Acute Vascular Occlusion in Horses: Effects on Skeletal Muscle Size and Blood Flow

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
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

Disciplines
Large or Food Animal and Equine Medicine | Medicine and Health Sciences | Rehabilitation and Therapy | Veterinary Medicine

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 Abe¹, CF Kearns², HC Manso Filho³, Y Sato⁴, M Sleeper⁵ and KH McKeever⁴

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

Corresponding author: abebe@comp.metro-u.ac.jp

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Abstract
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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 training¹,². 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.

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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. 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.

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 initial 2–3 min of each occlusion and 10 min following the last occlusion.

Measurements of muscle thickness and circumference
Ulnaris lateralis/flexor digitorum profundus (ULFDP) and extensor digitorum communis (EDC) muscle thicknesses were measured using B-mode ultrasound (Aloka SSD-250, Tokyo, Japan). The measurement site for the ULFDP 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 pre-chilled 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). 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. 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.
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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.

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. 1](image1.png)

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

![Fig. 2](image2.png)

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. ***$P < 0.01$**
The aetiology of equine laminitis is not well understood, nor is there a consensus in the data regarding its cause. Studies assessing lactate acidosis following carbohydrate feeding have reported much higher (~8 times larger) central lactate levels 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. 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 laminitis.

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. 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. 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.

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

<table>
<thead>
<tr>
<th></th>
<th>FVI (m)</th>
<th>PFV (m s⁻¹)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.10 ± 0.01</td>
<td>0.38 ± 0.04</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>Occlusion</td>
<td>0.10 ± 0.01</td>
<td>0.37 ± 0.04</td>
<td>34 ± 1</td>
</tr>
</tbody>
</table>

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

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
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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.

Acknowledgements

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