods and procedures were conducted with the prior approval of the Rutgers University Institutional Review Board for the Care and Use of Animals.

Six healthy, unfit Standardbred mares (age 14 ± 4 years, body mass 529 ± 49 kg, mean ± standard deviation) were used for the study. During the experiment, the mares were housed in 3 m × 3 m stalls and fed a diet of commercially available grain pellet (Brown's and Sons, Inc., Birdsboro, PA) and alfalfa/grass hay. Water and salt were given *ad libitum*.

General experimental design

Before the experiments, horses were trained to wear an occlusion cuff at the most proximal position of the forelimb. During the acclimatization period, the occlusion pressure (130–160 mmHg) was selected with regard to their resting blood pressure and after reviewing the human data[1–3]. Neither heart rate nor respiration rate was changed by this occlusion pressure (data not shown). The experiment was composed of two parts: blood flow and muscle/girth measurements. The total period of this experiment was for 5 days, including the 2 days of acclimatization.

Experiment 1

Changes in limb girth and muscle thickness were measured following vascular occlusion. Horses (standing at rest) wore an occlusion cuff at the most proximal position of the left forelimb. The right forelimb was used as control. The set of occlusive stimuli consisted of an occlusion for 5 min followed by the removal of occlusion pressure for 2 min. Three sets of occlusions were given to the horses. During the occlusion session the occlusive pressure was set at 200 mmHg (range: 195 to 205 mmHg). The occlusion pressure, duration of the stimulus and rest time are based on similar protocols in humans[1–3].

Experiment 2

The effect of vascular occlusion on arterial blood flow was tested. The occlusion cuff procedure was the same as in experiment 1. Measurements were taken during the final 2–3 min of each occlusion and 10 min following the last occlusion.

Measurements of muscle thickness and circumference

Ulnaris lateralis/flexor digitorum profundus (UL-FDP) and *extensor digitorum communis* (EDC) muscle thicknesses were measured using B-mode ultrasound (Aloka SSD-250, Tokyo, Japan)[4]. The measurement site for the UL-FDP and EDC was midway between the olecranon and the accessory carpal bone. Measurements were made using a 5 MHz scanning head that was coated with vegetable oil and was placed perpendicular to the tissue interface. The subcutaneous adipose tissue–muscle interface and the inter-muscular interface were identified from the ultrasonic image. The distance from the adipose tissue–muscle interface to the inter-muscular interface was accepted as the muscle thickness. The coefficient of variation (CV) for the measurement of muscle thickness with this method, calculated from test–retest (six samples), was 1.0%.

Forelimb circumference at the midpoint between the olecranon and the accessory carpal bone was measured using a flexible tape measure. Circumference of the first phalanx was measured by the same technique. The calculated CV for the measurement of forelimb and first phalanx circumference was 0.6%.

Blood chemistry

Blood samples (2–3 ml) were taken from the jugular vein and the cephalic vein of the forelimb before, at the end of the each 5-min occlusion and 15 min after the last occlusion. Blood samples were placed in prechilled tubes containing lithium heparin and placed on ice. One tube was used to measure packed cell volume and total plasma protein concentration. The other samples were centrifuged at 4°C and the plasma collected and stored at −80°C. Packed cell volume and total plasma protein concentration were measured in duplicate using the microhaematocrit technique and refractometry, respectively. Plasma lactate concentration was measured in triplicate using a lactate analyser (Sport 1500; YSI, Inc., Yellow Springs, OH)[5]. The CV for the measurement of lactate, calculated from test–retest (five samples), was 1.7%.

Measurements of arterial blood flow

The *radialis* artery was located medial to the accessory carpal bone with two-dimensional real-time ultrasonography using a Caris ultrasound machine (Bedford Falls, NY). Pulsed-wave Doppler was performed with a 5–10 MHz mechanical transducer three times at baseline and twice during each occlusion. A final measurement was also obtained 2–3 min after removal of the cuff. Heart rate, peak flow velocity (PFV) and the flow velocity integral (FVI) were measured each time[6]. Doppler ultrasonography measures blood flow velocity by using the change in frequency of the ultrasound beam that is reflected back to the transducer from moving blood cells (Doppler Principle).

Statistical analysis

Results are expressed as mean ± standard error. Analysis of variance (ANOVA) was used to determine any significant differences in muscle thickness or packed cell volume before and after occlusion measures. For the measures of arterial blood flow and lactate, an ANOVA with test for repeated measures was used. The *a priori* level of statistical significance was set at $P < 0.05$ for all tests.

Results

Limb girth and muscle thickness

Mid-forelimb circumference of the control limb did not change ($P > 0.05$). In the occluded forelimb, the circumference increased ($P < 0.05$) gradually from the first to the third occlusion (Fig. 1). The increased limb size was maintained for 15 min following removal of the occlusion. There was no change ($P > 0.05$) in circumference of the first phalanx during or after occlusion sessions in either forelimb (occlusion and control). In the occluded leg, muscle thickness increased ($P < 0.01$) by 16.2% and 5.5% in the extensor (EDC muscle) and flexor (UL-FDP muscle), respectively, following occlusion (Fig. 2a and 2b). Muscle thickness did not change ($P > 0.05$) in the control limb.

Blood chemistry

Plasma lactate level in the forelimb was three times higher than in the jugular (Fig. 3a). There was no change ($P > 0.05$) in the jugular plasma lactate level during the three occlusions and 15 min after removal of the occlusion. However, plasma lactate in the forelimb was higher ($P < 0.05$) at the end of the first and second occlusions compared with pre-occlusion. Following the third occlusion, the forelimb lactate level was not different ($P > 0.05$) from the pre-occlusion lactate level. Plasma lactate 15 min after removal of the third occlusion was not different ($P > 0.05$) from pre-occlusion lactate levels.

Packed cell volume increased ($P < 0.05$) at the end of the third occlusion in the jugular and returned to pre-occlusion level 15 min following occlusion (Fig. 3b). Plasma total protein (data not shown) concentration was not changed ($P > 0.05$) by the occlusive stimulus, although the value in the forelimb was higher ($P < 0.05$) than that in the jugular.

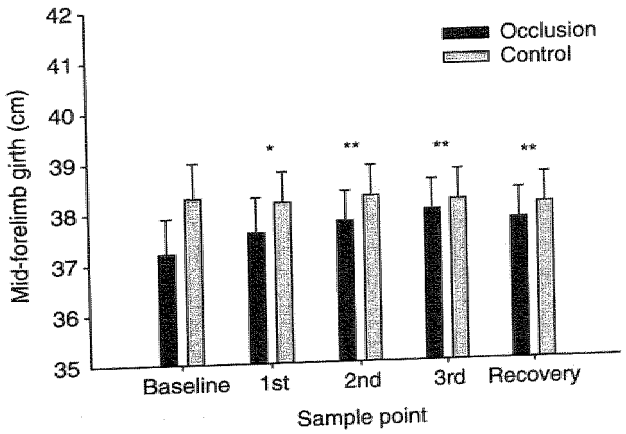

FIG. 1 Girth measurements of the occluded and control forelimbs, taken at the midpoint between the olecranon and the accessory carpal bone $^{**}P < 0.05$, $^{*}P < 0.01$

Doppler blood flow

There were no significant changes in Doppler-measured FVI or PFV in the blood flow of the *radialis* artery at any measurement time point (Table 1). Heart rate was also unchanged ($P > 0.05$) at all measurement time points (Table 1).

Discussion

The primary finding of the present study is that acute vascular occlusion produced an increased limb circumference and muscle thickness but did not result in any abnormalities in blood flow likely to result in possible reperfusion injuries. These data indicate that vascular occlusion represents a safe model that may be used in horses.

Doppler-derived blood flow measurements did not reveal any significant changes in arterial blood flow distal to the occlusion, either during or after the occlusion. There was a mild, but non-significant, increase in FVI and PFV following the first occlusion, with all measurements returning to their pre-occlusion state

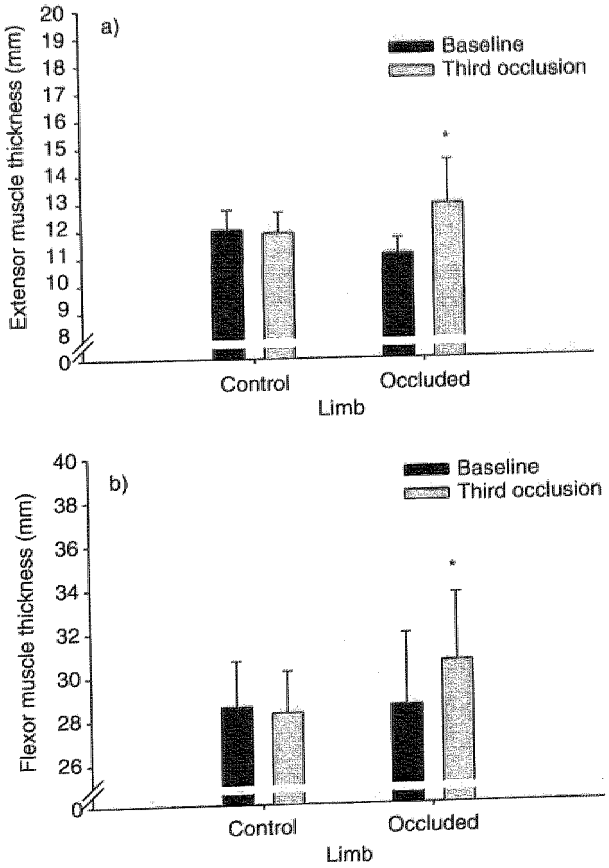

FIG. 2 Ultrasound-measured muscle thickness of (a) the *ulnaris lateralis/flexor digitorum profundus* (UL-FDP) and (b) the *extensor digitorum communis* (EDC). Measurement site for the UL-FDP and EDC was midway between the olecranon and the accessory carpal bone, taken before the first occlusion and immediately following the third occlusion $^{*}P < 0.01$

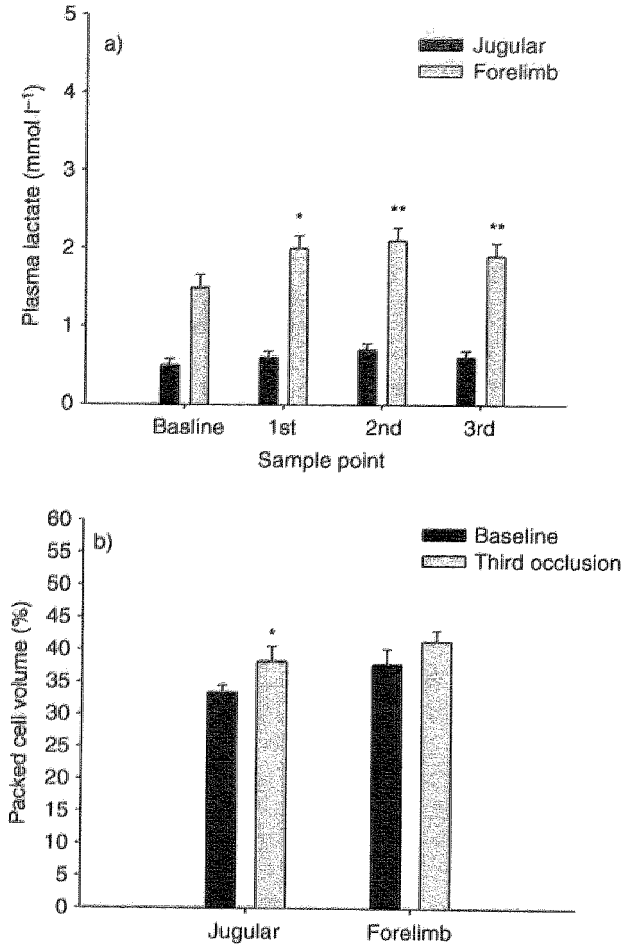

FIG. 3 Haematological variables. (a) Plasma lactate concentration in the jugular vein and the forelimb taken during the three successive occlusion stimuli. (b) Packed cell volume taken in the jugular vein and the forelimb before the first occlusion and immediately following the third occlusion $^{*}P < 0.05$, $^{**}P < 0.01$

for each subsequent measurement. These findings suggest that perfusion is maintained to the distal limb in spite of the occlusion. In humans using an occlusion pressure of 110 mmHg, it is believed that while this increase in local pressure may cause pooling in the capacitance vessels and decrease blood flow[7], it is not sufficient to completely suppress arterial blood flow distal to the cuff. Therefore, the blood flow results of the present study imply that the occlusion pressure of 200 mmHg in the horse is unlikely to result in significant blood flow aberrations, and so inflammatory side-effects in the lower limb (such as laminitis) are unlikely.

Table 1 Effect of moderate vascular occlusion on Doppler-derived arterial blood flow and heart rate

	FVI (m)	PFV ($m\,s^{-1}$)	HR (beats/min)
Baseline	0.10 ± 0.01	0.38 ± 0.04	36 ± 3
Occlusion (200 mmHg)	0.10 ± 0.01	0.37 ± 0.04	34 ± 1

FVI – flow velocity integral; PFV – peak flow velocity; HR – heart rate. Values are expressed as mean ± standard error.

The aetiology of equine laminitis is not well understood, nor is there a consensus in the data regarding its cause[8]. Studies assessing lactate acidosis following carbohydrate feeding have reported much higher (~8 times larger) central lactate levels[9,10] than reported in the current study. Also, we are unaware of any published investigations regarding equine laminitis that have reported plasma lactate in the digital blood. It is believed, however, that ischaemia of the digital digit and resulting oedema play a role in equine laminitis[8]. There were no changes in limb girth measured at the first phalanx of the occluded limb. Taken together with lack of change in Doppler-measured blood flow or change in central lactate, these data would indicate that the vascular occlusion stimulus of the present study should not result in equine laminitus.

To our knowledge, this is the first paper to measure muscle thickness following acute vascular occlusion in horses and/or humans. The resultant change in girth (~3%) was similar to that seen in human lower limbs (2–3%) after a single occlusion of similar pressure[3]. In humans, the increased muscle girth is maintained for a couple of hours (approximately 50% of peak thickness, 1.5 h post-occlusion) after a single occlusion[3]. In the present study, the muscle thickness and circumference were measured only 15 min following the release of the final occlusion. However, the increased muscle thickness may persist for a couple of hours following the occlusion. Most likely the change in limb size and muscle thickness was related to a fluid shift that resulted due to an increase in the local pressure. Care must be taken while interpreting these data, however, since it is not known whether these acute physiological responses seen in the present study will result in limb muscle hypertrophy following repeated occlusion (analogous to chronic training) in horses. Therefore further experiments are warranted.

In the present study, there was an increase in packed cell volume following acute vascular occlusion. Two possible explanations exist. First, the increase in local pressure may have resulted in plasma extravasation. The increases in limb girth and muscle size are consistent with fluid shifts into the muscle and/or interstitial area. The loss of circulating fluid into the muscle could then produce a haemoconcentration. Alternatively, in response to cardiovascular stressors, horses respond by mobilizing their reserve of red blood cell-rich splenic blood into the cardiovascular system[11]. Catecholamines were not measured and it is not inconceivable that splenic contraction may have occurred in response to the occlusion. The difference in local *versus* centrally drawn blood is most likely due to a dilution of the haemoconcentrating fluid shifts happening at the muscle below the

occlusion. Since occlusion caused an increase in limb muscle thickness and limb lactate, these data suggest that perturbations to the local environment were taking place.

Conclusion

Acute limb occlusion produces significant limb muscle thickness increases in horses but does not result in any blood flow abnormalities that may result in possible reperfusion injuries. Therefore, vascular occlusion may be a suitable and safe model to use in horses. In addition, vascular occlusion has been shown to be beneficial to those populations who could not otherwise tolerate high-intensity training. Since musculoskeletal (orthopaedic) problems are a prime cause of poor race performance in horses, vascular occlusion may have significant therapeutic/rehabilitative value for the equine athlete.

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