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Behavioral studies in healthy Standardbred Trotters subjected to short term forced recumbency aiming at an adjunctive treatment in an acute attack of laminitis

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FOREWORD

Firstly, I would like to express my deep gratitude and appreciation to my supervisor Ove Wattle for guidance, inspiration and enthusiasm during the years it took to complete this work.

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SUMMARY

Laminitis is a debilitating disease causing much suffering to horses and ponies all over the world. It is manifested in the hooves as a mechanical failure of the suspensory apparatus between the hoof capsule and the distal phalanx. Due to the weight of the horse the disease may result in a dislocation of third phalanx.

Six healthy standardbred trotters were used in this study on whether standardbreds tolerate forced recumbency as well as Shetland ponies. Recumbency was induced pharmacologically but maintained only by lowering the ceiling height of the box to a height of approximately 125 – 140 % of the horse's thoracic height. Behavior and bodily functions were observed and recorded during and after the study periods of up to two days. Blood was sampled for serum analyses of CK and ASAT to detect muscle tissue damage.

The results of this study indicate that the increased body weight of Standardbred Trotters when compared to Shetland ponies does not have a negative effect on bodily functions during a limited period of recumbency. All horses accepted the treatment well and no adverse effects were seen during or after the study.

SAMMANFATTNING

Fång är en vanlig sjukdom som ofta medför ett stort lidande för den drabbade hästen och inte sällan resulterar i att individens framtida användbarhet påverkas radikalt eller att hästen behöver avlivas av djurskyddsskäl. Sjukdomen medför en hållfasthet – belastningsproblematik i det att det vävnadslager, lamellagret, som binder ihop hovväggen med hovbenet av någon anledning blir försvagat så att det inte längre klarar av att bära hästens vikt. Detta resulterar i att vävnaden slits sönder och hovbenet, som bär hästens vikt, sjunker/roterar ner mot marken. Det har föreslagits att hästar som inte bär vikt på sina hovar under det akuta skedet kan undvika en lägesförändring av hovbenet och därigenom få en betydligt bättre prognos för tillfrisknad.

I denna studie undersöktes om friska varmblodiga travhästar, psykiskt och fysiskt, klarar att ligga i en välhalmad box med sänkt tak i upp till 2 dygn. De 6 hästarna lades omkull med hjälp av dissociativ anestesi och därefter sänktes taket ner till 125 – 140 % av respektive hästs bröstorgshöjd. Beteende och kroppsfunktioner studerades kontinuerligt. För bedömning av muskelpåverkan togs blodprov innan hästen gick in i boxen, direkt efter det att den rest sig upp och 1-2 dagar efter boxvistelsen och blodserumets nivåer muskelenzymerna CK och ASAT analyserades.

I överensstämmelse med resultaten i en tidigare studie, som utfördes på shetlandsponnier, bedömdes travhästarnas kroppsfunktioner inte påverkas negativt av att de hålls liggande under en begränsad tidsperiod. Alla försökshästarna accepterade behandlingen väl och inga allvarliga komplikationer uppstod under eller efter försöksperioderna.

INTRODUCTION

Laminitis is a debilitating disease causing much suffering to horses and ponies all over the world. It is manifested in the hooves as a failure of the suspensory apparatus between the hoof capsule and the distal phalanx (Pollitt 1999). The pathophysiology is complex and still not completely understood (Eades 2010). There are numerous theories on etiologies behind the disease, but only a few have proven effective and reproducible in experimental models (Heymering 2010).

Laminitis has been divided into three phases; developmental, acute and chronic (Hood and Stephens 1981). The developmental phase is defined as the time passing between the initial exposure to the laminitis causing agent(s) and the first clinical sign of lameness. The acute phase begins with the first sign of lameness and last either until 72 hours of lameness has passed without mechanical failure or until there are clinical and/or radiological evidence of displacement of the distal phalanx and thereby progression into the chronic phase (Hood 1999a).

The earliest histopathological changes in laminitis are seen in the basal cells of the epidermis (Linford 1987; Obel 1948; Wattle 2000). These early changes are seen not only in the basal cells of the lamellar layer, but also in the periople, hoof wall and chestnut (Obel 1948; Wattle 2000; Ekfalk 1991). Changes in the lamellar corium appear later in the course of the disease, in areas of epidermal damage (Linford 1987; Obel 1948; Wattle 2000). There are no histopathological changes in the corium of the non-weight-bearing epidermal tissues (Obel 1948; Wattle 2000).

Even though the degree of tissue damage can vary in different hooves from the same horse (Pollitt 1996) and at different sampling levels of one digit (Wattle 2000), the lamellar layer of all four hooves is always affected by laminitis with a systemic origin (Wattle 2000). When standing, normal horses distribute more weight on their front than their hind limbs, which has been proposed as the explanation to why the front hooves often are seemingly more severely affected by laminitis (Hood *et al.* 2001).

Based on his findings, Obel (1948) proposed that the primary change in laminitis is a direct disturbance in the process of cornification in the epidermal cells, causing a weakening of the lamellar tissue until it no longer is able to support the weight of the horse, resulting in secondary tissue damage and pedal bone displacement.

It was later suggested by Wattle *et al.* (1995) that the disabling effects of laminitis could be counteracted by keeping the horse recumbent during the acute phase of the disease, as a supplementary treatment irrespective of the initial cause. The recumbency would eliminate the stress from the body's weight on the lamellar layer, thereby preventing secondary tissue damage, giving the tissue time to regenerate and regain its weight-bearing properties.

Wattle *et al.* (1995) proved short-term forced recumbency to be well tolerated by healthy Shetland ponies. Recumbency was achieved pharmacologically, but maintained only by reducing the ceiling height of the box. The ponies were kept in recumbency for periods of up to 48 consecutive hours, during which they consumed normal amounts of hay and water and were able to pass both faeces and

urine. The ponies showed signs of discomfort only if they, when attempting to rise, got themselves into an uncomfortable position in one of the four corners of the experimental box. They were however always able to shift into a more comfortable position without assistance. All three ponies rose immediately when aware that the ceiling had been removed and none of them showed any long term adverse effects from the treatment.

In studies on the normal sleep and rest behavior of horses, Steinhart (1937) and Dallaire (1986) reported that horses divide their sleep between several periods per day. The total time spent in recumbency ranged from 10 minutes to 5 hours and 15 minutes per day, the longest observed period of uninterrupted recumbency lasted 3 hours and 30 minutes.

Time spent in recumbency is often used as a variable when measuring welfare and preferences in studies comparing different housing conditions, such as bedding materials, in horses (Chaplin and Gretgrix 2010; Hunter and Houpt 1989; Mills *et. al.* 2000; Pedersen *et. al.* 2004; Raabymagle and Ladewig 2005; Thompson 1995; Werhahn *et. al.* 2010;). These studies mostly consist of night time observations only, but total periods of up to 4,2 hours of recumbency per night are reported. Bertone (2006) observed one horse lying down continuously for 12 hours after a period of sleep deprivation.

When trying to determine the effects of systemic anesthetics versus recumbency on circulation and respiration, ponies trained to lay down in left lateral recumbency without the use of any sedatives, have been used in two different studies, one by Hall (194) and one by Rough et al (1984). Based on the results from their respective studies, they made somewhat contradictory conclusions regarding the influence of anesthetic agents versus the body position. Hall (1984) attributed changes to the recumbency rather than the anesthetic agent, although acknowledging that drugs may influence their magnitude. Rough et al (1984) found no significant cardiopulmonary changes among the conscious ponies, suggesting that depression during anesthesia may be primarily caused by the drugs.

The objective of the present study was to determine whether Standardbred Trotters tolerate forced recumbency by lowering the ceiling height of the box as well as ponies, and whether their increased body size has a negative influence on bodily functions during and after two days of uninterrupted recumbency.

MATERIALS AND METHODS

The horses used in this study were all healthy Standardbred Trotters (see table1). Prior to the study, the horses were housed and fed in accordance with standard routines of the department of Clinical sciences, SLU. Hence, they were not in any way prepared for the study by special tending or feeding.

Table 1: Experimental horses used in the study

	Gender	Age (years)	BW (kg)	Height (cm)	Experimental period (days)
Horse 1	Mare	3	416	154	1
Horse 2	Gelding	4	379	153	1
Horse 3	Gelding	5	420	153	1,5
Horse 4	Gelding	10	420	153	1,5
Horse 5	Gelding	11	510	153	2
Horse 6	Gelding	11	550	167	2

The experimental box used in this study was constructed as an even eight-square, with an area of 8,3 square meters. Each of the 8 wall segment contained an independent door, allowing easy access from all directions (figure 1 a - b). The ceiling of the box was by wires connected to a winch to enable the height adjustments needed for the study. During the study periods, the ceiling was locked in place by bolts to each corner pole. The corner poles were in turn secured to the ground by land ties.



Figure 1a and b: The experimental box.

The box was placed in an indoor hall, on an area of mixed sand and fine gravel. Generous amounts of straw were used as bedding material (Figure 1b). The room was always well lit during the studies and had a temperature of 15-25 degrees Celsius, depending on ambient temperatures.

Before the experimental periods, the horses were given acepromazin, 0,03 mg/kg BW, intramuscularly approximately 30 minutes prior to surgical preparation of the jugular areas of both sides. A mila Venflon was inserted into each jugular vein, to facilitate access from both sides, and in turn connected to extension tubes.

Sedatives, romofidin 0,1-0,12 mg/kg BW and butorfanol 0,025 mg/kg BW, were given 60 to 90 minutes after the acepromazin dosing and before walking the horses into the experimental box. A protective padded face-mask was used to minimize the risk of injury to the head during falling (Figure 2a). Recumbency was achieved through dissociative anesthesia, diazepam 0,03-0,05 mg/kg BW and ketamin 2,2 mg/kg BW. When the horses lay in lateral recumbency, the protective

mask was removed and a fabric sheet was loosely placed over the horses' eyes. The box ceiling was lowered to a height of approximately 125% of the horse's thoracic height and locked in place before the horse regained consciousness. Approximately 12 hours into the experiment, the ceiling height was increased to 130 – 140%, depending on the individual horses' temperament and ability to change between left and right recumbency.



Figure 2 a: Horse with protective padded face-mask before induction of anesthesia. b: The roof was initially lowered to a height of approximately 125% of the horse's thoracic height.

The horses were continuously monitored and cared for during the experimental periods. Body position, behavior, rising attempts, consumption of hay and water and passing of urine and faeces were observed and registered continuously. Heart rate and respiratory rate and character were measured through palpation, auscultation or observation at least every 4th hour. The horses were regularly offered water and always had easy access to hay during the studies.

Sternal recumbency was defined as the horse lying with head and neck upright and the front legs flexed under or stretched in front of the body with one hind leg under and the other besides the abdomen. Lateral recumbency being a horse lying on its side, head and neck resting on the bedding and all four legs one side of the body. Shifting was defined as when a horse shifted between sternal and lateral recumbency more than twice within a two minute period. An attempt to rise was defined as when a horse tried to lift the box ceiling, involving both front and hind quarters. Hence, light contact between mere head and ceiling were not included.

Within 30 minutes after the experimental period, the horses underwent thorough physical examinations and were checked for lameness at walk and trot before returned to their usual stables. Behavior, physical status and bodily functions were closely monitored for at least 4 days following the end of the study period. According to their previous owners' wishes, two of the horses were then later euthanized.

Blood was sampled for serum analyses of muscle enzymes, Serum Aspartate Aminotransferase (ASAT) and Creatine kinase (CK) prior to anesthesia, within 30 minutes post forced recumbency, and one day after the end of the trial. ASAT and CK were analyzed at the University animal hospital laboratory for clinical pathology.

This study and experimental design was approved by the Uppsala Committee for Ethical Review of Animal Experiments at a meeting on February 26th 2010.

RESULTS

Body positions and rising attempts

After induction of anesthesia, the horses spent between 24 and 80 minutes in lateral recumbency before shifting themselves into sternal recumbency. Four horses stayed in this position for 21 - 46 minutes, while 2 attempted to rise immediately. All horses first attempt to raise occurred 27 - 92 minutes post induction.

All horses accepted the treatment after an initial period of more or less frequent and forceful attempts to rise. One horse initially sometimes pawed with its front hooves as if frustrated by the situation, but settled down and this behavior became much less frequent after a couple of hours. In general, the horses became less and less interactive during the experimental periods.

When being recumbent, the horses frequently shifted between the same side lateral and sternal recumbency, on average 15 times per hour. They rarely spent more than 20 minutes in the same position and periodically they shifted several times per minute. These episodes of frequent shifting often took place shortly before changing between left and right recumbency, which occurred more seldom.

Technique and frequency of turning between left and right recumbency varied among the horses. The first 5 horses turned sides over sternum, sometimes during attempts to rise.

Horse 1 turned itself regularly every 2-6th hours. Horse 2 turned only once, after first having spent 22 hours on one side. Horse 3 turned sides 3 times, spending at most 20 consecutive hours on one side. Horse 4 turned sides every 5-14th hours, a total of 4 times during the experimental period. Horse 5 turned sides 5 times. Four of these turning were assisted by tying a rope around the fetlock of the upper hind leg, sliding it under the horse and pulling the leg under the horse, which thereby tilted over to the other side.

Horse 6 was the only horse which turned between left and right recumbency by rolling over on its back. The horse performed this rolling behavior a total of 32 times during the study period, after first having spent 18 hours on one side. Occasionally, this method of turning resulted in uncomfortable positions with the legs too close to the box walls, but the horse always managed to shift into a more comfortable position on its own.

For time distribution between the different recumbent positions and number of rising attempts, see table 2.

Table 2: Individual time distribution between the different positions in % of the total time spent in recumbency and number of rising attempts

	Left sternal	Left lateral	Right sternal	Right lateral	Shifting	Number of rising attempts
Horse 1	17	32	17	32	3	41
Horse 2	3	5	53	37	3	39
Horse 3	7	2	24	54	2	41
Horse 4	29	27	19	18	7	73
Horse 5	10	27	17	23	22	40
Horse 6	16	31	11	34	8	9

Water/hay consumption

All horses started to eat within between 40 – 111 minutes post induction and consumed normal amounts of hay and water during the study periods. Hay was consumed mostly in sternal position, but sometimes also in lateral recumbency.

No muzzle was used and all horses began eating hay while still under influence from the sedatives and anesthetics used to induce recumbency. Two hours into the experiment, horse no. 5 began showing signs of esophageal impaction, i.e. frequent swallowing and nasal discharge. The horse was given a low dose of sedative (Detomidin) and spasmolyticum (Metamizol) and the impaction could be resolved by nasogastric tubing while the horse was still recumbent in the experimental box, without further complications during or after the experimental period.

None of the other horses needed any medication post anesthesia.

Urination

Horse 1, 2 and 3 did not urinate while recumbent in the experimental box. Horses 4, 5 and 6 urinated twice during the recumbency period, first after approximately 3 to 9 hours and again after 18 to 40 hours. All horses urinated within 30 minutes after rising. The three horses that did not urinate while recumbent did so within 10 minutes after rising.

Defecation

The first passing of faeces occurred after 4 to 16 hours of recumbency, followed by regular defecation with intervals of 0,6 - 15 hours. All horses also defecated within 30 minutes after rising.

Breathing

Respiratory rate (RR) varied between 12 – 16 breaths per minutes, except for shorter sequences with RR of 16-32 per minutes. The later rates were mostly seen before changing position between left and right and were regarded as a sign of discomfort. All horses were periodically observed holding their breath a couple of seconds before exhaling during the study periods, sometimes in combination with making a groaning sound during exhalation. This was also regarded as a sign of

discomfort since it rarely occurred the first hour after changing sides or after urination.

Heart rate

Heart rates were consistently within normal reference ranges of 24-48 beats per minutes during the entire study period in horses 1, 3, 4, and 6. In horse 2 and 5 heart rates increased during the last 4-6 hours of recumbency, up to a maximum of 60 beats per minutes, but normalized again within an hour after rising.

Rising and status after experiment

All horses rose without any difficulties within 11 minutes after elevation of the box ceiling. Immediately after the experimental periods, the horses moved willingly but had a somewhat stiff gait with a shortened length of stride both at walking pace and trot. Stride length increased markedly after walking the horses 10-15 minutes and normalized entirely within the following 24 hours.

None of the horses showed any signs of gastrointestinal problems or neuropathy during or after the study. Only mild and transient myopathies were seen as tremors in the triceps- and quadriceps areas and elevated CK levels, lasting for up to 24 hours after the experimental periods in all horses (Table 3).

Cutaneous edema and soreness in areas over knees and elbows were observed in all horses after the experimental periods, persisting for up to 48 hours after the experimental period.

Appetite, defecation, urination, and social behavior were normal for all horses following the experimental period.

Blood samples

Table 3: Serum CK and ASAT analysis results

	CK (2-12 μ kat/l)			ASAT (2,5-13 μ kat/l)		
	Before study	After study	1 day after study	Before study	After study	1 day after study
Horse 1	3,5	9	2,9	5,9	7,6	10,6
Horse 2	-	60,1	5	-	10,8	10,9
Horse 3	4,7	12,5	6,7	5,3	8	8
Horse 4	4,6	16,9	3,1	4,5	10,4	9,6
Horse 5	9,6	16,6	9,5	8,4	11	9,8
Horse 6	4,3	11	6,3	6	8,9	8,7

DISCUSSION

The floor area of the experimental box was spacious enough to allow even the largest of the horses to lie comfortably on its side with legs and neck fully extended. All horses accepted well to lie down in the box after an initial period of more or less frequent and forceful attempts to rise indicating that the octagonal construction is better tolerated by horses than the quadrangle box used by Wattle *et al.* (1995). Since all eight sides of the experimental box could be opened, examination and tending to the horses during the experiments was easily

manageable. One horse initially sometimes pawed with its front hooves as if frustrated by the situation, but settled down and this behavior became much less frequent after a couple of hours. In general, the horses became less and less interactive during the experimental periods. Animals differ in the way they cope with challenges in their environment but with increasing anxiety it is common that active coping shifts to a more passive mode. The behavior to be still and wait for an opportunity to fly is a basic instinct/passive behavior, to preserve energy to a last shake off or escape. However, this coping style depends on environmental conditions such as space, density and food availability (Koolhaas 2007). Regarding equine laminitis, it seems less cruel to keep the horse under the stressful situation of forced recumbency for 48 hours to prevent a severe dislocation of the distal phalanx followed by months – years of rehabilitation or euthanasia, than to use the current “state of art” treatments for this disease. All horses included in this study returned to a normal social behavior within hours after rising and normal physiological status within a couple of days.

Since one of the intended areas of use for the box is treatment of horses with acute spontaneous laminitis in an ambulatory practice (Wattle *et. al.* 1995), the box had to meet several constructional requirements. For example, easy transportation and assemblage while maintaining the stability to withstand rising attempts and the flexibility to stand on uneven grounds without the risk of ceiling or doors getting stuck. These requirements were satisfyingly met by the construction used, but the box area needs to be increased by 1 m² before it can be used for warmbloods.

The rolling behavior of horse 6 was not associated with any signs of pain, such as increased heart or respiratory rates, and was interpreted simply as an alternative means of changing sides. Even if the rolling sometimes led to the horse getting stuck half way on its back, the horse never panicked but simply tilted itself back to the original side again.

No explanation to the increased heart rates in horse 2 and 5 was found after allowing the horses to rise. Therefore it was interpreted as a sign of physical or mental distress. In case of horse 2, most probably from having a filled urinary bladder since the heart rate went down directly after it had urinated.

The horse which acquired an esophageal impaction did not begin to eat hay earlier or in greater amounts than the other horses in the study. The clinical signs began to show after the horse having eaten only a couple of mouthfuls of hay. Why this occurred in this specific individual was not explained by this study. But, the fact that this horse only had been resting in a paddock the previous 4 years might have contributed to this malady.

The muscle tremors seen in all horses after the recumbency periods were not more severe or long-lasting the more time the horses spent in recumbency. For example, in the horse which spent 22 consecutive hours on its right side, tremors were only slightly more pronounced on the right side, but still clearly present on the left side which had only been lowermost for two hours.

The increase above reference ranges in serum CK seen only immediately following the experimental period in four of the horses, indicate a mild degree of transient acute muscle damage. This could be expected simply because of the

horses' body masses. The greatest increase was seen in horse 3, which could be explained by the fact that it spent a large amount of the experimental period on one side and it had a relatively large muscle mass compared to the other horses. It had participated in its last racing competition less than 3 weeks prior to the study. When under general anesthesia, horses can acquire severe myopathies after as little as 1 hour, with duration of anesthesia and hypotension pointed out as risk factors (Richey *et al.* 1990). No such adverse complications occurred in this study. The horses were not given any medication influencing cardiovascular function after the initial induction and they were able to change position when uncomfortable. The possibility to frequently shift body position, i.e. change pressure points, and thereby allow blood flow to different areas of the body is crucial for avoiding pressure sores.

Based on the limited number of horses used in this study, increased body size and weight does not seem to have a negative effect on physical tolerance for forced recumbency by lowering the ceiling height of the box. From a human point of view, the horses also accepted and handled the treatment well mentally.

It is currently not known if 48 hours of forced recumbency is enough to prevent horses with acute laminitis from turning into chronic laminitic horses. However, since none of the horses suffered any adverse or lasting effects from the experiments, the period of recumbency could probably be extended further if needed, as indicated by Wattle *et al.* (1995). Before this short term forced recumbency method can be used as an adjunctive treatment in clinical cases of acute laminitis further research and testing are needed. The next step will be to study the clinical and histopathological effects of recumbency on the hoof weight bearing tissues, in horses with experimentally induced laminitis.

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