

Glycogen Content in Different Muscle Fibre Types Before and After Lengthy Anaesthesia in Horses

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Abstract

General anaesthesia in horses is often followed by postoperative complications, including myopathy. Many colic patients are compromised by preoperative stressors, such as starvation, trailer rides, physical stress and pain, plus postoperative physical stress in connection with standing up after recumbency. These factors may influence muscle glycogen stores, since glycogen is an important substrate source in metabolism. The aim of this study was to investigate muscle glycogen content with histochemical techniques, before and after lengthy anaesthesia in both healthy horses and in colic patients.

Seven colic patients and 5 healthy warmblooded trotters were anaesthetized (duration > 3 hr). Patients did not eat (9-51 hr) prior to surgery and had been transported large distances to surgery (53 -320 km). Healthy horses were denied food (12 h) prior to anaesthesia. Choice of premedication and induction agents was left to the anaesthetist. Anaesthesia was maintained with isoflurane with horses in dorsal recumbency. *M. gluteus medius* was biopsied in all horses at induction, end of anaesthesia and in some horses, 24 hours after anaesthesia. Samples were cut in a cryostat and stained for myosin ATP-ase activity after pre-incubation at pH 4.6, to identify type I, IIA and IIB fibres, and with Periodic Acid-Schiff's reagent, in order to subjectively evaluate 'low' or 'high' glycogen content in fibres.

Healthy horses and two patients had a high glycogen content in all fibre types at all biopsy times. Three patients had a high glycogen content in most fibres at induction of anaesthesia, but had a few low staining fibres at end of anaesthesia. A low glycogen content in most fibres (primarily type I fibres) was seen at induction of anaesthesia in the other four patients. In three of these horses, type IIB fibres also had a low glycogen content. Changes in glycogen content were still evident at end of anaesthesia and even 24 hours after standing up in recovery. The horses with a poor preoperative physical status had many fibres with low glycogen content.

This study has shown that glycogen availability in muscle fibres may be an important factor to consider in anaesthesia and in the postoperative recovery period, and that this could be related to preoperative stress factors.

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Introduction

Postoperative myopathy

General anaesthesia in horses poses more risks than in man. The perioperative death rate in surgery in man has been shown to be 0.01% (Arbous *et al.*, 2001), versus 2.7% (Tevik, 1983) and 1.6% (Johnston *et al.*, 1995) in two studies of equine anaesthesia complications. Emergency surgery is almost 5 times more likely to lead to mortality compared to elective procedures. Colic surgery involves additional risk, being almost 10 times more likely to lead to mortality versus elective surgery (Mee, Cripps & Jones, 1998). Patients at low risk for anaesthetic complications, i.e. candidates for elective surgery with good preoperative physical status, also experience anaesthesia-related complications. Muscle related complications are not uncommon, and are often due to postoperative myopathy. In a study by Tevik (1983) of mortality related to anaesthesia, 24.2% of deaths were due to postoperative myopathy.

Postoperative myopathy is thought to be a result of ischemia caused by inadequate intraoperative muscle perfusion over long periods of time. Contributing factors are hypotension produced by anesthetic drugs and the intrinsic weight of muscle, which causes compressive forces restricting capillary perfusion and limits venous return (Taylor & Clarke, 1999). Prevention of the development of myopathy involves minimizing the length of anaesthesia and avoiding hypotension by strategic fluid and drug therapy, thoughtful positioning of limbs and ample padding.

Signs of myopathy may be evident as soon as the horse tries to stand after the end of anaesthesia or may debut after the horse has stood up in recovery. Signs vary from mild lameness to extreme pain, copious sweating, tachycardia, tachypnea, and impaired function or ability to stand on various affected limbs. There may be myoglobinuria, raised creatine kinase or aspartate aminotransferase levels in serum (Radostits *et al.*, 2000). Histopathological findings in horses with postoperative myopathy are: loss of cross-striations, massive, acute, hyaline degeneration and swollen, homogeneous, extremely eosinophilic muscle fibres (Friend, 1981). Fibres can be fragmented, disorganized, mineralized or vacuolated. Oedema, fibrin, erythrocytes, neutrophils and mononuclear cells may be found in the nearby proximity. There may be signs of ischemic necrosis with extensive bleeding and thrombotic vessels. Biopsies taken during anaesthetic recumbency in otherwise healthy horses also have similar lesions to those described above. However, 24 hours after anaesthesia, lesions have disappeared in healthy horses but persist in horses with postoperative myopathy (White, 1982). Most horses recover from postoperative myopathy from within a few days up to one week. In severe cases, the patient may never be able to regain the standing position and is finally euthanized.

Many studies of postoperative myopathy have focused on factors which decrease muscle tissue perfusion during anaesthesia, e.g. hypotensive anaesthetic agents and external physical factors such as placement on the operating table and length of

anaesthesia (Dodman *et al.*, 1988; Klein, 1978, 1990; Tevik, 1983; etc.). White & Short (1978) found qualitative changes in muscle glycogen content in muscle biopsy material during the course of anaesthesia, in horses which later showed signs of postoperative myopathy. The present study focuses on investigating glycogen content in muscle fibres before and after equine anaesthesia. To our knowledge there is little information in the literature on substrate availability in muscle, in the context of anaesthesia complications in the horse. A review of fuel utilization in the horse and some basic muscle physiology is necessary as a background to this study.

Fuel utilization in the horse

Horses use carbohydrates, lipids and proteins for fuel (for review, see Lawrence, 1990; Valberg, 1986). The relative proportion of fuels used depends on availability of substrate, hormonal effects, exercise duration and intensity. At rest and low-intensity exercise of long duration, lipids are the dominant fuel. As exercise intensity increases, the proportion of carbohydrate metabolism increases and muscle glycogen is the dominant fuel used at high intensity work. Sources of carbohydrate are muscle glycogen or blood glucose. At rest, most carbohydrate is oxidized to carbon dioxide and water. As exercise intensity increases, oxidation is incomplete due to inadequate oxygen availability and lactate is formed, which can at very high intensities lead to a drop in muscle pH and fatigue. The metabolism of protein for energy production is of minor importance compared to lipid and carbohydrate metabolism.

Muscle fibre types and recruitment

Muscle consists of different kinds of fibres equipped to meet various postural and locomotive demands. Motor neurons of varying size innervate the different types of muscle fibres, and depending upon the level of excitation, different types of fibres will be activated (for review, see Hultman, 1995; Lindholm, 1974; Valberg, 1986). Type I fibres have the smallest motor neurons, which contract easiest and at low levels of excitation. These fibres take a long time to reach peak tension (slow twitch) and are fatigue resistant. They have a high oxidative capacity, an abundance of mitochondria and capillaries, low glycolytic capacity, and low creatine kinase and ATP-ase activity (i.e. type I fibres are used in low intensity work of long duration). Type II fibres have larger motor neurons and thus require more excitation for contraction to occur. They take a short time to reach peak tension (fast twitch) and have a low fatigue index, high glycolytic capacity, high creatine kinase content and high ATP-ase activity. Type II fibres are divided into two subtypes, type IIA and type IIB. Type IIA fibres are more oxidative, have a higher fatigue resistance and smaller motor neurons than type IIB fibres. Type IIB fibres have the largest motor neurons, and are most glycolytic of all fibre types. The ratio of type IIA/IIB fibres increases with training (Essén-Gustavsson & Lindholm, 1985). Type IIB fibres are used in work of high intensity and short duration, whereas type IIA fibres allow for better performance during work of high intensity and longer duration. Histochemical analysis of myofibrillar adenosine

triphosphatase activity, identifies fibre types I, IIA and IIB through pre-incubation of muscle samples at pH 4.6 (Brooke & Kaiser, 1970).

Glycogen substrate availability

There are many factors, such as: starvation, exertion, stress and tissue hypoxia during anaesthesia, which could be of potential importance for glycogen metabolism in colic patients and healthy horses when anaesthetized. Prior to surgery, colic patients may have gone long periods of time without food, which would create a catabolic situation and deplete energy stores. Colic patients roll, thrash around and are walked, often under the course of many hours, before being transported to surgery. Riding in the horse trailer requires the use of postural muscles to balance when the trailer bounces and turns. The horse may panic, releasing catecholamines, which cause an increase in glycogenolysis. The colic patient, in addition, may suffer from impaired cardiovascular function due to dehydration and hypovolemic shock. This may contribute additionally to reduced perfusion and hypoxia in muscle tissue. Increased concentrations of lactate and a resultant acidosis are seen in many colic patients because of poor tissue perfusion (Short *et al.*, 1981; Svendsen, Hjortkaer & Hesselholt, 1979). Postoperatively, the horse may have difficulty standing up in recovery, making multiple attempts to stand, crashing and flailing around in the recovery box. If the horse shows signs of postoperative complications, the introduction of food may be delayed, and the horse may be maintained on infusion of electrolytes and glucose. The summation of all factors appears intuitively to present a considerable metabolic challenge for colic surgery candidates.

Aim

The aim of this study was to study muscle glycogen staining pattern before and after abdominal surgery in healthy horses and compare this with horses undergoing prolonged general anaesthesia without surgery. A better understanding of muscle physiology and metabolism before and after anaesthesia could ultimately provide more understanding of the aetiology of postoperative myopathy.

Material and methods

Horses

Seven colic patients (4 warmblooded riding horses, 2 warmblooded trotters, and 1 Shetland pony) and 5 healthy warmblooded trotters were studied. Five patients were geldings, one a mare and one a stallion. Three healthy horses were mares and 2 geldings. Patients weighed 230-630 kg and were 3-15 years of age (mean 10 yr), whereas healthy horses weighed 411-578 kg and were 3-17 years old (mean 8 yr). Training status in patients varied from excellent to poor, whereas all healthy horses were kept at pasture at the university and trained minimally. Patients refused or

were denied food 9-51 hours preoperatively and were transported 53-320 km to surgery. In healthy horses, food was denied 12 hours prior to anaesthesia, which lasted 250-322 min (mean 283 min) in healthy horses and 190-330 minutes (mean 243 min) in colic patients. Healthy horses did not undergo any surgical intervention apart from muscle biopsy sampling. Physical status for horses was determined preoperatively and horses ranked on a scale of I-V, according to the American Society of Anesthesiologists, where I represents a healthy horse and V is moribund. Large weight was placed on general health, heart rate, capillary refill time and peripheral circulation (mucosal appearance, temperature of extremities). Table 1 shows relevant preoperative clinical information.

Table 1. *Preoperative clinical information for healthy horses (1a-e) and patients (2a-g). Physical status is classified as I-V (I, healthy; V, moribund), in horses of different breeds: Swedish warmblooded trotter (Swt), Polish warmblood (Pw), Swedish warmblood (Sw), Shetland pony (Sp), and sex: mare (m), gelding (g), stallion (s)*

Horse id	Breed	Sex	Age (yr)	Weight (kg)	Transport distance (km)	Physical status
1a	Swt	m	6	578	0	I
1b	Swt	m	17	489	0	I
1c	Swt	g	3	411	0	I
1d	Swt	m	4	470	0	I
1e	Swt	g	12	522	0	I
2a	Pw	g	15	620	93	III
2b	Sw	g	11	609	70	III
2c	Sw	g	11	630	240	III
2d	Swt	s	3	450	320	III
2e	Sp	g	11	230	210	IV
2f	Swt	m	11	580	53	IV
2g	Sw	g	5	516	130	V

Anaesthesia

Healthy horses were premedicated with detomidine (Domosedan 10 mg/ml, Orion Pharma AB, Animal Health, Sollentuna), <10 ug/kg iv. Colic patients were premedicated varyingly. Two patients were anaesthetised without premedication, due to poor physical status in one horse, and a systolic heart murmur in the other. One patient received solely romifidine (Sedivet 10 mg/ml, Boehringer Ingelheim Vetmedica, Copenhagen, Denmark), and two were given romifidine combined with butorphanol (Torbugesic 10 mg/ml, Fort Dodge Animal Health, Iowa, USA). Another horse was given detomidine and the last horse was given acepromazine (Plegicil 100 mg/ml, Pharmacia Animal Health, Helsingborg, Sweden) as premedication.

Anaesthesia was induced in all healthy horses and four patients with guaiphenesin (Myolaxin 150 mg/ml, Bayer AB, Gothenburg) in a 7.5% iv solution

given symptomatically, plus thiopentone (Pentothal Natrium, 500 mg powder mixed to 12.5%, Abbott Scandinavia AB, Solna), 3-6 mg/kg iv. Three patients received ketamine (Ketaminol 100mg/ml, VetPharma AB, Lund) and diazepam (Diazepam 5 mg/ml, Nordic Drugs AB, Limhamn) as iv induction agents instead of guaiphenesin. Anaesthesia was maintained in all study horses with isoflurane (Isoflo vet, Schering-Plough AB, Stockholm) at a target surgical MAC of 1.57%. Horses were positioned in dorsal recumbency. Breathing was mechanically controlled by intermittent positive pressure ventilation in healthy horses and one patient, whereas all other horses breathed spontaneously. An intravenous electrolyte infusion (Ringer-acetat, 270 mosm/kg, Fresenius Kabi, Uppsala) was administered to healthy horses at a target rate of 3 ml/kg/h. Hypotension (< 60 mmHg) was corrected in healthy horses by increased speed of electrolyte infusion or dobutamine (Dobutrex 12.5 mg/ml, Eli Lilly Sweden AB, Stockholm) infusion. In patients, intravenous electrolyte infusion was given at maximum speed and hypotension was primarily corrected by an infusion of dextran (Macrodex 60 mg/ml with sodium chloride, Pharmalink AB, Upplands Väsby) or, after 30 minutes without effect, dobutamine (Dobutrex 12.5 mg/ml, Eli Lilly Sweden AB, Stockholm).

Sampling techniques

In all horses, *M. gluteus medius* was biopsied according to the method described by Lindholm & Piehl (1974). Biopsies were taken at induction of anaesthesia (start), end of inhalation anaesthesia (end) and in the five surviving patients, 24 h after the end of anaesthesia (day 2). Samples were rolled in talcum powder, immersed in liquid nitrogen and stored at -80°C until time of analysis. Serial transverse sections were cut in a cryostat microtome at -20°C and stained for myosin ATP-ase activity after pre-incubation at pH 4.6 (Brooke & Kaiser 1970), in order to identify type I, type IIA and type IIB fibres. Staining intensity for glycogen was evaluated with periodic acid-Schiff's (PAS) reagent at a minimum of 200 fibres per biopsy. Fibres were subjectively evaluated as having low (white-light pink) or high (pink) staining intensity.

Before anaesthesia, plasma lactate concentration was determined through collection of heparinized venous blood samples, which were centrifuged immediately after sampling. Plasma was frozen and stored at -80 °C until time of analysis (Analox GM-7, Analox Instruments Ltd, London).

During anaesthesia, samples for blood gas analysis were collected anaerobically in heparinized 2ml syringes from a facial artery (in most cases, *A. faciei transversa*) and stored on ice a maximum of 10 minutes, until time of analysis (ABL 5, Radiometer, Copenhagen, Denmark). Arterial oxygen tension (PaO₂), carbon dioxide tension (PaCO₂) and pH were measured. Oxygen saturation, bicarbonate concentration and base excess (ABE) were calculated. Corrections were made for blood temperature and species.

Results

Anaesthesia complications

Anaesthesia complications were classified as severe, moderate, slight, or none, based on values for mean arterial blood pressure, oxygen saturation and base excess. Table 2 summarizes anaesthesia information. Normal values for mean arterial blood pressure are 80-120 mmHg and for oxygen saturation, >95% (Thurmon, Tranquilli & Benson, 1996). Difficulty getting up to a standing position and postoperative muscle or gait disturbances were noted.

Table 2. *Length of anaesthesia, anaesthesia complications, mean arterial blood pressure (MAP) and oxygen saturation in horses*

Horse id	Length of anaesthesia (min)	Anaesthesia complications	MAP (mmHg)	Oxygen saturation (%)
1a	250	slight	58-84	90-99
1b	300	slight	52-76	94-100
1c	322	none	83-100	100
1d	265	none	66-87	99-100
1e	280	moderate	47-74	100
2a	230	slight	52-81	95-98
2b	330	moderate	75-94	47-69
2c	225	slight	73-96	82-93
2d	256	slight	59-68	100
2e	190	severe	75-134*	43
2f	190	slight	60-82	91-94
2g	280	severe	32-55	79-91

*, indirect measurement using a tail cuff

Fibre type composition

Healthy horses were all warmblooded trotters, with an average of 23% type I, 38% type IIA and 39% type IIB fibres. Warmblooded riding horses in the patient group had an average fibre composition of 10% type I, 42% type IIA and 48% type IIB fibres. Warmblooded trotters in the patient group had an average of 21% type I, 42% type IIA and 36% type IIB fibres. The Shetland pony had 22% type I fibres, 26% type IIA fibres and 52% type IIB fibres. Average fibre composition in individual horses can be found in Table 3.

Table 3. Mean muscle fibre composition (type I, IIA and IIB fibres) evaluated from biopsies taken at start, end and day 2

Horse id	Type I fibres (%)	Type IIA fibres (%)	Type IIB fibres (%)
1a	33	35	32
1b	18	37	45
1c	21	43	36
1d	18	36	46
1e	25	37	38
2a	12	37	51
2b	11	48	41
2c	10	40	50
2d	27	51	22
2e	22	26	52
2f	16	34	50
2g	6	44	50

Pre-operative plasma lactate

Mean plasma lactate (Tab. 4) for the five healthy horses at the start of anaesthesia was 0.7 mmol/l, compared to 4.9 mmol/l in patient horses. Four of the patients had lactate values above the healthy horses.

Table 4. Plasma lactate values in mmol/l

Horse id	1a	1b	1c	1d	1e	2a	2b	2c	2d	2e	2f	2g
Plasma lactate value	0.3	0.9	-*	1.1	0.4	0.8	5.1	1.7	0.4	6.5	4.5	15.4

*, -, horse 1c was not sampled

Glycogen staining pattern

The appearance of different muscle fibre types in myosin ATP-ase and PAS stains in one healthy and in one patient horse is shown in Fig. 1 and 2.

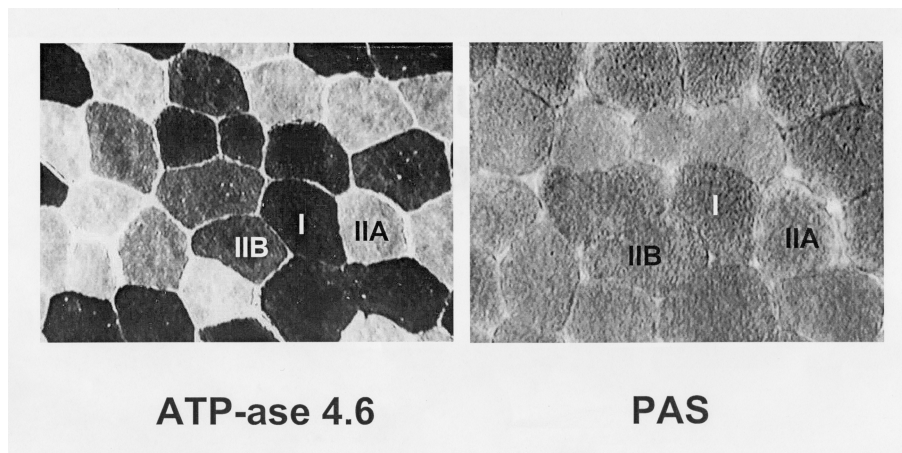


Fig. 1. Type I, type IIA and type IIB fibres in a myosin ATP-ase stain at pH 4.6, and the appearance of the same fibres in a PAS stain. Muscle fibres are from a healthy horse (1e) at start of anaesthesia; with uniformly high glycogen staining intensity.

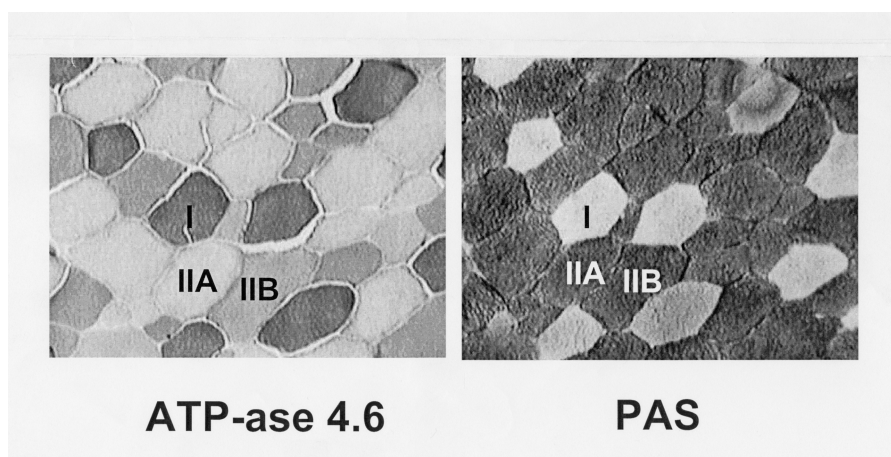


Fig. 2. Type I, type IIA and type IIB fibres in a myosin ATP-ase stain at pH 4.6, and the appearance of the same fibres in a PAS stain. Muscle fibres are from a patient (2d) at start of anaesthesia; many type I fibres have low glycogen staining intensity, whereas type IIA and type IIB fibres have high glycogen staining intensity.

The percentage of type I, IIA and IIB fibres with high and low staining intensity is shown in Fig. 3 and 4. All healthy horses had a good general glycogen status, i.e. most fibres of all fibre types had a high PAS staining intensity at both start and end of anaesthesia.

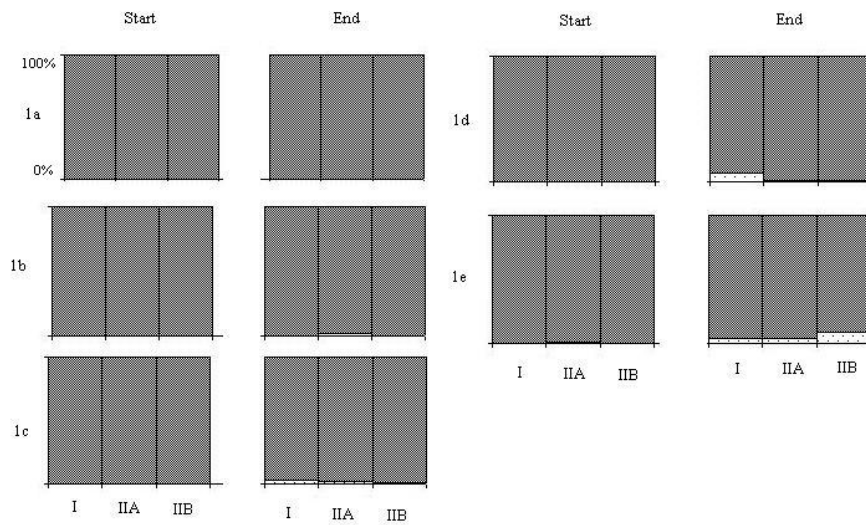


Fig. 3. Glycogen staining intensity in healthy horses (1a-1e) for different fibre types (I, IIA, and IIB) at start and end of anaesthesia. Grey colouring represents high glycogen staining intensity, white colouring with grey spots represents low, and histogram scale is 0-100%.

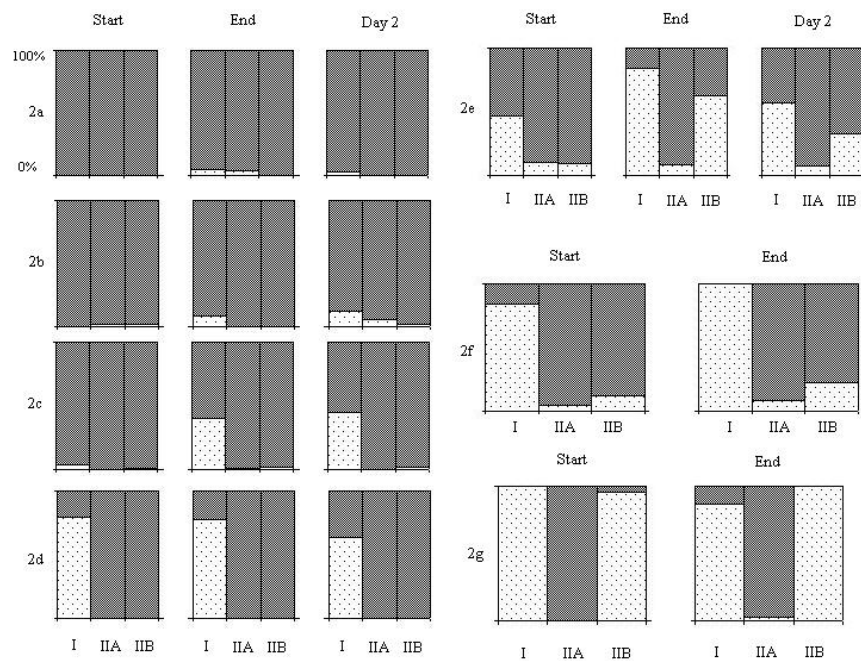


Fig. 4. Glycogen staining intensity in patients (2a-2g) for different fibre types (I, IIA, and IIB) at start, end and day 2 (2f and 2g were not biopsied on day 2). Grey colouring represents high glycogen staining intensity, white colouring with grey spots represents low, and histogram scale is 0-100%.

Two of the seven patients had glycogen staining patterns much like healthy horses, i.e. most fibres had high PAS staining intensity at all biopsy times. The other five patients had more varied glycogen staining patterns than healthy horses. Horse 2c had a large proportion of low staining type I fibres at end and on day 2, with almost all fibres staining high at start of anaesthesia. Type IIA and type IIB fibres stained mostly high at all times. Horse 2d had a large proportion of low staining type I fibres at all times, and no type IIA nor type IIB fibres stained low at any time. Three horses had a more extensive general glycogen emptying pattern compared to the other patients. In these horses (2e, 2f, 2g), a large proportion of type I fibres stained low at all times, and in addition, type IIB fibres also had low staining intensity.

Discussion

Some horses in the patient group had low staining intensity at start of anaesthesia. This indicates that glycogen had been used as an energy substrate in these fibres. The reason for this may be related to factors such as starvation, stress, physical exertion and limited oxygen availability in muscle tissue.

Starvation is a possible factor which could explain lack of glycogen in patient horses, because the starvation period averaged 26 hours compared to only 12 hours in healthy horses. In humans, healthy, starved individuals burn fatty acids as a primary fuel and little glucose is metabolised, whereas in critically ill human patients, insulin resistance occurs and blood glucose cannot access muscle cells (Wolfe & Martini, 2000). It is plausible that intracellular stores of glycogen would be used up and a muscle glycogen deficit would ensue. This situation could also occur in horses and may have contributed to low glycogen staining results in severely ill horses in this study (2e, 2f, and 2g). Other possible factors which could explain glycogen emptying patterns in study horses are: stress, decreased muscle perfusion and physical exertion.

Horses in the patient group were all stressed preoperatively by colic symptoms and transport to surgery. Stress promotes the release of adrenaline, which prioritizes 'fight and flight' mechanisms in the body, including increased rates of glycolysis in the liver and muscles, increased blood glucose concentration, increased levels of metabolism in all cells in the body (Guyton & Hall, 1997). Hultman (1995) showed that the rate of glycogenolysis increased in human leg muscle tissue in type I fibres after adrenaline infusion, but was unaffected in type II fibres. Stress could therefore have been a factor contributing to low glycogen substrate availability at the start of anaesthesia, especially in type I fibres.

Physical exertion is another possible contributing factor to lack of substrate in type I and to a lesser extent, in type IIB fibres. Colic patients are often walked, under the course of many hours, before being transported to surgery. This kind of low intensity exercise could be responsible for glycogen depletion in type I fibres. Postural muscles, important in balancing in the trailer and standing up in the recovery box, contain relatively large proportions of type I fibres (Karlström,

1995), and therefore, transport to surgery could further deplete muscle glycogen reserves. Type IIB fibres can be thought to be active during panic preoperatively, e.g. when rolling and thrashing in pain. These fibres may even be involved postoperatively, when the horse is trying to stand up. Type IIA fibres are recruited primarily during moderate intensity exercise, and therefore it is not surprising that almost no low staining type IIA fibres were observed in any of the horses in this study at any time.

Another possible explanation for lack of substrate in type I fibres in patients could be decreased oxygen availability in muscle tissue. Hultman (1995) found that the rate of glycogenolysis increased in type I fibres (but not in type II fibres) after 30 seconds of anoxia. Many patients in this study had preoperative signs of compromised circulation, resulting from strangulating intestinal lesions, which could possibly have influenced glycogenolysis in peripheral muscle tissue during this time.

Horse 2g had the poorest physical status (V) of all patients, and concurrently, a plasma lactate value of 15.4 mmol/l at the start of anaesthesia, indicative of a poor prognosis. During anaesthesia, severe complications occurred and the horse died in recovery. A value of >7 mmol/l for plasma lactate indicates a poor prognosis (Donawick *et al.*, 1975) in colic patients. Plasma lactate values at start of anaesthesia (*cf.* Table 4) were raised in the four patients with most depressed physical status at arrival (2b, 2e, 2f, 2g). Svendsen, Hjortkaer & Hesselholt (1979) described plasma lactate as being a reliable indicator of poor surgical prognosis, as appears to be confirmed by this study.

Glycogen was available in ample quantities in all healthy horses as a substrate for energy release both during anaesthesia and in the recovery period, when much energy is needed to get up into a standing position after recumbency.

Three patients had minimal fibres of low staining intensity at start. This may be due to: a relatively short transportation distance (2a, 2b), good preoperative physical status (2a, 2c), or a short starvation period (2b) (*cf.* Table 1). The four remaining patients had a large percentage of fibres of low glycogen staining intensity at start in type I fibres and in one horse, even in type IIB fibres. Lack of substrate in these fibres may be a result of preoperative factors. Horse 2d had a poor appetite the last month due to a gastric ulcer, a race start one week prior to surgery with lameness thereafter, transport 320 km to surgery and 51 hours of preoperative starvation. Horses 2e, 2f and 2g had the poorest physical status of all patients. Horse 2f had foaled 4 days prior to surgery and developed peritonitis as a result of foaling complications. Horse 2g had severe signs of circulatory shock at the preoperative clinical examination.

After surgery (end biopsy), fairly similar glycogen staining patterns were seen in most patients compared to the start biopsies. In horse 2e, the Shetland pony, there was an increase in low staining type I and type IIB fibres between start and end of anaesthesia. For this horse, this may have been related to severe anaesthesia complications, including extremely low tissue oxygen saturation.

For those horses biopsied on day 2, no large changes in muscle glycogen content occurred compared to start and end biopsies. The three patients with many low staining fibres at end of anaesthesia showed minimal changes on day 2, which may have been related to low food intake after surgery. These results are consistent with studies of recovery of muscle glycogen substrate after racing (Hyypä, Räsänen & Pösö, 1997; Snow *et al.*, 1987). They have shown that more than 3 days are required for total recovery of muscle glycogen content. Furthermore, it was shown that low oral carbohydrate intake after racing delayed recovery of glycogen supply in muscle (Snow *et al.*, 1987). After surgery, colic patients are usually denied hay for at least 24 hours postoperatively, implying that a similar delay in glycogen recovery could occur, due to lack of carbohydrate supply.

One healthy horse contracted myositis in *M. triceps brachii* postoperatively, without signs from *M. gluteus medius* or evidence of lack of substrate in biopsies taken. Two other healthy horses had difficulty standing up in recovery, and all these horses were without notable depletion of glycogen substrate in any fibre types. Lack of glycogen substrate seen in some fibres in some of the patients was not related to any development of myopathy.

This study has shown that colic patients can have compromised energy substrate depots in muscle tissue, which could be a factor to consider during anaesthesia and in postoperative care.

Sammanfattning

Postoperativa komplikationer efter allmännarkos är vanligare hos häst jämfört med andra djurslag. En ofta förekommande komplikation är postoperativ myopati. Före en operation kan många kolikpatienter ha varit utan foder en längre tid, åkt långt i hästtransport, upplevt fysisk stress och smärta. Postoperativt kan fysisk stress förekomma i samband med att hästarna reser sig efter narkosen. Dessa faktorer skulle kunna påverka glykogenförråden i muskeln, eftersom glykogen är ett viktigt energisubstrat. Syftet med studien var att undersöka glykogeninnehållet i muskulaturens fibrer med histokemiska tekniker, innan och efter långvarig narkos, hos friska hästar och kolikpatienter.

Sju kolikpatienter och 5 friska hästar ingick i studien (narkoslängd >3 h). Patienterna hade ej utfodrats (9-51 h) innan operationen och de hade åkt hästtransport (53-320 km). De friska hästarna hade ej utfodrats (12 h) innan sövningen. Anestesi inducerades med guaifenesin/thiopental eller ketamin/diazepam och underhölls med isofluran och syrgas. Hästarna låg i ryggläge och andades spontant eller ventilerades mekaniskt. Intravenösa elektrolyter administrerades och vid hypotension gavs dextran eller dobutamin. Biopsier från *M. gluteus medius* togs vid induktion och vid slutet av narkos samt hos vissa hästar, 24h efter narkosens slut. Prover snittades i kryostat och färgades dels för myosin ATPas aktivitet efter preinkubering vid pH 4,6, för att identifiera typ I, IIA och IIB fibrer, och dels med Periodic Acid-Schiff's reagens, för att subjektivt utvärdera högt respektive lågt glykogeninnehåll.

De friska hästarna hade ett högt glykogeninnehåll i alla fibertyper vid alla biopsitagningsstillfällena. Tre av patienterna hade ett högt glykogeninnehåll i de flesta fibrer vid anestesiens induktion. Ett lågt glykogeninnehåll i ett flertal fibrer (framförallt typ I fibrer) sågs vid anestesiens induktion hos fyra patienter. Hos tre av dessa hästar hade också en del typ IIB fibrer ett lågt glykogeninnehåll. Förändringarna i glykogeninnehåll kvarstod direkt efter narkosens slut samt även 24 timmar efter resning. De hästar som hade sämst

preoperativ status var också de som uppvisade ett flertal fibrer med lågt glykogeninnehåll vid alla biopsitagningstillfällen.

Den begränsade glykogentillgången i fibrerna hos vissa av kolikpatienterna är troligen relaterat till preoperativa stressfaktorer. Denna studie har visat att glykogentillgången i en del muskelfibrer är begränsad hos vissa kolikpatienter inför narkos, vilket kan vara en faktor av betydelse för narkosens förlopp och den postoperativa återhämtningen.

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