Effects of Anaesthesia on Haemodynamics and Metabolism in Horses

Evaluated by Laser Doppler Flowmetry, Microdialysis and Muscle Biopsy Techniques

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Abstract

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Complications related to the musculo-skeletal system (fractures and post-anaesthetic myopathy; PAM) after anaesthesia are more common in the horse than in other species. PAM has been shown to be related to hypotension during anaesthesia, which results in hypoperfusion of the muscle and probably hypoxia or ischaemia.
In the present study peripheral perfusion in relation to central circulation was investigated during anaesthesia and in response to pharmacological provocations and during different modes of ventilation. The muscle metabolic response to anaesthesia was also evaluated. The effect of severe illness (colic) on metabolic parameters was studied before, during and after anaesthesia. Peripheral perfusion was assessed using laser Doppler flowmetry (LDF), and central circulation by conventional techniques. Muscle metabolism was studied with microdialysis, analysis of muscle biopsy specimens and biochemical markers in venous blood samples.
Peripheral perfusion was more closely related to cardiac output than to arterial blood pressure. Compared to spontaneous breathing, intermittent positive-pressure ventilation negatively affected both central and peripheral perfusion. Central circulation was better preserved but respiration was more depressed during propofol-ketamine anaesthesia than during isoflurane anaesthesia. In clinically healthy horses both inhalation anaesthesia and propofol-ketamine anaesthesia were associated with an anaerobic metabolic response characterised by increased lactate in plasma, muscle and dialysate and in some cases by decreased muscle phosphagen stores. No muscle metabolic differences were detected between the dependent and non-dependent muscles (muscle biopsy and microdialysis) during lateral recumbency. Many colic horses entered anaesthesia with profoundly affected metabolism and activation of both the carbohydrate and lipid metabolic pathways due to increased sympathetic output resulting from pain and insufficient circulation. The content of ATP in these horses was low at start of anaesthesia but anaesthesia did not further deteriorate the metabolic stress. Horses that died or were euthanised at an early stage had the highest concentrations of lactate in plasma, muscle and dialysate before and during anaesthesia. During the first week after anaesthesia the metabolism and electrolyte balance were affected in both healthy and colic horses, but to a greater extent in colic horses.

Keywords: anaesthesia, horse, perfusion, LDF, muscle, metabolism, microdialysis, colic

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”Think before you start to work and, while at work, keep thinking.”
Guido Gezelle (Belgium)

Contents

Abbreviations

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ASA	American Society of Anesthesiologists
ATP	Adenosine triphosphate
$C(a-\bar{v})O_2$	Arterio-venous oxygen content difference
CK	Creatine kinase
CP	Creatine phosphate
Cr	Creatine (muscle)
DO_2	Delivery of oxygen
d.w.	Dry weight
FFA	Free fatty acids
Hb	Haemoglobin
Hct	Haematocrit
HR	Heart rate
ICMP	Intracompartmental muscle pressure
IPPV	Intermittent positive-pressure ventilation
La/Py ratio	Lactate-to-pyruvate ratio
LDF	Laser Doppler flowmetry
MSAP	Mean systemic arterial pressure
MPAP	Mean pulmonary arterial pressure
PaO_2	Partial pressure of oxygen
$PaCO_2$	Partial pressure of carbon dioxide
PAM/PAL	Post-anaesthetic myopathy/lameness
PAP	Pulmonary arterial pressure
PVR	Pulmonary vascular resistance
$\dot{Q}t$	Cardiac output
$Qs/\dot{Q}t$	Venous admixture
RR	Respiratory rate
SAP	Systemic arterial pressure
SB	Spontaneous breathing
SV	Stroke volume
SVR	Systemic vascular resistance
V_E	Minute ventilation

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I: Anna Edner, Birgitta Essén-Gustavsson and Görel Nyman. 2005. Muscle metabolic changes associated with long-term inhalation anaesthesia in the horse analysed by muscle biopsy and microdialysis techniques.
Journal of Veterinary Medicine A 52, 99-107.

II: Anna Edner, Görel Nyman and Birgitta Essén-Gustavsson. 2002. The relationship of muscle perfusion and metabolism with cardiovascular variables before and after detomidine injection during propofol-ketamine anaesthesia in horses. *Veterinary Anaesthesia and Analgesia* 29, 182-199.

III: Anna Edner, Görel Nyman and Birgitta Essén-Gustavsson. 2005. The effects of spontaneous and mechanical ventilation on central cardiovascular function and peripheral perfusion during isoflurane anaesthesia in horses. *Veterinary Anaesthesia and Analgesia* 32, 136-146.

IV: Anna Edner, Görel Nyman and Birgitta Essén-Gustavsson. 2005. The metabolic responses to anesthesia and recovery in healthy horses in comparison to colic horses subjected to abdominal surgery. (Manuscript).

V: Anna Edner, Birgitta Essén-Gustavsson and Görel Nyman. 2005. The metabolic responses to anesthesia and recovery in healthy horses in comparison to colic horses subjected to abdominal surgery: A microdialysis study. (Manuscript).

Introduction

General background

Through evolution, the horse has become an amazing athlete. Its versatility allows it to perform successfully in the wide range of activities that its human caretakers invent. It runs fast in different gaits and for various distances, it jumps over obstacles sometimes as high as or higher than itself and it may be trained to do centred figures of dressage. The horse's ability to do all this originates from the need to be able to escape from predators and to protect itself and its offspring. Being a potential prey, the wild, adult horse rarely lies down and if it does, it is only for very short periods. But even the domestic horse spends only short periods lying down and prefers to rest on its sternum rather than lying in the lateral position. A healthy horse never lies on its back and only spends short periods (mean 23 minutes) in lateral recumbency (Littlejohn & Munro, 1972). Hall (1984) found that ponies that had been trained to lie down in the lateral position on command resisted lying down for more than 20 minutes in lateral recumbency and got up on their feet despite opposite instructions. Hall's own theory as to why the ponies refused to continue lying down was that possible changes in lung volume or the development of muscle pain due to compression ischaemia in the lowermost, dependent muscles might have caused discomfort.

There are situations, however, when we are forced to place the horse on its side or on its back, sometimes for some hours, for example when we anaesthetise the horse for special medical investigations such as for radiology or for surgery. Not surprisingly, general anaesthesia is associated with a higher risk of complications in the horse than in other domestic species that are routinely anaesthetised. In the healthy horse, anaesthesia is associated with a mortality of approximately 0.6-0.9% (Tevik, 1983; Young & Taylor, 1993; Mee et al., 1998a; Johnston et al., 2002). In horses undergoing emergency abdominal procedures the corresponding figures are 4.3 to 7.9% (Mee et al., 1998b; Johnston et al., 2002). These figures may be compared with the anaesthetic mortality in dogs and cats, which ranges from 0.01-0.4% in different studies, with the lower mortality in cats (Clarke & Hall, 1990; Gaynor et al., 1999; Dyson, 2000). Non-fatal complications in the horse are supposedly much more frequent than fatal complications, but they are much more difficult to assess. Common intra-operative complications in the horse include hypotension, hypoxaemia and hypercapnia. In the recovery phase, myopathies, fractures and cardiac arrest predominate. The total frequency of post-anaesthetic myopathy (PAM) or lameness (PAL) varies between 0.9 and 8% in different reports (Johnson et al., 1978; Klein, 1978; Serteyn, 1988; Richey et al., 1990; Rijkenhuizen & van Dijk, 1998). Further, approximately one-third of the anaesthesia-related mortality may be assigned to fractures and myopathies occurring during recovery (Johnston et al., 2002).

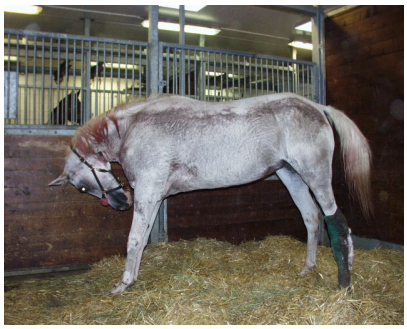



Figure 1A: A Thoroughbred stallion with severe post-anaesthetic myopathy (PAM) in the left triceps, infraspinate and supraspinate muscles and with raised plaques on the chest. The horse has adopted the characteristic pose for a horse with forelimb PAM: the shoulder is dropped and the forefoot is non-weightbearing.
Figure 1B: An Arabian mare with PAM in the triceps muscles of both forelimbs. The horse is in severe pain and has adopted a saw-horse position.

Post-anaesthetic myopathy

Post-anaesthetic myopathy (Figure 1A-B) in the horse was described in the literature nearly 100 years ago (Dollar, 1920) and has since been documented in several reports (Trim & Mason, 1973; Waldron-Mease, 1977; Johnson et al., 1978; Klein, 1978; White, 1982; Grandy et al., 1987; Serteyn et al., 1987b; Dodman et al., 1988; Lindsay et al., 1989; Richey et al., 1990). The most commonly affected muscles in lateral recumbency are the triceps, supra- and infraspinate and masseter muscles and the muscles covering the rib cage, and in dorsal recumbency the gluteus and the longissimus. The gluteus may, however, be affected during lateral recumbency (Branson et al., 1992) and the triceps during dorsal recumbency (Rijkenhuizen & van Dijk, 1998). Other affected muscles are the adductor muscles of the hind limbs and the pectoral muscles (Friend, 1981; Dodman et al., 1988). It is often but not always the muscles facing the table (dependent muscles) that are affected (Branson et al., 1992; Rijkenhuizen & van Dijk, 1998).

PAM may develop in any type of horse, but there are few reports of its occurrence in the very young horse (Short & White, 1978; Manning et al., 1995). The symptoms depend on the extent of the muscular lesions, but include a reduced ability to bear weight on the affected limb, swelling, pain and distress. Single or several muscle groups may be affected and a generalised condition may develop (Waldron-Mease, 1978; Friend, 1981). Most often, the horse still manages to stand up after anaesthesia, but some cases end with the horse being euthanised because of inability to regain the standing position. The treatment is symptomatic and aims at keeping the horse calm and free from pain and maintaining the circulation. The prognosis is usually good and most symptoms resolve within a few days. But since PAM increases suffering and the cost and prolongs hospitalisation, much effort has been made to elucidate the course of events leading to this complication.

Several contributing factors have been encountered, including malignant hyperthermia, hypotension, reduced muscle perfusion, the weight of the horse, the feeding regimen, the muscle mass (breed, training status), patient positioning and

padding, prolonged recumbency, and reperfusion after a period of hypoperfusion (Waldron-Mease, 1977; Waldron-Mease, 1978; Lindsay et al., 1980; Grandy et al., 1987; Lindsay et al., 1989; Richey et al., 1990; Serteyn et al., 1990; Taylor & Young, 1990; Serteyn et al., 1991; Serteyn et al., 1994). At present the main hypothesis is that of a reduced blood flow through the muscles during anaesthesia, leading in some instances to a hypoxic or ischaemic condition. Anaerobic metabolism becomes the source of energy with subsequent production of lactate and a lowered intracellular pH, eventually resulting in membrane damage. In some cases the most serious cellular lesions develop during reperfusion rather than during the ischaemic period (Ames et al., 1968; Sjöström et al., 1982). During reperfusion, free radicals may be formed and account for some of the membrane damage (Ames et al., 1968) and there are equine studies that support this latter theory (Serteyn et al., 1990; Serteyn et al., 1994).

One of the intriguing questions is how and why muscular ischaemia develops in some horses but not in others, which seemingly have had identical anaesthetic procedures. Another question is why horses that have had extremely low partial pressures of oxygen in the arterial blood may recover uneventfully from anaesthesia while other well-oxygenated horses may develop myopathy. A common denominator in many cases of equine myopathy seems to be the occurrence of prolonged periods of hypotension. Several studies have shown a higher incidence of PAM after hypotensive anaesthesia (Klein, 1978; Grandy et al., 1987; Lindsay et al., 1989; Richey et al., 1990; Branson et al., 1992).

Halothane and isoflurane, which are the most commonly used anaesthetics for maintenance anaesthesia in horses, profoundly affect the central circulation by decreasing cardiac output and affecting the vascular bed (Stevens et al., 1971; Steffey & Howland, 1978; Steffey & Howland, 1980; Franke & Endrich, 1983; Greenblatt et al., 1992G). From this follows that hypotension is a common clinical complication during inhalation anaesthesia in the horse (Steffey & Howland, 1978; Taylor & Young, 1993).

Hypotension results in a reduced driving pressure for blood flow through the capillary bed. If the pressure within the muscle (intracompartmental muscle pressure; ICMP) increases while the mean arterial blood pressure remains constant or decreases, the capillary blood flow through the tissue will eventually become restricted and a compartment syndrome will evolve (Heppenstall et al., 1988). An increase in ICMP and a resultant compartment syndrome may develop after trauma to the muscle, local bleeding or oedema, or reduction of venous drainage, or during compression of the muscle (Heppenstall et al., 1988; Mubarak et al., 1989). The two latter events may occur during equine anaesthesia, either in the dependent muscle groups due to poor padding or in any muscle due to poor positioning of the horse (Redfern et al., 1973; Lindsay et al., 1985; White & Suarez, 1986; Norman et al., 1989; Taylor & Young, 1990). Hypotension which results in a reduced capillary driving pressure will render the muscle even more vulnerable to the compressive forces exerted by the horse's own weight (Matsen et al., 1979).

Measurement of intramuscular blood flow

During equine anaesthesia, intramuscular blood flow has been measured or estimated by means of Xe^{133} clearance (Weaver & Lunn, 1984), injection of radio-labelled microspheres (Staddon et al., 1979; Manohar et al., 1987a; Manohar et al., 1987b) and laser Doppler flowmetry (LDF) (Serteyn et al., 1986; Serteyn et al., 1987a; Still et al., 1996; Lee et al., 1998a; Lee et al., 1998b; Raisis et al., 2000a; Raisis et al., 2000b; Raisis et al., 2000c).

The Xe^{133} clearance technique is based on injection of the radioactive substance into the organ of interest and measurement of the substance clearance rate (Lassen et al., 1964; Kjellmer et al., 1967). The readings are continuous and yield absolute values for local blood flow in mL/min/100 g tissue and are therefore directly comparable. However, a radioactive substance has to be handled.

For measurement of organ blood flow by the radioactive microsphere technique, the animal has to be sacrificed to obtain the results, limiting the use of this method. The method involves injection of radio-labelled microspheres into the left ventricle of the heart, where they mix with the blood before being distributed to tissues. The microspheres are trapped in the capillaries in proportion to the tissue's rate of perfusion (Wagner et al., 1969). The concentration of microspheres in the tissue is determined by measuring the radioactivity. If cardiac output is measured simultaneously, calculation of the tissue perfusion in mL/min/100g tissue is possible. The blood flow is measured over a whole muscle or organ, and by using differently labelled microspheres several measurements may be performed (Manohar et al., 1987a). The disadvantage of this technique is that it is an invasive and terminal procedure requiring the handling of a radioactive substance.

Laser Doppler flowmetry is a relatively novel method for measuring blood flow (Holloway & Eatkins, 1977; Öberg et al., 1984). It is technically easy and the blood flow can be measured either on organ surfaces or within an organ. Depending on the tissue of interest, the technique can be minimally invasive and readings are obtained continuously and on line. It is further described below.

Laser Doppler flowmetry

The technique relies on the phenomenon of Doppler shift. A beam of laser light (780 nm), carried by a fibre-optic probe, is widely scattered and partly absorbed by the tissues being studied. Light hitting moving cells undergoes a shift in wavelength (Doppler shift), while light hitting static objects remains unchanged. The magnitude and frequency distribution of these changes in wavelength are directly related to the number and velocity of blood cells, but unrelated to their direction of movements. The information is picked up by a returning fibre, converted into an electronic signal and analysed (Nilsson et al., 1980; Öberg et al., 1984). The principle of laser Doppler measurement is illustrated schematically in Figure 2. The recordings are relative and are expressed on an arbitrary scale. LDF does not therefore provide an absolute value of perfusion in mL blood/mg tissue/unit of time. To allow comparison of different measurements, the probes can be calibrated in a standard motility solution before use. The measured volume of

tissue is approximately 1 mm^3 around the probe end, but this depends on the probe used and the properties of the tissue.

For measurement of skin perfusion, the probe is placed directly onto the hairless, cleaned skin. When measuring intramuscular perfusion a probe extension is used, consisting of a thin, optic fibre, which will act as a very narrow but thick window allowing light to pass in both directions. Recordings are transferred to a computer and visualised as on-line graphs with commercial software provided by the manufacturer. The software is later used for analysis of data.

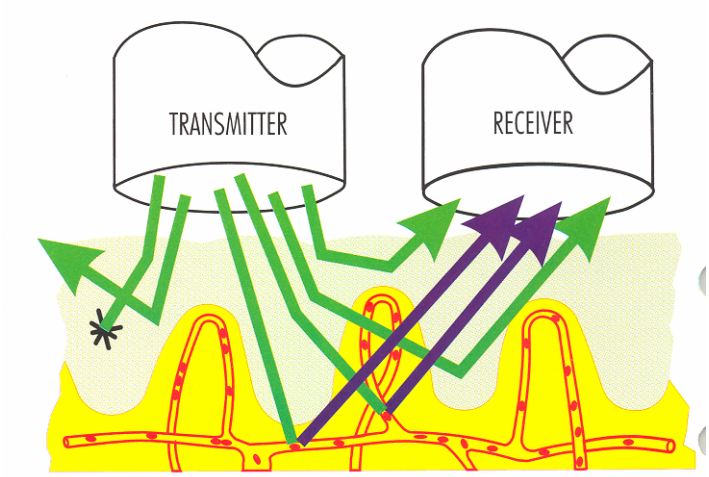


Figure 2: The principle of laser Doppler flowmetry. A beam of laser light carried by a fibre-optic probe is widely scattered and partly absorbed by the tissue. Light hitting the moving red blood cells undergo a Doppler shift (purple arrows), while light hitting static objects is unchanged. The information is picked up by a returning fibre, converted into an electronic signal and analysed. Figure from Perimed, Järfälla, Sweden.

Previous results from muscle blood flow measurements

With the microsphere and Xe^{133} clearance techniques, the muscular blood flow during inhalation anaesthesia in the horse has been found to be reduced compared to that in the awake horse (Weaver & Lunn, 1984; Manohar et al., 1987a; Manohar et al., 1987b; Goetz et al., 1989). Isoflurane reduces muscular blood flow to a lesser extent than halothane (Serteyn et al., 1987a; Sigurdsson et al., 1994). In a study by Lee et al. (1998a), a change from isoflurane to halothane resulted in a decrease in blood flow in the triceps brachii, while no alteration in blood flow was seen when isoflurane replaced halothane. Some intravenous agents used for induction of anaesthesia have less depressant effects on the circulation compared to inhalation agents (Wong & Jenkins, 1974; Hubbell et al., 1980; Matthews et al., 1991; Tanaka & Nishikawa, 1994), but since most of the potentially stimulatory physiological effects of different induction agents will be overridden by the depressant effects of the subsequent inhalation agent (Taylor, 1991; Taylor & Young, 1993), total intravenous anaesthesia (TIVA) has gained increasing attention during the last decades.

In many ways TIVA has been reported to be superior to inhalation anaesthesia. To a degree depending on the combination of anaesthetic agents used, TIVA induces less stress (Taylor, 1989a; Taylor, 1990; Luna et al., 1996) and may offer additional analgesia (Mayer et al., 1990; Guit et al., 1991), and compromises the central circulation to a lesser extent (Young et al., 1993; Nolan et al., 1995; Luna et al., 1996; Marntell & Nyman, 1996). Also, there are indications that the mortality risk is reduced during TIVA as compared to that during inhalation anaesthesia (Johnston et al., 2002). Many different combinations and infusion rates of anaesthetics have been tried in TIVA. Common in various combinations are alpha-2 receptor agonists, ketamine, guaifenesin, propofol and benzodiazepines. Up to now and to the best of my knowledge, however, there are no reports on intramuscular blood flow during TIVA.

ICMP has been found to be higher in dependent than in non-dependent muscles in the anaesthetised horse (Lindsay et al., 1980; Lindsay et al., 1985; McDonell et al., 1985; Lindsay et al., 1989), and it has been hypothesised that the muscle microcirculation is reduced to a greater extent in dependent muscles. Results from comparisons of blood flow between dependent and non-dependent muscle groups have been conflicting. Differences have been found in some studies (Serteyn et al., 1986; Serteyn et al., 1991), but not in others (Staddon et al., 1979; Weaver & Lunn, 1984; Manohar et al., 1987a; Goetz et al., 1989).

Muscle metabolism during anaesthesia

Blood sampling and measurements of metabolites and other markers is a widely used method for evaluating muscle metabolism and damage (Clarke, 1973; Johnson et al., 1978; Stjernström et al., 1981b; Fiacchino et al., 1989; Manfredi et al., 1991; Aktas et al., 1997). Most often samples are withdrawn from a large vein. This is a simple method and because of the size of the horse, sampling frequency is usually not a restricting factor. The drawback is that the measured analytes represent a mean of the whole body and significant changes in limited areas may go unrecognised or will be underestimated. Unless a tissue-specific analyte is measured, its origin is not clear. Despite the fact that PAM is a muscular problem, few studies have actually addressed the muscle metabolic events in association with anaesthesia by examination of the muscle itself. Elevated plasma concentrations of lactate during and after equine anaesthesia have frequently been observed (Robertson, 1987; Lindsay et al., 1989; Taylor, 1989b; Serteyn et al., 1991; Taylor, 1991; Serteyn et al., 1994; Luna et al., 1996) and most authors have considered that this lactate is produced in muscle. Hitherto no one has actually measured the lactate concentration within the muscle itself. It is therefore of prime interest to investigate the biochemical changes in muscle during and after anaesthesia.

Analyses of muscle biopsy samples and of muscle interstitial fluid through microdialysis are two methods by which muscle metabolism may be studied more directly.

Muscle biopsy sampling

Muscle biopsy sampling with measurements of metabolites and markers of energy balance is a technique that is frequently employed in equine exercise research (Essén-Gustavsson et al., 1984; Gottlieb et al., 1988; Harris et al., 1991; Hyyppä et al., 1997; Schuback & Essén-Gustavsson, 1998; Essén-Gustavsson & Jensen-Waern, 2002) and has been used to some extent in anaesthesia studies in humans (Bergström, 1962; Bergström et al., 1965; Stjernström et al., 1981a). Muscle specimens have also been analysed in anaesthesia studies in rats (McLoughlin et al., 1987; Ferreira et al., 1998). Most biopsy samples that have been obtained in horses in association with anaesthesia have been obtained from myopathy cases and prepared only for histological evaluation. The findings were focal and included acute oedema, hyaline degeneration, vacuolisation, fibre necrosis and mineralisation (Friend, 1981; White, 1982; Dodman et al., 1988; Norman et al., 1989), and resembled alterations seen during rhabdomyolysis (Lindholm et al., 1974). There is only one report of results from biochemical analysis (glycogen) of muscle samples in association with anaesthesia (White & Short, 1978). That study was performed on three horses and the findings were inconclusive.

The needle biopsy technique (Figure 3) is usually well tolerated by the awake horse and may be performed after local analgesia of the skin and fascia (Lindholm & Piehl, 1974; Larsson & Hultman, 1979).

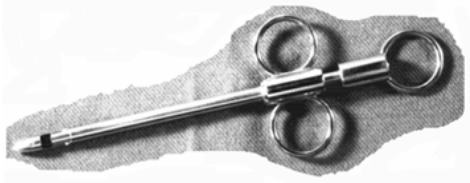


Figure 3: A biopsy needle for percutaneous sampling of muscle tissue.

In studies performed at the equine clinic at SLU, repeated biopsies have been obtained from the same incision without untoward effects (Valberg et al., 1985; Gottlieb, 1989; Schuback & Essén-Gustavsson, 1998). Needle biopsy samples allow direct interpretation but only give a snapshot view of the muscle metabolic events. Therefore, biopsy sampling is often performed repeatedly. To be able to compare results, biopsy sampling should be standardised regarding area and depth (Kline & Bechtel, 1988). The gluteal muscle is one of the most frequently studied muscles in the horse (Lindholm & Piehl, 1974; Snow & Guy, 1980; Essén-Gustavsson et al., 1984; Valberg et al., 1985; Snow et al., 1986; Hyyppä et al., 1997; Schuback & Essén-Gustavsson, 1998; Essén-Gustavsson & Jensen-Waern, 2002), since it is an extensor of the hip and together with the longissimus dorsi muscle is a major muscle in propulsion (Sisson, 1975; Snow & Guy, 1980). The gluteus muscle is probably of great importance for the horse when it has to regain the standing position after lying down during anaesthesia (Sisson, 1975). In addition, the gluteus medius is affected by PAM (Branson et al., 1992; Rijkenhuizen & van Dijk, 1998).

Microdialysis

Microdialysis offers a possibility of studying the metabolic events of the muscle interstitium through the fact that extracellular fluid is sampled almost continuously and for many hours with a minimal amount of trauma (Ungerstedt, 1991). It has become a frequently used research tool for studies of many different tissues in animals (Ingvast-Larsson et al., 1992; Fuchi et al., 1994; Stallknecht et al., 1999; Ungerstedt et al., 2003) and humans (Ungerstedt & Hallström, 1987; Rosdahl et al., 1998; Ettinger et al., 2001) and especially in research on ischaemia (Müller et al., 1995; Jansson et al., 2003; Ungerstedt et al., 2003; Östman et al., 2004).

Microdialysis was originally developed as a method for measuring neurotransmitters of the brain (Delgado et al., 1972; Ungerstedt & Hallström, 1987; Ungerstedt, 1991). Since then, a wide range of probe types have been introduced, allowing its use in several different organs and body compartments (Ingvast-Larsson et al., 1991; Ungerstedt, 1991Rosdahl, 1993 #408; Henriksson, 1999; Stallknecht et al., 1999). The principle of microdialysis is that the function of a blood capillary is mimicked by passive exchange of molecules across a membrane and along a concentration gradient (Figure 4A).

The microdialysis catheter is continuously perfused with a solution (termed perfusate) by means of a syringe pump. Dialysis occurs at the membrane end of the catheter and the returning fluid, called dialysate, is collected in microvials attached to the outlet tubing (Figure 4B). The pore size of the dialysis membrane determines which molecules may pass through the membrane. Since substances added to the perfusate will diffuse into the interstitial space, the local effect of pharmacological substances can also be studied (Hagström-Toft et al., 1998). The achieved concentration of the analytes in the microdialysis sample is dependent on the degree of equilibration between the perfusate and interstitial fluid. At complete equilibration the concentration in the dialysate will equal that in the interstitium. The recovery of substances depends on the rate of perfusion, membrane length, cut-off point and the diffusion characteristics of the substance and tissue (Ungerstedt, 1991). At 0.3 μL/min the recovery relative to the interstitial fluid concentration approaches 100% for lactate, glycerol and urea and 90% for glucose (Rosdahl et al., 1998). With very low perfusion rates, fluid loss may be substantial. By adding a colloid such as dextran to the perfusate, this problem may be overcome (Rosdahl et al., 1997; Hamrin et al., 2002).

Microdialysis seems to be a valuable tool in studies of metabolism in association with anaesthesia, since it can be used in clinical situations with a minimum of trauma. The advantages of this technique are that metabolic changes can be measured almost continuously and basically on line for extended periods without adverse effects. Up to now there have been no reports on the use of microdialysis in muscle in the anaesthetised horse.

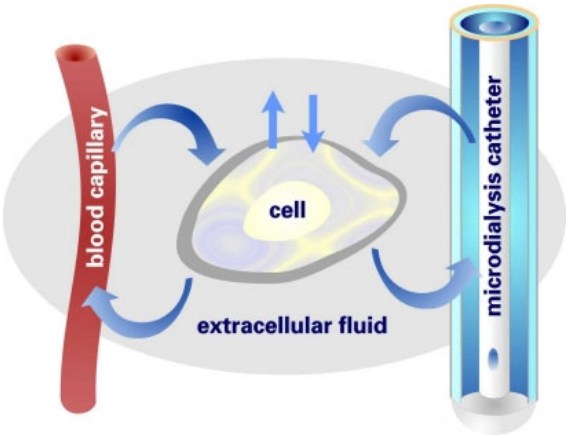


Figure 4A: The principle of microdialysis is to mimic the function of a blood capillary. The membrane is perfused with a solution that resembles the interstitial fluid. Water-soluble molecules in the interstitial (extracellular) fluid diffuse across the dialysis membrane into the perfusate. Diffusion occurs along a concentration gradient of the molecules in the interstitial fluid and the perfusate. With a long enough membrane and a slow rate of perfusion, the two compartments will equilibrate in regard to the concentration of water-soluble molecules. Figure from CMA/Microdialysis AB, Stockholm, Sweden.

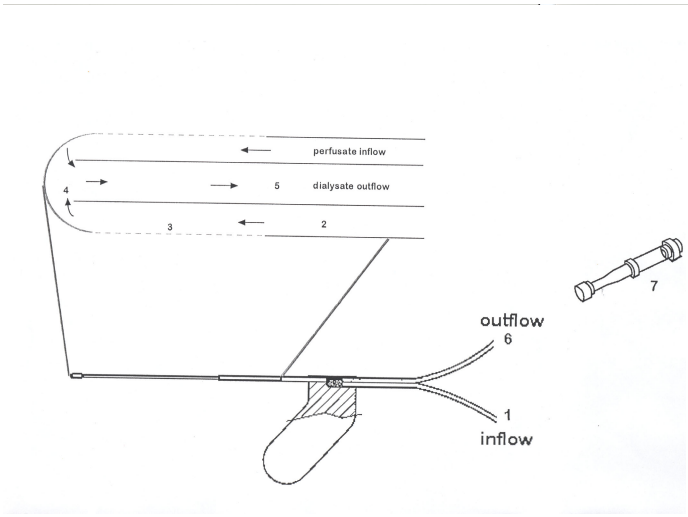


Figure 4B: A schematic illustration of a microdialysis catheter as used in the present study. Perfusate enters the catheter through the inflow tubing (1) and flows between the outer dialysis membrane and the inner shaft tubing (arrows pointing left). At the proximal end the perfusate enters through a hole in the inner shaft tubing (arrows pointing right) and is then propelled through the outflow tubing and collected in a microvial (7). Reproduced with kind permission of Hans Rosdahl (Rosdahl, 1998).

Aims of the investigation

The principal aim of this work was to study peripheral perfusion and muscle metabolism during and after anaesthesia in horses. A further aim was to determine whether peripheral changes in perfusion and metabolism were reflected by corresponding changes in central circulation and blood analytes.

The specific aims were:

- to analyse muscle biopsy samples and use the muscle microdialysis technique as complements to measurements of analytes in the blood as means of studying muscle metabolism (Studies I, II, IV and V);

- to use laser Doppler flowmetry as a means of studying peripheral perfusion in muscle and skin during anaesthesia (Studies I, II and III);

- to study muscle perfusion
  - in the dependent and non-dependent gluteus with the horse placed in lateral recumbency (Studies I and II);
  - in relation to central circulation during anaesthesia in response to pharmacologically induced cardiovascular provocations and with different modes of ventilation (Studies I, II and III);

- to study muscle metabolism
  - in the dependent and non-dependent gluteus with the horse placed in lateral recumbency (Studies I and II);
  - to examine the possible effects of inhalation anaesthesia and total intravenous anaesthesia on muscle metabolism (Studies I and II);

- to study the effects of abdominal surgery on metabolism in colic horses in comparison to the effects of anaesthesia alone in healthy horses, and to examine the effects of surgery or anaesthesia alone on recovery up to seven days after anaesthesia (Studies IV and V).

Materials and methods

Horses

The horses in Studies I-III were all healthy Standardbred trotters owned by the Department of Large Animal Clinical Sciences, SLU, Uppsala, Sweden. Study IV comprised 20 healthy University-owned Standardbred trotters and 20 privately owned horses of various breeds, from a Shetland pony to large Swedish Warmblooded horses. These were referred to the university equine clinic because of unresolved colic. After arrival at the clinic, they were examined and treated according to the decision of the clinician on duty. When surgery had been decided upon, the horse owner was asked for permission to allow the horse to enter the study and written consent was obtained. There was no selection of cases and the study was performed with as little interference as possible with the treatment and care of the horses. Study IV was performed in parallel with Study III such that 9 of the horses in Study III underwent sampling at the same time for participation in Study IV. Study V was performed on 10 of the healthy and 10 of the colic horses included in Study IV.

Table 1. Summarised data for the horses investigated in the respective studies.

Study	Group	No.	Breed	Age years	Weight kg	Sex
I	Halo	7	Stb Tr.	9 (4-19)	489 (430-530)	1 mare, 6 geldings
	Iso	6	Stb Tr.	7 (2-13)	467 (375-548)	4 mares, 1 gelding, 1 stallion
II		7	Stb Tr.	7 (5-12)	492 (416-581)	3 mares, 4 geldings
III		10	Stb Tr.	5 (4-8)	492 (420-584)	5 mares, 5 geldings
IV	Healthy	20	Stb Tr.	8 (3-19)	495 (411-584)	12 mares, 8 geldings
	Colics	20	Various	11 (2-22)	527 (230-698)	10 mares, 8 geldings, 2 stallions
V	Healthy	10	Stb Tr.	7 (4-17)	503 (428-584)	5 mares, 5 geldings
	Colics	10	Various	10 (3-15)	520 (230-695)	4 mares, 5 geldings, 1 stallion

Age and weight are given as the group mean with the range within parentheses. Halo = halothane-anaesthetised horses; Iso = isoflurane-anaesthetised horses; Stb Tr. = Standardbred trotter. See respective papers for a specification of the different breeds of horses in Studies IV-V. A total of 53 horses were used in 61 anaesthetic procedures.

Procedures

In the present investigation LDF, microdialysis, and blood and muscle biopsy sampling were used to assess muscle and skin perfusion and muscle metabolism. In addition, central circulation and respiration and gas exchange were studied using conventional techniques.

Peripheral perfusion and central circulation were studied during inhalation anaesthesia and TIVA and during spontaneous breathing and intermittent positive-pressure ventilation (IPPV). Muscle metabolism was studied during and after inhalation anaesthesia and TIVA in lateral recumbency in healthy horses and after inhalation anaesthesia in dorsal recumbency in healthy and in colic horses subjected to abdominal surgery. The first studies allowed evaluation of muscle microdialysis and muscle biopsy sampling as methods of studying muscle metabolism during and after anaesthesia in horses and the feasibility of using these techniques in a patient material.

In Study **I** the effect on muscle metabolism of long-term inhalation anaesthesia using halothane in one group of horses and isoflurane in another group was investigated. All horses were placed in lateral recumbency and were breathing spontaneously. Both groups were subjected to students' surgical training and were euthanised at the end of anaesthesia. In the halothane group, surgery was performed during the study, while in the isoflurane group, experimental anaesthesia preceded surgical training. The horses of the latter group were therefore kept anaesthetised after the end of the research protocol. In the halothane group muscle biopsy specimens were taken and venous blood was sampled after induction and at the end of the anaesthetic procedure. In the isoflurane horses similar samples were obtained before induction and at the end of the experimental procedure (in Table 2 given as Anaesthesia duration) and repeated blood samples were taken during anaesthesia. In this group microdialysis was performed, starting before induction and continuing until the horses were euthanised (i.e. beyond true experimental time). During experimental anaesthesia cardiovascular provocations were performed. The first provocation was a 30-minute infusion of dobutamine (0.5 μg/kg/min) beginning 60 minutes after the start of inhalation anaesthesia. The second provocation was an intravenous (IV) injection of detomidine (10 μg/kg) given 60 minutes after the end of dobutamine infusion. The last provocation was an IV injection of acepromazine (0.05 mg/kg) administered 60 minutes after detomidine. Central cardiovascular measurements and measurements of peripheral perfusion in the gluteal muscle and on the skin using LDF were also performed. These results are not reported in Paper I but are given in the results section of this thesis.

Study **II** aimed at achieving a holistic view of the effects of propofol-ketamine anaesthesia on the central circulation, peripheral perfusion and muscle metabolism. The effects of a cardiovascular provocation with detomidine were also investigated. Muscle biopsy samples were obtained the day before anaesthesia, at the end of anaesthesia and the day after the experimental procedure. Blood was sampled and central haemodynamic and respiratory measurements were made at set time points before and during anaesthesia, after the provocation, during

recovery when the horse was still recumbent, and after recovery to standing. Blood was also sampled the day after anaesthesia. Peripheral perfusion was measured continuously during anaesthesia, using laser Doppler flowmetry.

Table 2. An overview of the different procedures of each study.

Study	Premed	Induction	Anaesthesia	Anaesthesia duration	Provocation	Samples
I Halo	Ace Rom Meth	GG+Thio	Halothane	436±25		Muscle Blood
I Iso	None	GG+Thio	Isoflurane	321±15	Dobutamine Detomidine Ace	Muscle Blood M.d LDF
II	None	GG+Prop	Ketamine-Propofol infusion	117±7	Detomidine	Muscle Blood LDF Central circulation
III	Det	GG+Thio	Isoflurane	249±13	SB/IPPV	Blood LDF Central circulation
IV Healthy	Det	GG+Thio	Isoflurane	228±26	None	Muscle Blood
IV Colics	Various	GG+Thio GG+Ket α2+Ket	Isoflurane	183±62 (45-300)	Abdominal surgery	Muscle Blood
V Healthy	Det	GG+Thio	Isoflurane	230± 23 (193-273)	None	Blood M.d
V Colics	Various	GG+Thio GG+Ket α2+Ket	Isoflurane	208± 53 (145-300)	Abdominal surgery	Blood M.d

The duration of anaesthesia is given in minutes with mean ± standard deviation, range within parenthesis. Abbreviations: Ace=acepromazine; α2=α2-receptor agonist; Det=detomidine; Meth=methadone; GG=guaifenesin; Thio=thiopentone; Prop=propofol; Ket=ketamine; SB=spontaneous breathing; IPPV=intermittent positive-pressure ventilation; Muscle=muscle biopsy; Blood=blood sample; M.d=microdialysis; LDF=laser Doppler flowmetry; Halo = halothane-anaesthetised horses; Iso = isoflurane-anaesthetised horses.

In Study **III** the effects of spontaneous breathing and mechanical ventilation on the central circulation and peripheral perfusion were investigated. Horses were allocated to receive either IPPV before spontaneous breathing (SB) or SB before IPPV. Venous blood for determination of Hb, Hct and lactate was obtained during anaesthesia.

Studies **IV** and **V** addressed the effects of colic and colic surgery on metabolic parameters in comparison with the effect of anaesthesia in dorsal recumbency in

healthy horses. Metabolic parameters were studied in the blood during the period from before anaesthesia to the end of one week post-anaesthesia. Muscle biopsy samples were obtained at the beginning and end of anaesthesia, and one hour and one day after recovery. Microdialysis of the gluteal muscle was performed during anaesthesia and for as long as the catheter was functioning up to 24 hours after recovery from anaesthesia.
The horses were classified according to the system of the American Society of Anesthesiologists (ASA; 1-5) where ASA 1 describes a clinically healthy individual and ASA 5, a moribund patient, not expected to survive 24 hours with or without surgery.

In the healthy horses in Studies I-V food was withheld for 10-12 hours prior to anaesthesia, but access was given to straw bedding and water. The colic horses of Studies IV and V were starved or fasted for a period of time that depended on their diseased state. The total known duration of colic varied between 3 hours and 1.5 days, with a median of 14 hours.

Instrumentation and measurements of central haemodynamic parameters

The horses in Study II were instrumented prior to induction of anaesthesia, while in those of the other studies instrumentation was performed after the commencement of inhalation anaesthesia.

Awake instrumentation was carried out aseptically in the unsedated, standing horse after local analgesia with lidocaine (Xylocain® 2%, Astra, Södertälje, Sweden). An arterial catheter was placed in a facial artery (Hydrocath™ arterial catheter, Ohmeda, UK). A 7F thermodilution catheter (Swan-Ganz, Edwards Laboratory, Santa Ana, CA, USA) was introduced percutaneously into the right jugular vein using the Seldinger technique (Seldinger, 1953) and advanced until the tip reached the pulmonary artery. A pig-tail catheter (Cook Europe A/S, Söborg, Denmark) was introduced approximately 8 cm proximal to the thermodilution catheter, advanced until the tip reached the right ventricles and then retracted into the right atrium. The positions of the catheters were verified by the observation of characteristic pressure waves. The catheters were locked in position with Luer-lock adapters. In the isoflurane-anaesthetised horses in Studies I and III a facial artery catheter, a pig-tail catheter and a thermodilution catheter were placed as described during the first 30-40 minutes of inhalation anaesthesia. In Studies IV and V a facial artery catheter (Insyte-W, Becton Dickinson, Infusion Therapy AB, SE –251 06 Helsingborg, Sweden) was placed after the beginning of inhalation anaesthesia.

Cardiac output ($\dot{Q}$ t) was determined by the thermodilution technique. A bolus of ice-cold 0.9% saline was rapidly injected and $\dot{Q}$ t was calculated by a cardiac output computer (Edwards Cardiac Output Computer 9520 A, Santa Ana, CA, USA).

Systemic and pulmonary arterial blood pressures (SAP and PAP) were measured invasively by connecting the transverse facial and pulmonary arterial catheters to pressure transducers (Baxter Medical AB, Eskilstuna, Sweden) positioned at the level of the scapulo-humeral joint in the standing horse, the sternal manubrium in the laterally recumbent horse and the thoracic inlet in the dorsally recumbent horse.

The pressure transducers were calibrated beforehand at atmospheric pressure (0 mmHg), and at 100 mmHg using a pressure manometer.

In Studies II and III and in the isoflurane group in Study I blood pressures and an electrocardiogram (ECG: base-apex lead) were recorded and the heart rate (HR) was determined on a Sirecust 730 (Siemens-Elema, Solna, Sweden). Arterial blood pressure and ECG in Studies IV-V were monitored on a Datex Light (Datex Engström Instrumentation Corporation, Helsinki, Finland).

Systemic (SVR) and pulmonary (PVR) vascular resistances and stroke volume (SV) were calculated as follows:
SVR (mmHg L/min)= mean SAP (mmHg)/ $\dot{Q}$ t (L/min)
PVR (mmHg L/min)= [mean PAP-diastolic PAP (mmHg)]/$\dot{Q}$ t (L/min).
Diastolic PAP was used in the formula as a substitute for wedge pressure (Bonagura & Muir, 1991).
SV (L/beat)= $\dot{Q}$ t/HR.

Respiratory and blood gas measurements

Respiratory rate (RR) was measured by observing costo-abdominal movements. In Study II minute volume (V_E) was determined by collecting exhaled air into a weather balloon over one minute for measurement with a Tissot spirometer (Collins Inc., Braintree, MA, USA). Inspiratory and end-tidal expiratory carbon dioxide ($ETCO_2$) were sampled from the mouthpiece of the tracheal tube and their concentrations were measured with an anaesthetic and O_2 -gas analyser (Servo Gas Monitor 120, Siemens-Elema, Sundbyberg, Sweden). In Studies III-V tidal volume (V_T), inspiratory oxygen, $ETCO_2$ and peak inspiratory pressure were measured using side stream spirometry (Capnomac Ultima, Datex Engström Instrumentation Corporation, Helsinki, Finland). The samples were collected at a customer-designed mouthpiece (Erkki Heinonen) attached to the end of the endotracheal tube. The gas analyser was calibrated prior to each experiment for O_2, CO_2 and isoflurane, using room air and the manufacturer's calibration gas mixture. Minute ventilation (V_E) was calculated from V_T x RR. Measurements were made at body temperature.

Blood for blood-gas analysis was drawn into heparinised syringes and analysed for oxygen and carbon dioxide partial pressures (PO_2, PCO_2) and pH with a standard electrode technique within 10 minutes of sampling (ABL™5, Radiometer, Copenhagen, Denmark). Oxygen saturation ($SatO_2$) and bicarbonate (HCO_3^-) were calculated. A correction for current blood temperature was made. In the isoflurane-anaesthetised horses in Study I and in Studies II and III arterial (a) and mixed venous ($\bar{v}$) blood from the pulmonary artery was analysed, while in Studies IV-V arterial and venous blood from the jugular vein was used for the analyses.

Formulas

The following calculations were made (Lentner, 1990):
Alveolar oxygen partial pressure:
P_AO_2 (kPa)= (P_IO_2 (kPa)-$PaCO_2$)/0.8 (respiratory exchange ratio), where P_IO_2 = partial pressure of inspired O_2.
Content of oxygen in arterial, mixed venous and end-capillary pulmonary blood:
CzO_2 (mL/L)= [Hb concentration (g/L) x 1.39 x oxygen saturation of Hb (fraction)] + [PzO_2 (kPa) x 0.225 (mL/L)], where z = a, $\bar{v}$, ć. $P\acute{c}O_2 \approx P_AO_2$.
O_2 delivery: DO_2 (L/min)= CaO_2 (mL/L) x $\dot{Q}$ t (L/min)/1000.
Oxygen consumption: $\dot{V}O_2$ (mL/min)= C(a-$\bar{v}$)O_2 (mL/L) x $\dot{Q}$ t (L/min).
Venous admixture: $\dot{Q}$ s/$\dot{Q}$ t= [(C$\acute{c}O_2$)-CaO_2]/[C$\acute{c}O_2$-C$\bar{v}$ O_2].
For these calculations blood gas values at 37º C were used.

Instrumentation and measurements of muscle and skin perfusion: Laser Doppler flowmetry

Peripheral perfusion was measured using a commercially available LDF apparatus (Periflux 4001, Perimed, Järfälla, Sweden).

In Study II muscle perfusion was measured during anaesthesia in the right and left middle gluteal muscles (Figure 5) and in the right and left semimembranosus muscles in Study III. In six horses in Study III, perfusion was also measured in the left medial gluteal muscle. Skin perfusion was measured on the skin on the croup on the dependent side in Study II and on the hairless, ventral aspect of the tail (Figure 5) in both Study II and Study III.

As soon as the horse was placed on the surgical table, the skin covering the sites of muscle measurements was surgically prepared. A 0.7 x 40 mm hypodermic needle was inserted 3 cm into the muscle as an introducer for the microtip, which was a thin, optic fibre (Straight microtip with slanted tip, MT A500-0.120 mm, 0.5 mm diameter, Perimed). After placement of the tip, the hypodermic needle was retracted and the microtip was connected to the probe (Master Probe; Probe 418-x, Perimed). Skin perfusion was measured on the surface of the skin using a skin probe (Probe 407, Perimed) applied directly to the skin after cleansing with alcohol (Figure 5).

Figure 5: Laser Doppler flowmetry recordings of perfusion in the non-dependent gluteus (left) and on the tail skin (right) in the laterally recumbent anaesthetised horse.

Skin and muscle perfusion was recorded continuously until the end of anaesthesia on line in a computer, using software provided by the manufacturer (Perisoft 1;14, Perimed). Perfusion is expressed as Perfusion Units (PU), which are arbitrary. The LDF probes were calibrated before use in a standard motility solution provided by the manufacturer.

In one pilot study performed prior to Study II the perfusion value for biological zero was assessed in two university-owned horses. At the time of measurements the horses were anaesthetised in lateral recumbency and anaesthesia had been maintained with halothane in oxygen for several hours for participation in research and surgical training. Both horses were destined for euthanasia at the end of anaesthesia.

Muscle and skin perfusion was measured by means of LDF before and after euthanasia (pentobarbitone sodium; Avlivningsvätska för djur, 100 mg/mL, Apoteksbolaget AB, Umeå, Sweden). In one horse muscle perfusion was measured with single-fibre LDF in three spots in the long digital extensor muscle of the non-dependent hindlimb and skin perfusion was measured on the skin in the same area. In the other horse muscle perfusion was measured in one spot in the non-dependent middle gluteal muscle and skin perfusion was measured on the hairless, ventral aspect of the tail base.

In another pilot study muscle perfusion was measured with LDF in four standing horses placed in stocks. Muscle microtips were inserted into the right and left gluteal muscles as described above, but here two microtips were placed in each muscle approximately 4 cm apart. Perfusion was measured with the horse unsedated and after intravenous administration of detomidine (0.01 mg/kg) or acepromazine (0.05 mg/kg) in two horses respectively.

Instrumentation, sampling and analyses of muscle interstitial fluid: Microdialysis

For sampling of muscle interstitial fluid, commercially available microdialysis catheters (CMA 70 Brain Microdialysis Catheter, CMA/Microdialysis AB, Solna, Sweden) were used. The membrane length in all instances was 30 mm and the shaft was either 600 or 800 mm long. In Study I, two hours before induction of anaesthesia a microdialysis catheter was introduced into each of the right and left gluteal muscles 4-5 cm from the point of muscle biopsy. In the first two horses a small-animal intravenous catheter (Venflon 1.2 x 40 mm, Becton Dickinson Infusion Therapy AB, SE –251 06 Helsingborg, Sweden) was used as an introducer. After insertion of the microdialysis catheter the plastic sheath of the intravenous catheter was retracted 3 cm to leave the membrane end of the microdialysis catheter free. As the microdialysis membranes in these two horses were damaged, a specially designed pull-away catheter (provided by CMA) that could be completely removed after insertion of the microdialysis catheter was used thereafter. In Study V one microdialysis catheter was introduced into the right gluteal muscle after the anaesthetised horse had been placed in dorsal recumbency on the surgery table. To allow insertion of the catheter, the horse was tilted to the right.

In all instances, a small, portable, battery-powered syringe pump (CMA 106 Microdialysis pump) was secured with self-adhesive wrap to the horse's tail. Using this pump a modified Krebs-Henseleit buffer, with the addition of a colloid (40 g/L dextran-70), was perfused through the microdialysis catheter at a rate of 0.3 μL/min. The catheter was flushed with perfusate for 20-40 minutes before insertion to purge the tubing and probe of air bubbles. The microvial was secured with adhesive tape to the mattress of the surgery table or, after termination of anaesthesia, to the croup of the horse at a site approximately level with the microdialysis catheter (Figure 6). All vials were weighed before and after sampling to allow estimation of fluid loss or gain. An equilibration period of ninety minutes was allowed after introduction of the catheter and before the first sample was collected and saved for analysis. After sampling, the vials were kept in iced water before storage at -20 °C pending analyses.

In both Studies I and V, the samples were analysed for the concentrations of lactate, glucose and urea. In Study I all samples were analysed for glycerol, while in the last five colic horses in Study V, pyruvate was measured instead. The analyses were performed with enzymatic fluorometric methods, using a commercially available sample analyser (CMA/600, CMA Microdialysis AB, Solna, Sweden). The length of time taken for the dialysate to travel the distance from the tip of the membrane to the microvial depended on the length of the tubing system, but was taken into account when calculating the sampling time. Sampling time was set as the time of discontinuation of sampling.

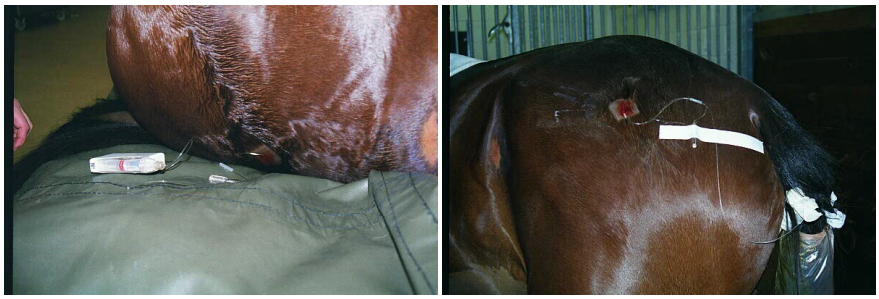


Figure 6: Microdialysis in the left gluteal muscle of an anaesthetised horse in dorsal recumbency (left) and in a standing horse (right) after recovery from anaesthesia and colic surgery. The dorsally recumbent horse has been tilted slightly to its right side to visualise the catheter entry site into the muscle. The syringe pump lies on the mattress beside the anaesthetised horse or is secured to the tail of the standing horse (protected from faeces with a plastic bag). The adhesive tape securing the catheter in place has been removed before photography.

Muscle biopsy sampling and analyses

Muscle biopsy specimens were obtained from the gluteus medius with the needle-biopsy technique as described by Lindholm and Piehl (Lindholm & Piehl, 1974).

The biopsy samples were taken from a site half-way on a midline between the tuber coxae and the tail base. Samples were obtained with a muscle biopsy needle

(external diameter 5 mm) after surgical preparation and, in the awake horse, after local analgesia with 2 mL of 2% lidocaine (Xylocain, Astra, Sweden) instilled subcutaneously and under the fascia. A 10-mm incision was made through the skin and fascia with a scalpel and muscle samples were obtained from a site 5-6 cm deep in the muscle belly. Subsequent biopsy samples were obtained through the same incision. The biopsy incision was protected with a wad of cotton wool and adhesive tape. When muscle biopsies, microdialysis and LDF were performed in the same muscle, 4-6 cm was always allowed between the sites of muscle biopsy sampling, the microdialysis catheter and the LDF probe. In Studies IV-V muscle biopsy samples were obtained in the right gluteus while microdialysis was performed in the left gluteus muscle.

In the standing horse, biopsy samples were taken with the horse standing in stocks and with a twitch applied. In the anaesthetised and dorsally recumbent horse, the horse had to be tilted to the left to give access to the biopsy site. In the laterally recumbent horse, when a biopsy sample was to be taken from the dependent gluteus, the horse was turned into the opposite lateral position immediately before sampling.

In the halothane-anaesthetised horses of Study I samples were taken from the non-dependent gluteus after induction and from both gluteus medius muscles at the end of anaesthesia. In Study II and in the isoflurane-anaesthetised horses of Study I, samples were taken from the non-dependent and dependent gluteus before and at the end of the anaesthesia. In Study II samples were also taken 24 h after recovery from anaesthesia. In Study IV biopsy samples were taken from the right gluteus after induction and at the end of anaesthesia in all horses. In 13 colic horses and in seven healthy horses, a biopsy sample was obtained the day after anaesthesia, and in six colic horses and in the seven healthy horses a biopsy sample was also obtained one hour after recovery to standing.

The samples were immediately frozen in liquid nitrogen and stored at -80º until analysed. They were freeze-dried, dissected free from connective tissue, blood and fat, and then weighed (1-2 mg dry weight;d.w.) and extracted in perchloric acid before being neutralised with potassium hydroxide. The concentrations of adenine nucleotides (ATP, ADP, AMP) and inosine monophosphate (IMP) were determined by a modified HPLC technique using a C:18 (250x4.6; 5 µm) column (Sellevold et al., 1986). Muscle creatine phosphate (CP) was determined with the HPLC technique (Dunnett et al., 1991). Muscle lactate and glycogen concentrations were assayed with fluorometric methods (Lowry & Passonneau, 1973).

Muscle temperature measurements

Muscle temperature was measured in Study II and in six horses in Study III. In Study II needle probes (thermistor: DM852; probe MKA08050 A, ELLAB, Rødovre, Denmark) were inserted 5 cm into the muscle bellies of the right and left longissimus muscles. In Study III soft thermistor probes (442-PI, Perimed) were used and inserted 4 cm into the muscle bellies of the right and left semimembranosus muscles through 0.7x40 mm hypodermic needles.

Blood chemistry and haematology

Blood samples were collected through a catheter in the jugular vein (Studies I, IV and V), or the catheter in the pulmonary artery (Studies II and III). Vials containing EDTA were used for determination of haemoglobin (Hb), haematocrit (Hct) and white blood cell count (WBC). Vials containing no additive (serum vials) were used for measurement of electrolytes (Na, Ca, K, Cl), inorganic phosphate (Pi), total protein, albumin, and aspartate aminotransferase (ASAT). Heparin vials were used for determination of lactate, glycerol, glucose, free fatty acids (FFA), cortisol, creatinine (Crea), hypoxanthine (Hx), xanthine (X) and uric acid. Creatine kinase (CK) was measured in serum in Study II and in plasma in Study IV. Plasma and serum were harvested after centrifugation and stored at -80°C pending analysis.

Blood analytes were assayed according to routine methods used at the clinical pathology laboratory at the Department of Biomedical Sciences and Veterinary Public Health, and at the muscle metabolic laboratory at the Department of Clinical Sciences. Further details of exact methods for analyses are given in Papers I-V.

Statistical analysis

Minitab® Statistical software (Minitab Inc., State College, PA, USA) was used for statistical analysis in Studies I- III, Statistica (Statsoft Inc., Tulsa, OK, USA) was used in Studies IV-V and SAS was used for certain analyses in Studies I, III and IV.

Generally, temporal changes in physiological data and blood and microdialysate analytes were analysed by one way or two way ANOVA for repeated measurements or by general linear model (GLM) in Minitab or Statistica statistical software (Minitab® Inc., State College, PA, USA; Statistica 7.0, StatSoft® Inc. Tulsa OK, USA). When ANOVA or GLM indicated a significant difference or interaction, the Tukey HSD post hoc test or planned comparison was applied to describe the patterns of differences. The post-anaesthesia changes in blood analytes in Study IV were analysed using the mixed procedure in SAS (SAS® System 9.1, SAS Institute Inc., Cary, NC, USA). A paired t-test was used in Study III to analyse the effects of transition between modes of ventilation and in Study II to compare muscle metabolic and relative perfusion changes between dependent and non-dependent muscles. Muscle biopsy samples were otherwise compared using non-parametric tests, where a Wilcoxon test or Friedman ANOVA was applied to detect within-group differences and the Mann-Whitney U test for differences between groups.

A p value of ≤ 0.05 was considered significant in all tests. Results are given as mean values ± standard deviation (SD) or as median values and range. In Study V the mean values ± standard error of the mean (SEM) are presented.

Results and discussion

Peripheral perfusion and central circulation

Measurement of peripheral perfusion

Laser Doppler flowmetry was chosen for measurement of muscle and skin perfusion, since the technique had shown promising results in other equine, human and laboratory animal studies (Svensson et al., 1985; Serteyn et al., 1986; Lee et al., 1993; Gustafsson et al., 1994; Erni et al., 1995; Hennig et al., 1995) and because it yields continuous, on-line information with a minimum of trauma. Peripheral perfusion was measured over time in response to pharmacological provocations and changes of the ventilatory mode during different anaesthetic protocols. For such measurements, LDF is a technique well worth further use in the horse during anaesthesia. An initial intention was to measure perfusion and compare the awake perfusion values with those in the anaesthesised horse. In a pilot study, however, it was shown that the single-fibre LDF technique was too sensitive to movements to allow measurements in the awake state, without sedation. Another intention was to compare the perfusion in the dependent and non-dependent muscle, since this had been described in some previous studies (Serteyn et al., 1986; Serteyn et al., 1991). However, since the technique yields arbitrary values of perfusion, it is not recommended for comparison between subjects unless an increased number of recordings from each site are performed (Smits et al., 1986; Kvernebo et al., 1990). For use over time and for comparison of the relative response to different provocations, the technique is outstanding.

The findings in the pilot studies are presented below, but since data were obtained from only a few horses, the results should be interpreted with caution.

Pilot studies of perfusion changes during euthanasia and in the awake horse

Perfusion after euthanasia

Perfusion measurements after pharmacological euthanasia in two horses showed a sharp decline in perfusion immediately after injection of the drug, indicating circulatory failure (Figure 7). When the horses were clinically dead (no cardiac movements, no corneal reflex, corneal dilatation, ECG flat), muscle perfusion ranged from 1.3 to 4.5 perfusion units (PU), and skin perfusion was 1.1 and 2.2 PU in the two horses respectively. Flow pulsations indicative of intermittent changes in the number or velocity of red blood cells were no longer visible. These values thus represent biological zero, which is the lowest recording of perfusion that can be obtained in a tissue, even after complete cessation of blood flow, i.e. after tourniquet application or in a piece of meat (Caspary et al., 1988; Wahlberg et al., 1992). It is not known why some perfusion is still recorded, but Brownian motion of the red blood cells is one possible explanation (Caspary et al., 1988; Wahlberg et al., 1992). Another possibility is that it could correspond to a residual flux in the microvascular bed, generated by vasomotion (Holloway & Eatkins, 1977). Some authors subtract the value for biological zero from the perfusion recordings

(Caspary et al., 1988), but since this value may differ between tissues and during the experimental period and varies depending on temperature (Caspary et al., 1988; Wahlberg et al., 1992), subtraction of a standard value may not be optimal. In our experience with this technique, the perfusion recordings were in some cases very low and approached the aforementioned values for biological zero as measured in the dead horses, but as long as pulsations were seen and provocations still had an effect, these recordings were considered valid. When single-fibre tips and skin probes were placed, care was always taken to ensure a "good quality" recording, i.e. with visible pulsations but not in close contact with a larger vessel. The latter was indicated by extremely high perfusion values in combination with high-amplitude pulsations. Placing the single-fibre tips in the dependent muscle was always more delicate since the area was in close contact with the underlying mattress.

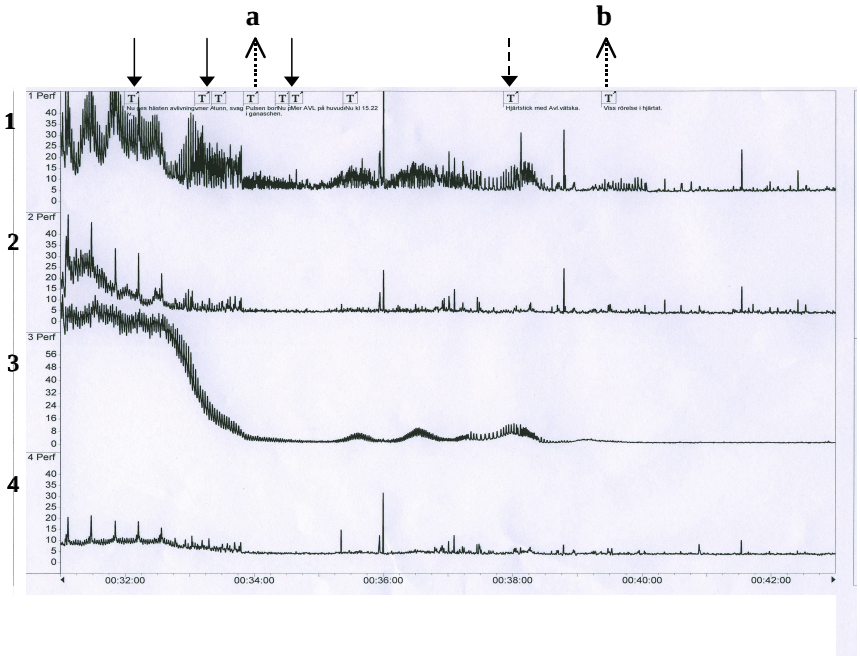


Figure 7: Recordings of perfusion in the long digital extensor muscle (channels 1-3) and in the nearby skin (channel 4) during euthanasia in a halothane-anaesthetised horse. Solid arrows indicate when pentobarbitone sodium was injected intravenously and the dashed arrow shows when it was injected intracardially. Comments: arrow **a**=no palpable pulse in the facial artery; arrow **b**= some cardiac movements still present. A time scale (hh:min:sec) is shown below the figure. The recordings are given in perfusion units.
After the first two injections of pentobarbitone, the perfusion decreased sharply. Pulsatile flow in channels 1, 2 and 4 persisted for approximately 10 minutes after the intracardiac injection, while in channel 3, the pulsations disappeared earlier.

The awake horse

The pilot study on muscle and skin perfusion using LDF in four standing horses showed decreased perfusion at all measured sites with a mean of -67% one minute

after detomidine injection. Fifteen minutes later the perfusion was still decreased compared to the baseline value at all measurement sites. After injection of acepromazine the changes in perfusion were variable (Table 3).

Table 3. Perfusion in four standing horses before and after sedation with detomidine or acepromazine

Horse	Site	-30	-15	0	Det 1	Det 5	Det 10	Det 15
1	R. gluteus	45	142	100	52	147	135	31
	L. gluteus		158	100	42	225	75	67
	R. skin	160	95	100	15	50	50	40
	L. skin	117	109	100	13	46	48	46
2	R. gluteus	93	113	100	21	12	24	6
	L. gluteus	68	95	100	63	37	82	44
	R. skin	215	125	100	26	33	49	33
	L. skin	84	155	100	29	1	1	1
	mean	112	124	100	33	69	58	34

Horse	Site	-30	-15	0	Ace 1	Ace 5	Ace 10	Ace 15
3	R. gluteus		51	100	58	108	61	77
	L. gluteus	112	41	100	98	102	115	128
	R. skin	54	99	100	59	120	117	92
	L. skin	71	129	100	66	110	105	80
4	R. gluteus		94	100	63	38	47	34
	L. gluteus		76	100	182	72	18	25
	R. skin		333	100	163	174	215	185
	L. skin		210	100	138	148	240	134
	mean		129	100	103	109	115	94

Perfusion is expressed as a percentage of that immediately before administration of the drug –30, -15 and 0 = 30 and 15 minutes and immediately before administration of the drug; Det = detomidine; Ace = acepromazine; 1, 5, 10 and 15 = minutes after administration of the drug; R. = right; L. = left.

Detomidine was expected to result in decreased perfusion, since this drug is known to induce peripheral vasoconstriction and bradycardia accompanied by a decrease in $\dot{Q}t$ (Ruffolo, 1985; Clarke & Taylor, 1986; Sarazan et al., 1989; Wagner et al., 1991). In a previous study with measurement of muscle surface perfusion in the standing horse, a maximum decrease in perfusion of 27% was noted after administration of xylazine (Hennig et al., 1995) In that study it took more than one hour for the perfusion to return to the baseline level. It was concluded that the muscle vascular autoregulation was unable to compensate for the anticipated decrease in cardiac output caused by xylazine. In the pilot study, perfusion was only measured for approximately 15 minutes following injection of detomidine, and thus the time taken for the perfusion to return to the baseline level is not known. The relative decrease in perfusion was more pronounced after detomidine injection in the present pilot study than after administration of xylazine in the study by Hennig et al. (1991), and the total duration of the decrease may have been similar or possibly even longer.

Acepromazine did not have a profound relaxing effect in the pilot study and no obvious changes in blood flow were observed. In previous studies acepromazine resulted in an increased digital (Hunt et al., 1994) and metatarsal artery (Walker & Geiser, 1986) blood flow in the standing horse. The increased blood flow was attributed to the antagonistic effect of acepromazine on alpha-1 adrenoceptors, causing vasodilation and decreased resistance to flow (Steffey et al., 1985; Baxter et al., 1989). It was therefore hypothesised in the pilot study that administration of acepromazine would increase blood perfusion in the muscle and skin, but this was not observed in either of the two horses studied. The different levels of the measured areas in relation to the level of the heart may have influenced the results (Seymour et al., 1993).

The perfusion measurements made by LDF in the standing, non-sedated horse in the pilot study were difficult to analyse, since the recordings were severely affected by so called movement artefacts, especially in muscle, caused by frequent, small contractions of the muscle. This is an acknowledged problem with use of this technique (Newson et al., 1986; Obeid et al., 1990) and most studies are therefore performed in non-moving or anaesthetised subjects. During sedation with detomidine, the horses relaxed and subsequently the recordings were less affected by movement artefacts. Perfusion studies using single-fibre LDF could possibly be performed in the awake but well-sedated horse.

Differences in blood flow between dependent and non-dependent muscles

During baseline measurements of gluteal muscle perfusion in the isoflurane-anaesthetised horses included in Study I (data are not reported in Paper I) and in the propofol-ketamine-anaesthetised horses in Study II, no consistent differences in perfusion (measured in PU) were found between the dependent and non-dependent muscles. There was a large variation in the perfusion levels, expressed as PU, between horses. However, since the LDF perfusion values are arbitrary, it is not really feasible to compare individual recordings between muscles or individuals. In line with the results from Studies II and III, LDF has been found to be better suited for comparisons of relative changes in blood flow under different experimental conditions within a subject (Smits et al., 1986; Obeid et al., 1990; Sigurdsson et al., 1994; Svedman et al., 1998; Pypendop & Verstegen, 2000; Raisis et al., 2000c). However, it is often desirable or necessary to make comparisons between subjects or tissues. A reasonable estimate of overall blood flow in an organ can be obtained by measurements at a number of points in the tissue (Smits et al., 1986), and comparisons between different muscles and measurement occasions will be made more reliable by increasing the number of studied objects (Smits et al., 1986; Kvernebo et al., 1990). This was not possible, however, in the present investigation and therefore in the following results and discussion sections only relative changes in response to provocations will be compared.

Earlier reports regarding possible differences in muscle microcirculation between dependent and non-dependent muscle groups are conflicting. Studies using the radio-labelled microsphere technique or Xe^{133} clearance have not revealed any differences in contralateral muscles (Staddon et al., 1979; Weaver & Lunn, 1984; Manohar et al., 1987a). In other studies, in which muscle surface

perfusion was measured using LDF, the perfusion was found to be lower in the dependent triceps muscle (in contact with the table), than in the non-dependent triceps (Serteyn et al., 1986). In one study where muscle surface perfusion in the triceps brachii and the biceps femoris was measured before and after induction of anaesthesia, the perfusion was slightly more reduced in the dependent than in the non-dependent muscles after induction of anaesthesia (Norman et al., 1992).

There may be several reasons for the different results, for example the different techniques used, the size of the measured area and possibly the size of the horses. Serteyn et al. (1986) and Norman et al. (1992) used LDF to measure the perfusion on the surface of the triceps brachii muscle, whereas in the other studies microcirculation was measured across the whole muscle and in a number of different muscles (Staddon et al., 1979; Weaver & Lunn, 1984; Manohar et al., 1987a). The studies varied regarding extra padding of the table. In Studies I and II perfusion was measured within the muscle belly of the gluteus with the horses resting on 18 cm-thick foam mattresses, and this could possibly have affected the results. The characteristics of the underlying surface have been found to affect the ICMP differently (Lindsay et al., 1985) and it is probable that perfusion is also affected differently. Since foam mattresses may also have different properties (Redfern et al., 1973), further comparisons between studies are uncertain.

The size of the studied horses may also have played a role, since 350-550 kg horses were studied by Serteyn et al. (1986), while the other studies were based on measurements obtained mainly in smaller ponies around 200 kg (maximum weight 407 kg). The horses of the present investigation weighed between 416 and 584 kg.

Measurements of blood flow in the femoral artery and vein and of perfusion in the semimembranosus muscle in the hindlimbs during isoflurane and halothane anaesthesia in horses have shown interesting results (Raisis et al., 2000a; Raisis et al., 2000b). Volumetric flow in the femoral arteries measured by Doppler ultrasonography was actually found to be lower in the non-dependent than in the dependent limb (Raisis et al., 2000a; Raisis et al., 2000b). Differences in peripheral haemodynamics between hindlimbs were also suggested by differences in the arterial waveform contours, with more reverse flow during diastole in the non-dependent femoral artery of horses anaesthetised with isoflurane (Raisis et al., 2000a). The decreased flow in the non-dependent limb may be explained by the altered position of this limb relative to the heart. Elevation of a limb above the level of the heart leads to a decreased hydrostatic pressure, which reduces the internal pressure. This results in an increased transmural pressure, possible vessel collapse and increased resistance to flow (Kamm & Pedley, 1989; Seymour et al., 1993; Turnbull et al., 2002). In humans, the compartment syndrome of the lower limb is a rare but serious complication of prolonged anaesthesia with the legs in an elevated position (Turnbull & Mills, 2001), and parallels between this syndrome and PAM occurring in the uppermost legs of the horse may be drawn. The studies of Raisis et al. (2000a and b) support this theory. Taylor and Young (1990) investigated the cephalic venous pressure and ICMP in the triceps muscle with the upper forelimb in different positions in laterally recumbent, anaesthetised horses. When the limb was pulled hard back or flexed in the carpus and pushed hard forward the ICMP or cephalic venous pressure, or both, were increased. In

combination with the above results, these findings imply that correct positioning of the limb is very important for reducing the likelihood of development of myopathy.

If the femoral blood flow differs between the non-dependent and the dependent leg, why then does not the microcirculation within the muscle differ similarly, and why is the clinical impression obtained that PAM occurs most frequently in the lowermost triceps?

ICMP has been reported to be higher in the dependent than in the non-dependent triceps and gluteus muscles of laterally recumbent horses (Lindsay et al., 1980; Lindsay et al., 1985; McDonell et al., 1985; Lindsay et al., 1989). If the external pressure increases, there will be an increase in transmural pressure, which, as mentioned above, may result in a collapse of vessels. In other species, capillary collapse associated with high transmural pressures opens up communications between arteries and veins. When arterio-venous shunts develop, arterial and venous blood flow can be maintained or even increased, while capillary perfusion is reduced (Lindbom & Arfors, 1984; Tangelder et al., 1984). The higher femoral arterial flow in the dependent limb that was observed in the studies by Raisis et al. (2000a; 2000b) may be just enough to keep the over-all dependent muscle microcirculation similar to that in the non-dependent limb during lateral recumbency. However, since the microcirculation within the muscle is not uniform (Smits et al., 1986; Kvernebo et al., 1990) and the Xe^{133} clearance and microsphere techniques measure perfusion across the whole muscle, spatial perfusion differences between dependent and non-dependent muscles may still exist.

The blood flow during anaesthesia has been reported to be higher in the dependent triceps than in the dependent gluteus (Dodman et al., 1985), and the decrease in blood flow from the awake to the anaesthetised state is more pronounced in the gluteus than in the triceps (Manohar et al., 1987b). It might therefore seem reasonable to believe that the gluteus would be just as prone as the triceps to develop PAM. Differences, for example, in the vascular structure between the triceps and gluteus muscles could possibly explain the difference in susceptibilities of these muscles to develop PAM.

Since the incidence of PAM is rather low and this disease may affect several different muscles, to be able to detect significant and clinically relevant differences in local blood flow it is possible that a different experimental design needs to be used. Blood flow and microcirculation, for example, could be studied in several sites in each muscle.

Central circulation during isoflurane and propofol-ketamine anaesthesia

There were few significant group differences in measured cardiovascular parameters between isoflurane- (Study I) and propofol-ketamine- (Study II) anaesthetised horses during the first hour of maintenance anaesthesia (AN60) (Figure 8). However, in the horses anaesthetised with propofol-ketamine $\dot{Q}t$ increased from the awake state to anaesthesia, while in the isoflurane-anaesthetised horses there was no change in $\dot{Q}t$ or rather a slight decrease. A better-preserved $\dot{Q}t$ during propofol-ketamine anaesthesia was also indicated by a higher DO_2. The extraction of oxygen did not differ between groups during anaesthesia, as seen

from a similar arterio-venous oxygen content difference ($C(a-\bar{v})O_2$). The increase in $\dot{Q}t$ in Study II was attributed mainly to the increased HR, since SV remained unchanged. HR did not increase in the isoflurane horses and SV decreased slightly. Both isoflurane and propofol lower the blood pressure, mainly through vasodilation (Stevens et al., 1971; Claeys et al., 1988; Park et al., 1992) and this effect resulted in the similar decreases in mean arterial systemic blood pressure (MSAP) and SVR.

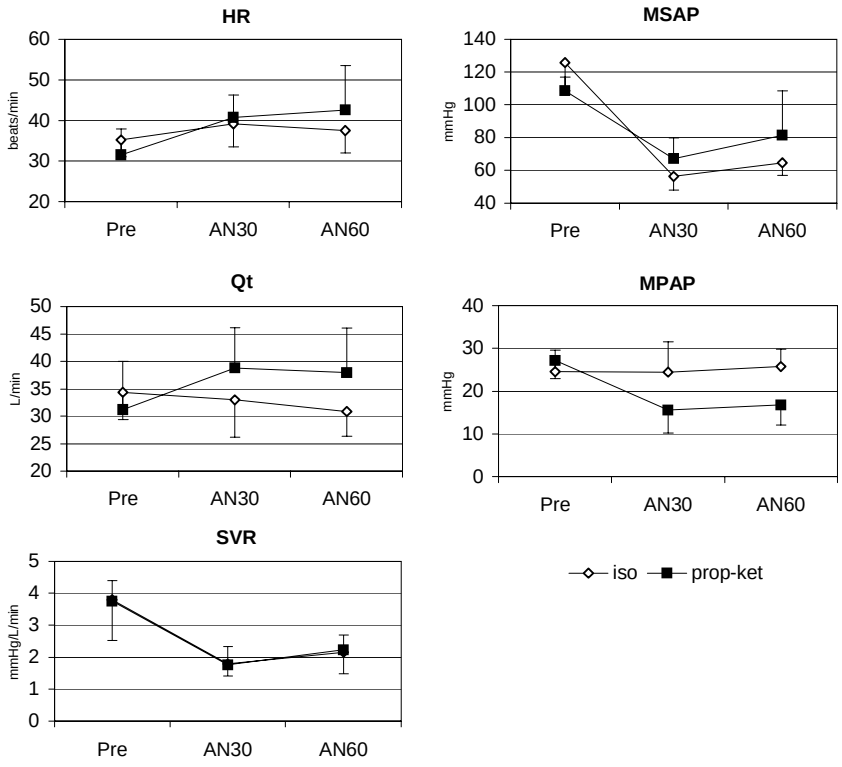


Figure 8. Circulatory data obtained before anaesthesia (Pre) and during the first hour of anaesthesia (AN30 and AN60) in the isoflurane-anaesthetised horses in Study I (iso) and in the propofol-ketamine-anaesthetised horses in Study II (prop-ket).
HR=heart rate; Q t= cardiac output; SVR=systemic vascular resistance; MSAP=mean systemic arterial blood pressure; MPAP= mean pulmonary arterial blood pressure.

While MSAP decreased similarly in both groups, mean pulmonary arterial pressure (MPAP) decreased significantly only in the propofol-ketamine-anaesthetised horses. The low MPAP may impair the ventilation-perfusion matching and explain why venous admixture ($\dot{Q}s/\dot{Q}t$) increased to a further extent in the propofol-ketamine-anaesthetised horses (Hedenstierna et al., 1987; Nyman & Hedenstierna, 1989).

An increased HR as seen during anaesthesia in Study II has been reported from other studies involving propofol (Mama et al., 1995; Duke et al., 1997). In one study in which the effects of propofol infusion at two different doses in

combination with xylazine were compared with ketamine in combination with xylazine, a higher heart rate was seen during the high-dose propofol + xylazine infusion (Mama et al., 1998). The increased HR in Study II was therefore probably associated with the infusion of propofol. This may be a direct effect of propofol, or possibly a reflex increase in response to the decreased blood pressure (Sellgren et al., 1992). In Study I (isoflurane), HR did not increase despite a decrease in blood pressure, which was of greater magnitude than that in Study II. This may be an effect of the greater attenuation of the baroreflex activity caused by isoflurane (Helleyer et al., 1989; Sellgren et al., 1992).

After 60 minutes of maintenance anaesthesia, pharmacological provocations aimed at inducing cardiovascular changes were performed in both groups, and therefore no further comparisons between groups can be made. It is possible that the induction drugs influenced cardiovascular function at AN60 in Study I, but it has been reported that the cardiovascular depressant effects of an inhalation anaesthetic overrides most of the effects of the induction agents (Taylor, 1991; Taylor & Young, 1993), which means that influence at AN60 was probably small. In addition, both induction protocols involved guaifenesin and no premedication with sedatives was given.

Clinical impression of propofol-ketamine anaesthesia

The combination of propofol and ketamine for maintenance anaesthesia was chosen since it has been proven to have many desirable properties. Propofol is rapidly cleared from the body (Nolan et al., 1996), recoveries are rapid and smooth (Nolan & Hall, 1985; Nolan & Chambers, 1989; Mama et al., 1995) and it induces less adrenocortical response as compared to halothane (Taylor, 1989a). Propofol in combination with ketamine provided excellent surgical conditions in ponies (Nolan et al., 1996) and ketamine has been recommended as an analgesic for TIVA with propofol in humans (Guit et al., 1991). Propofol alone at the doses necessary for surgical anaesthesia may result in hypotension (Claeys et al., 1988) and often respiratory depression (Taylor et al., 1986; Nolan & Chambers, 1989). By combining propofol with ketamine and by lowering the dose of propofol, some of the circulatory and respiratory depressant effects of propofol may be overcome, and well-preserved circulation has been reported with this combination (Guit et al., 1991; Nolan et al., 1996; Flaherty et al., 1997).

The induction quality with guaifenesin and propofol in the present study was good to excellent and intubation was performed with ease. No paddling was seen as has been reported previously after induction with propofol alone or after additional premedication (Nolan & Hall, 1985; Mama et al., 1995; Bettschart-Wolfensberger et al., 2001). The central circulation was well preserved, but marked respiratory depression was noted in some horses, necessitating occasional manual positive-pressure ventilation. Analgesia was sufficient to allow muscle biopsy sampling. The recoveries were not scored, but the overall impression was that they were satisfactory to good. Mentally the horses usually recovered very quickly and they were eager to get on their feet. They stood after a mean of 57 minutes, with a range of 32-98 minutes. One horse was very sensitive to noise and light and some ataxia was seen after recovery. Since norketamine, the active

breakdown compound of ketamine accumulates and the duration of ketamine infusion was rather long, this may have affected the recoveries (Nolan et al., 1996). It is possible that detomidine, given 30 minutes before the end of anaesthesia, may also have affected the recoveries, since it has been found to induce more ataxia after recovery from ketamine anaesthesia than the equivalent dose of xylazine (Clarke et al., 1986).

Peripheral perfusion in relation to central circulation in response to cardiovascular provocations during isoflurane and propofol-ketamine anaesthesia

To be able to compare changes in perfusion over time, all measurement points were compared with a baseline value which was set to 100% immediately before the pharmacological provocation with dobutamine (Study I), detomidine (Studies I and II) or acepromazine (Study I).

Provocation with dobutamine

Dobutamine infused in a small dose (0.5 μg/kg/min) induced minor central and peripheral changes in the isoflurane-anaesthetised horses in Study I and the results are similar to those of another study with an equivalent dose (Raisis et al., 2000b). In a study by Lee et al. (1998b), dobutamine infusion had to be increased above 2.5 μg/kg/min before a significant increase in cardiac index and in dependent and non-dependent triceps muscle perfusion was observed during halothane anaesthesia in ponies. Arterial blood pressure was reported to be significantly increased already at 1 μg/kg/min. Dobutamine is widely used during equine anaesthesia to improve blood pressure (Donaldsson, 1988), and infusion rates of 0.5-5 μg/kg/min are commonly recommended (Muir & Bednarski, 1983; Taylor & Clarke, 1999). In the above-mentioned study by Raisis et al (2000b), dobutamine at 0.5 μg/kg/min was found to result in an increased systemic arterial blood pressure. The infusion of dobutamine also resulted in an increase in the femoral arterial blood flow in both hindlimbs, but to a greater extent in the non-dependent than in the dependent limbs, leading to an equalised blood flow in the femoral arteries. However, no changes in muscle perfusion as measured by LDF were detected. This suggests that increased femoral flow is not necessarily associated with improved perfusion of skeletal muscle (2000b; 2000c).

The present investigation and others show that low dose rates of dobutamine (≤1 μg/kg/min) will improve blood pressure but not cardiac output or muscle perfusion. This again emphasises the importance of considering arterial blood pressure together with cardiac output and peripheral perfusion.

After discontinuation of dobutamine in Study I, there was a 47% increase in $\dot{Q}s/\dot{Q}t$ and a decrease in PaO_2 (-47%) and DO_2 (-32%). These changes may be a combined effect of a decrease in MPAP (-18%) and Hb (-17%) after the end of dobutamine infusion (Nyman & Hedenstierna, 1989). These effects raise the question whether the dobutamine infusion should be continued during the whole anaesthetic procedure once it has been instituted, in order to avoid the above

adverse changes. These changes in oxygen delivery to the tissues would also need to be addressed.

Provocation with detomidine

Detomidine induced a short-lasting but significant decrease in muscle perfusion during propofol-ketamine anaesthesia, which was of similar magnitude in the dependent and non-dependent gluteus (-35% and -33% respectively). Significant decreases were also observed in the tail (-58%) and back skin (-74%). During isoflurane anaesthesia detomidine induced significant decreases in tail skin (-51%) and back skin (-55%) and a non-significant decrease in the non-dependent muscle (-20%), but no change at all in the dependent muscle (+5%) (Figure 9).

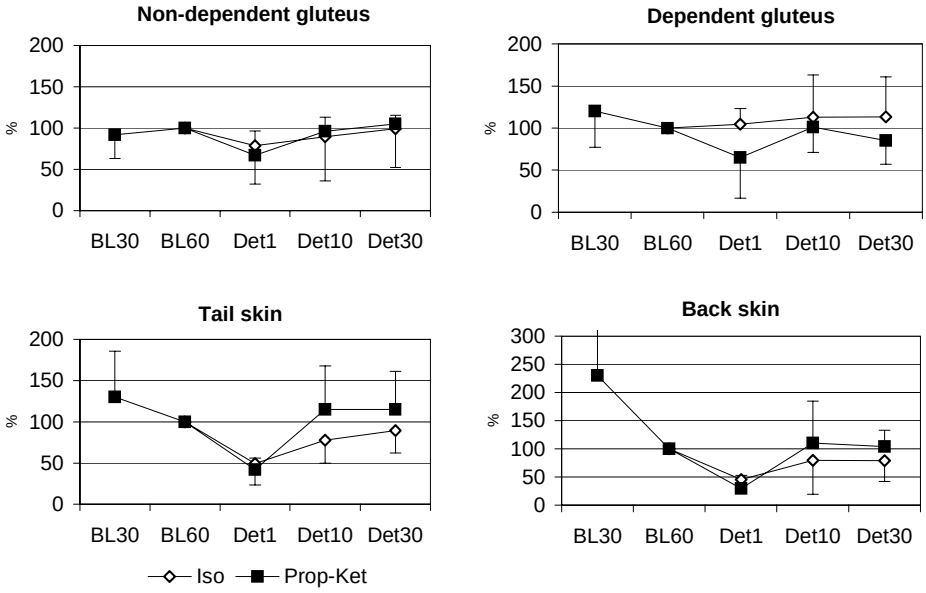


Figure 9: Perfusion (mean and SD) in the non-dependent and dependent gluteus and in the tail and back skin in the isoflurane-anaesthetised horses in Study I (Iso) and in the propofol-ketamine-anaesthetised horses in Study II (Prop-Ket) before and after administration of 10 µg/kg detomidine. Perfusion is shown as a percentage of that at BL60, which was set to 100%.
BL30 and 60 = baseline anaesthesia 30 and 60 minutes before the provocation; Det1, 10 and 30 = 1, 10 and 30 minutes after intravenous administration of detomidine.

The decreases in perfusion after detomidine injection during both propofol-ketamine and isoflurane anaesthesia were of shorter duration than had been shown by preliminary data from the pilot study in the awake horses. In addition, detomidine induced less decreases in perfusion in the muscle of the isoflurane-anaesthetised horses than in both awake and propofol-ketamine-anaesthetised horses.

The differences between the standing, awake horses and the anaesthetised horses may be due to chance, since only two standing horses were investigated. The

results may also be affected by different body positions. It is also possible that since both propofol and isoflurane are vasodilators (Eger, 1981; Hoka et al., 1998), this effect could partly have overridden the vasoconstrictive effect of detomidine. That this may have happened is also indicated by the finding that after 10 minutes the perfusion had returned to the same values as before detomidine injection in the propofol-ketamine- and isoflurane-anaesthetised horses but not in the standing horse.

Inhalation anaesthetics have been reported to blunt the vasopressor response to alpha-2 receptor agonists (Kenny et al., 1989), and this may possibly explain the slightly weaker response of perfusion to detomidine in the isoflurane-anaesthetised horses compared to the propofol-ketamine-anaesthetised horses in Study II or in the awake horses in the pilot study. This is in line with findings in earlier studies that alpha-2 receptor agonists induced no or variable changes in surface perfusion in non-dependent triceps muscle in isoflurane- (Still et al., 1996) and halothane- (Clarke et al., 1997) anaesthetised horses.

Heart rate, $\dot{Q}$ t and DO_2 decreased and SVR, MAP and $C(a\text{-}\bar{v})O_2$ increased after administration of detomidine in both Study I (isoflurane) and Study II (propofol-ketamine) (Figure 10). Thirty minutes after detomidine injection, $\dot{Q}$ t in the isoflurane horses had returned to a level not significantly different from the pre-detomidine value, while $\dot{Q}$ t in the propofol-ketamine-anaesthetised horses had not. However, there were no statistically significant differences between groups either before or 10 and 30 minutes after detomidine injection, and the lack of a significant time effect at 30 minutes in Study I may therefore be due to the limited number of horses. MAP had returned to the pre-detomidine level 30 minutes after detomidine injection but HR was still decreased in both studies. In Study I HR did not return to the pre-detomidine level until 60 minutes after detomidine. In Study II $\dot{Q}$ s/$\dot{Q}$ t had decreased 10 minutes after the injection of detomidine, which, as discussed previously, most likely was an effect of the simultaneous increase in MPAP (Nyman & Hedenstierna, 1989). Accordingly, the decreases in MPAP from 10 to 30 minutes after the administration of detomidine were accompanied by significant increases in $\dot{Q}$ s/$\dot{Q}$ t in both Studies I and II.

The responses of the central circulation and muscle and skin perfusion to detomidine were similar only in the immediate time period after detomidine injection. But 30 minutes after the injection, the perfusion in the muscle and skin had increased despite the continued low $\dot{Q}$ t and could possibly be an effect of autoregulation (Björnberg et al., 1988), or, as discussed above, an effect of the vasodilatory properties of the anaesthetic agents. This implies that not even measurement of $\dot{Q}$ t gives an accurate estimate of peripheral perfusion, a finding in line with the observation by Raisis et al. (2000b) that an increase in $\dot{Q}$ t was not paralleled by an increase in perfusion.

In conclusion, peripheral perfusion in Study II was found to correlate more closely with $\dot{Q}$ t than with MSAP. After administration of detomidine, the muscle and skin perfusion and $\dot{Q}$ t decreased, while the arterial pressure increased (Figure 11). This emphasises the importance of considering both central and peripheral

perfusion and not just arterial blood pressure when assessing the circulatory status of the anesthetised horse (Lee et al., 1998a).

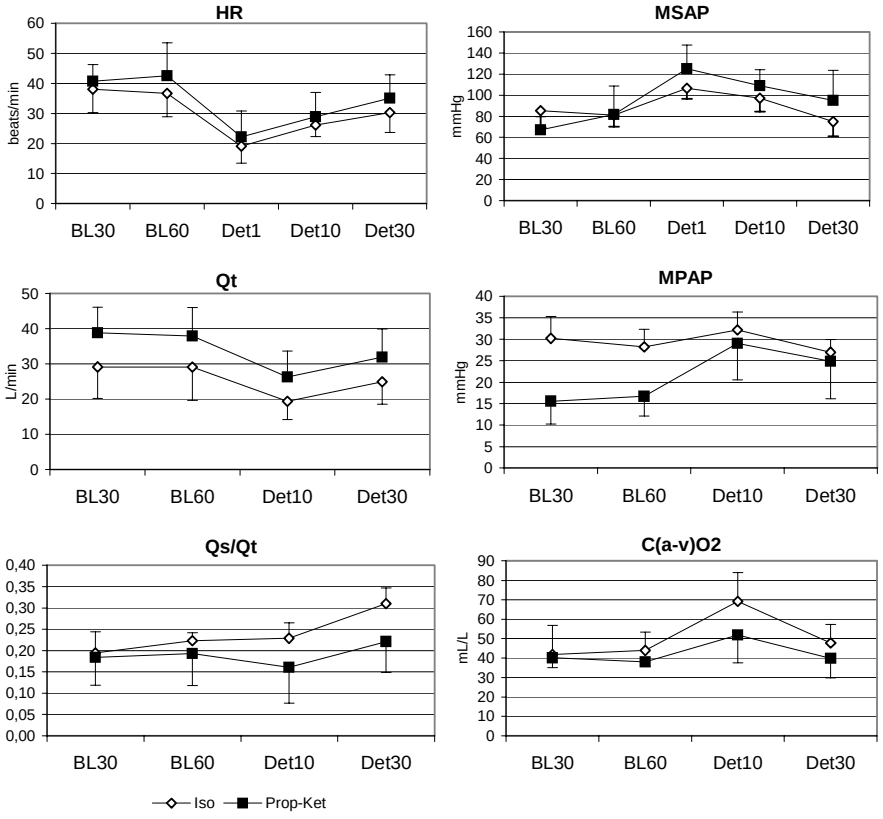


Figure 10: The response to detomidine of heart rate (HR), cardiac output (Qt), venous admixture (Qs/Qt), mean systemic arterial pressure (MSAP), mean pulmonary arterial pressure (MPAP) and arterio-venous oxygen content difference ($C(a\text{-}v)O_2$) during isoflurane (Study I) and propofol-ketamine anaesthesia (Study II). For key see Fig 9.

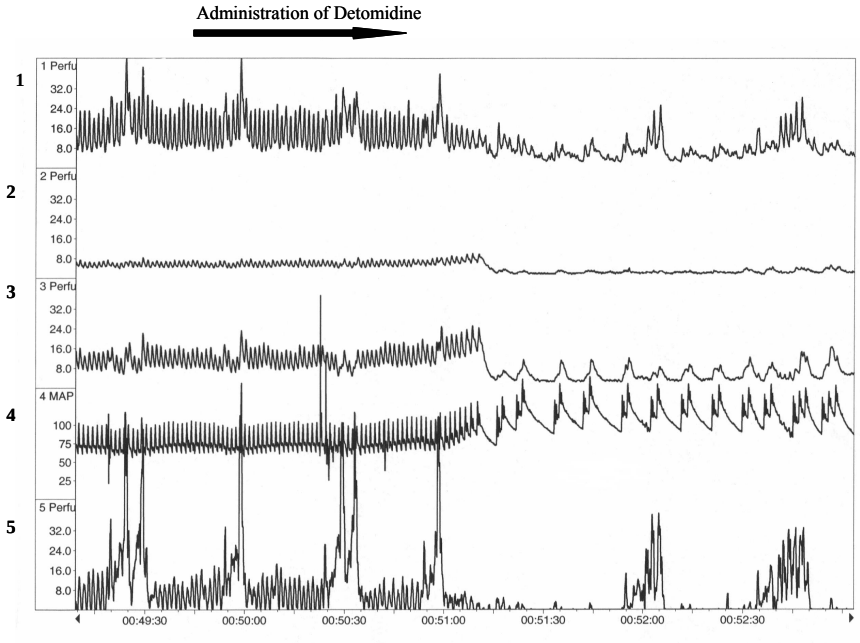


Figure 11: Changes in perfusion (perfusion units) and systemic arterial blood pressure (mmHg) induced by an intravenous injection of 10 µg/kg detomidine during propofol-ketamine anaesthesia in one horse. The recordings are 1: Perfusion in the non-dependent muscle; 2: perfusion in the skin on the back; 3: perfusion in the tail skin; 4: arterial blood pressure; 5: perfusion in the dependent muscle. Administration of detomidine is shown as a solid arrow. The regular spikes easily seen in recordings 1 and 5 are breathing artefacts.

Provocation with acepromazine

Acepromazine induced no further changes in perfusion, a finding in accordance with that in the muscle of the standing horse (pilot study). No significant changes were found in measured and calculated cardio-respiratory variables (HR, SAP, PAP, $\dot{Q}$ t, SVR, PVR, $\dot{Q}$ s/$\dot{Q}$ t, C(a-$\bar{v}$)O2, RR). In a study in which similar doses of acepromazine were administered during halothane anaesthesia, $\dot{Q}$ t increased as an effect of increased SV, but SAP decreased (Steffey et al., 1985), and in the standing horse acepromazine has been found to induce hypotension (Muir et al., 1979; Parry et al., 1982). The lack of effect of acepromazine in Study I may be due to the combined influence of time and the inhalation agent, since acepromazine was administered after approximately 3.5 hours of isoflurane anaesthesia.

In general, during isoflurane anaesthesia there were smaller changes in perfusion of the dependent than of the non-dependent gluteus in response to the cardiovascular provocations. No differences between muscles in the response to provocations were seen during propofol-ketamine anaesthesia. This may indicate that during isoflurane anaesthesia the perfusion in the dependent muscle was more affected by compression.

Peripheral perfusion and central circulation during spontaneous breathing and intermittent positive-pressure ventilation

In Study III both $\dot{Q}t$ and MAP were significantly lower during IPPV than during spontaneous breathing, indicating better preserved circulation during SB (Figure 12). These findings are in line with previous results (Steffey et al., 1977; Hodgson et al., 1986; Gasthuys et al., 1991; Mizuno et al., 1994) (Figure 12).

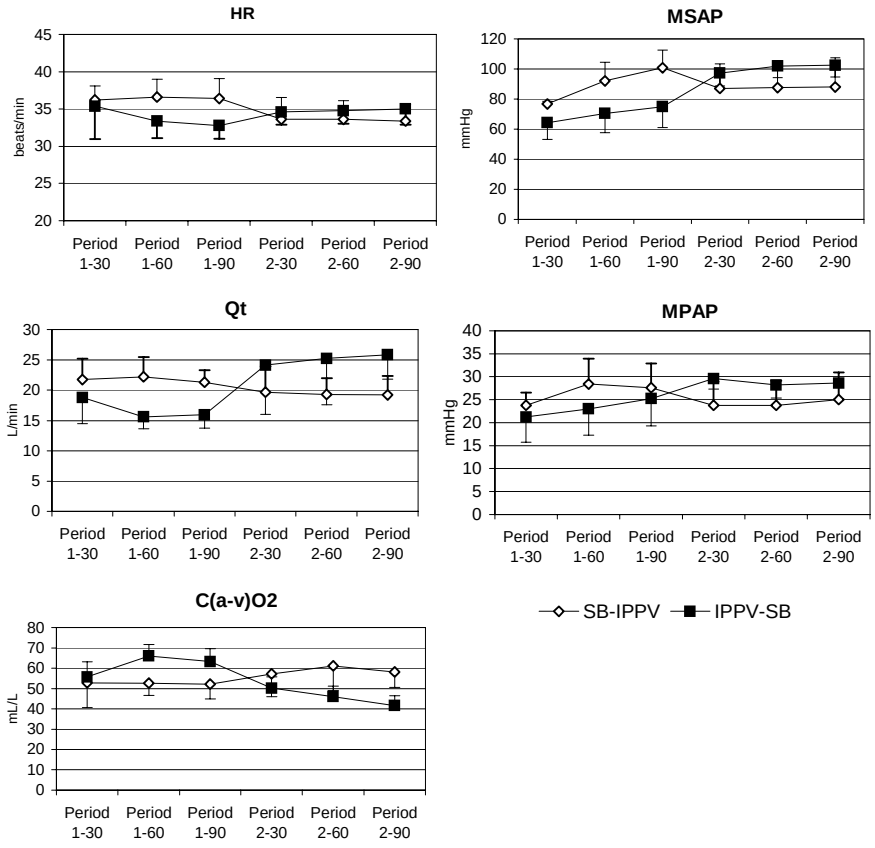


Figure 12: The effect of spontaneous breathing (SB) and intermittent positive-pressure ventilation (IPPV) on HR, $\dot{Q}t$, $C(a\text{-}\bar{v})O_2$, MSAP and MPAP.
SB-IPPV =spontaneous breathing (SB) preceeding intermittent-positive pressure ventilation (IPPV); IPPV-SB= IPPV preceeding SB; Period 1= the first 90-minute period; Period 2= the second 90-minute period; 30, 60 and 90=minutes during the first and second periods. Mean values and SD.

Contrary to the hypothesis, no significant differences were found in the quantitative measures (PU) of perfusion in muscle when the two successive 90-minute periods were compared. Changing the mode of ventilation did, however, affect the perfusion in the 30-minute time period immediately following the switch. When SB was changed to IPPV, the perfusion decreased significantly in the tail

skin and nearly significantly (p=0.056) in the left semimembranosus muscle, and when a change was made from IPPV to SB, there was a significant increase in perfusion in that muscle. Although short-lasting, these changes indicate that IPPV depresses peripheral perfusion. No immediate significant changes were found in $\dot{Q}$ t despite increases in SV and HR when IPPV was switched to SB.

There was a qualitative difference between the two modes of ventilation which again indicated better-preserved peripheral perfusion during SB. This difference consisted of so-called low frequency flow-motion, which was seen twice as often during IPPV as during SB (Figure 13). Flow-motion consists of blood flow fluctuations as a consequence of vasomotion, which is a spontaneous, rhythmical change in arteriolar diameter (Johnson & Wayland, 1967; Schmidt et al., 1993). Vasomotion occurring in frequencies of 1-3 cycles/min (low frequency) is thought to be a protective, compensatory mechanism that ensures tissue blood supply when the microcirculatory blood flow is low or when the driving pressure falls below a critical level (Schmidt et al., 1993; Rucker et al., 2000).

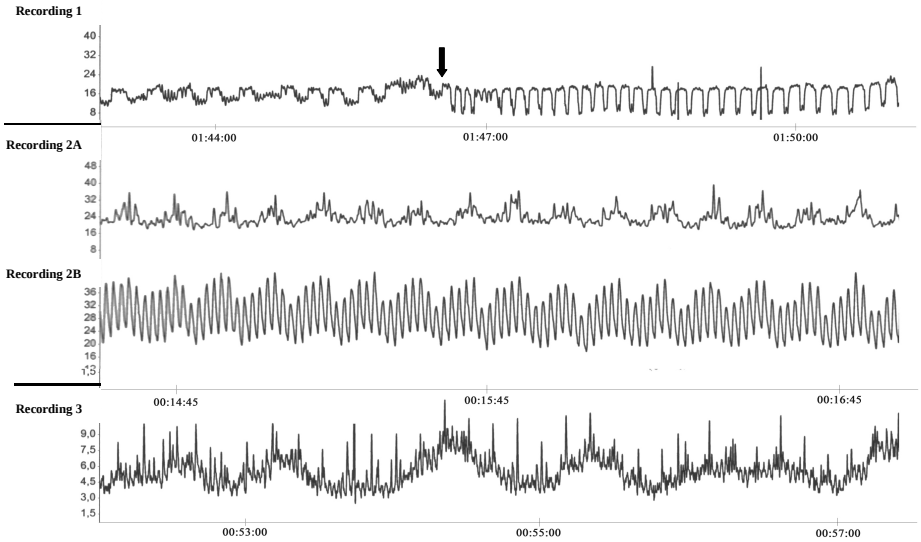


Figure 13: Perfusion recordings made on three different recording occasions, illustrating from above: Recording 1: Switching the mode of ventilation from spontaneous breathing to mechanical ventilation (arrow) with marked depression of perfusion during the inspiratory phase of mechanical ventilation. Recordings 2A and 2B: High-frequency flow-motion with a slightly different appearance between recordings. The high-frequency flow-motion is associated with breathing. Recording 3: Low-frequency flow-motion with a frequency of about 1 cycle per minute.
Note the different time scales for the different sequences.

That peripheral perfusion was reduced during IPPV as compared to SB was supported by a larger arterio-venous oxygen content difference during IPPV, indicating depressed circulation in relation to oxygen delivery or, alternatively, increased oxygen consumption. The latter did not occur, a finding in line with an

earlier observation that oxygen consumption did not differ between IPPV and SB (Hodgson et al., 1986). When Hb saturation with oxygen is adequate, the blood flow is proportionately more important than the oxygen content for tissue oxygen supply. The significantly higher PaO_2 found during IPPV was attributable to two horses in which PaO_2 doubled or tripled after a change from SB to IPPV. Thus instituting mechanical ventilation does not necessarily increase PaO_2 (Hodgson et al., 1986; Nyman & Hedenstierna, 1989; Day et al., 1995).

Positive-pressure ventilation in the horse results in a reduction of cardiac output (Steffey et al., 1977; Hodgson et al., 1986; Mizuno et al., 1994) due to impaired venous return caused by the positive pressure that is created in the thoracic cavity (Hall et al., 1968). Another cause of the further impairment of cardiac output in the mechanically ventilated as compared to the spontaneously breathing horse is a decrease in sympathetic tone following the reduction in $PaCO_2$ (Wagner et al., 1990; Khanna et al., 1995). Hypercapnia when seen during SB in Study III was mild to moderate (≤9.6 kPa), but may have led to increased sympathetic tone, since flow-motion was not as frequent during SB as during IPPV (Mikael Elam, personal communication, Sahlgrenska Hospital, Gothenburg, Sweden).

The results of Study III confirm earlier reports of increased depression of circulation during positive-pressure ventilation and they indicate, further, that peripheral perfusion is also affected.

The lack of significant period effects on quantitative measures of perfusion may be due to the sensitivity of the technique in combination with the small number of horses, or possibly to carry-over effects of the first period on the second. The initial intention was to measure peripheral perfusion during IPPV and SB on separate occasions in a cross-over design. With the results obtained during the first pilot studies, and as discussed above, that protocol was not possible and the change-over between ventilation modes during anaesthesia was therefore necessary.

In contrast to the LDF measurements in Studies I and II, where tail skin perfusion showed the most stable baseline recordings, but was also most reactive to provocations, the tail skin values in Study III were not stable until after approximately one hour of inhalation anaesthesia. Consequently, no comparisons of skin perfusion between the two time periods could be made. The values were initially very high and decreased slowly towards stable levels irrespective of which mode of ventilation was instituted first. The initially high values may have been an effect of the horse's position, since in Studies I and II the horses were placed in lateral recumbency and in Study III they were in dorsal recumbency. Thus gravity and possibly compression of the tail base may have influenced the perfusion differently. This finding was unexpected and prevented interpretation of the effect of different modes of ventilation on skin perfusion.

In healthy horses with a normal fluid balance and blood volume, the circulatory depression induced by IPPV may be of little clinical importance. On the other hand, in horses where fluid balance is affected by hypovolaemia or dehydration, a further reduction in circulation and perfusion may be detrimental.

Muscle metabolic response to anaesthesia: changes in blood, muscle biopsy and microdialysate samples

Since reduced muscle perfusion has been hypothesised to occur during anaesthesia and lead to the development of PAM, the needle biopsy sampling technique and microdialysis were used in the present investigation as complements to the more commonly used blood sampling to study muscle metabolic changes during and after anaesthesia. If hypoperfusion has caused an ischaemic situation in the muscle, this would be indicated by an increased content of lactate and a decreased content of phosphagen compounds (ATP, CP) in muscle biopsy specimens. To different extents in the various studies, such changes were found.

The results encourage further use of these techniques in research on metabolism during and after equine anaesthesia.

Dependent and non-dependent gluteal muscle: biopsy specimens and dialysate

There were no significant metabolic differences (lactate, CP, ATP) between the dependent and non-dependent gluteal muscle in either Study I or Study II. These findings are supported by the results of microdialysis in Study I, which also showed similar changes in both the dependent and non-dependent muscle (Figure 14). As with the perfusion measurements, differences between muscle groups were hypothesised and signs of more increased anaerobic metabolism in the dependent than in the non-dependent muscle were expected. The results obtained from muscle samples and microdialysis support earlier findings of no differences in perfusion between the dependent and non-dependent gluteal muscle (Manohar et al., 1987b; Goetz et al., 1989). The interpretation of the results from the muscle biopsy samples and dialysate are restricted, however, to the small area from which the sample was obtained, and the sampled number of horses was small. Spatial and individual differences in muscle metabolism may still exist, as was discussed for the perfusion measurements. The results of the present investigation show that in these horses and in the areas measured, there were no metabolic differences between the dependent and non-dependent muscle during anaesthesia and one day after recovery. Of the horses in Study II, none developed signs of muscle weakness or PAM.

Since no statistically significant differences between sides of muscle biopsy specimens were found, in this investigation the mean values of ATP, CP and lactate for the two sides are presented (Figure 15).

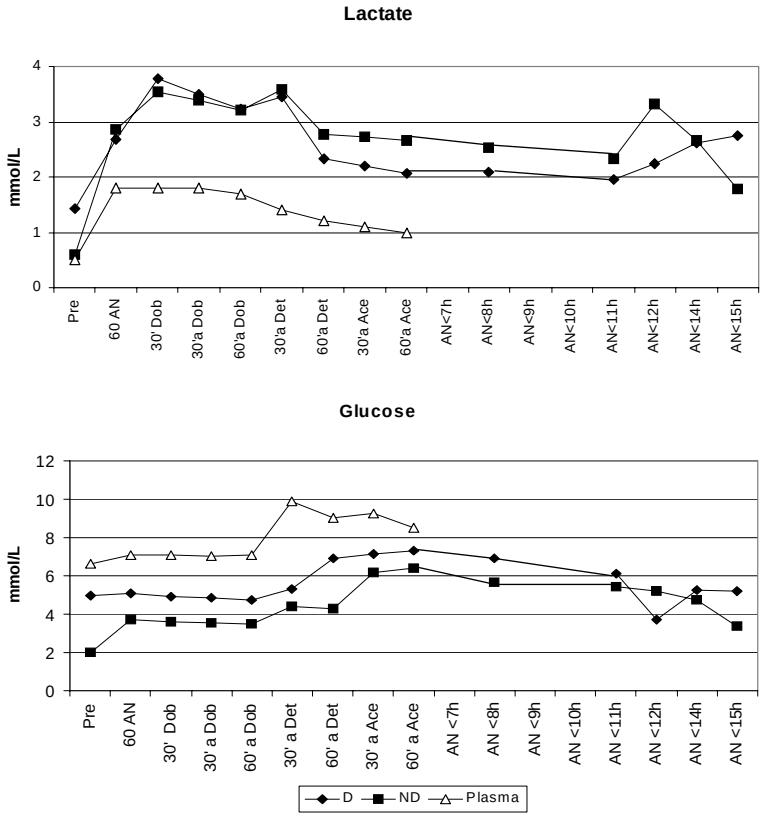
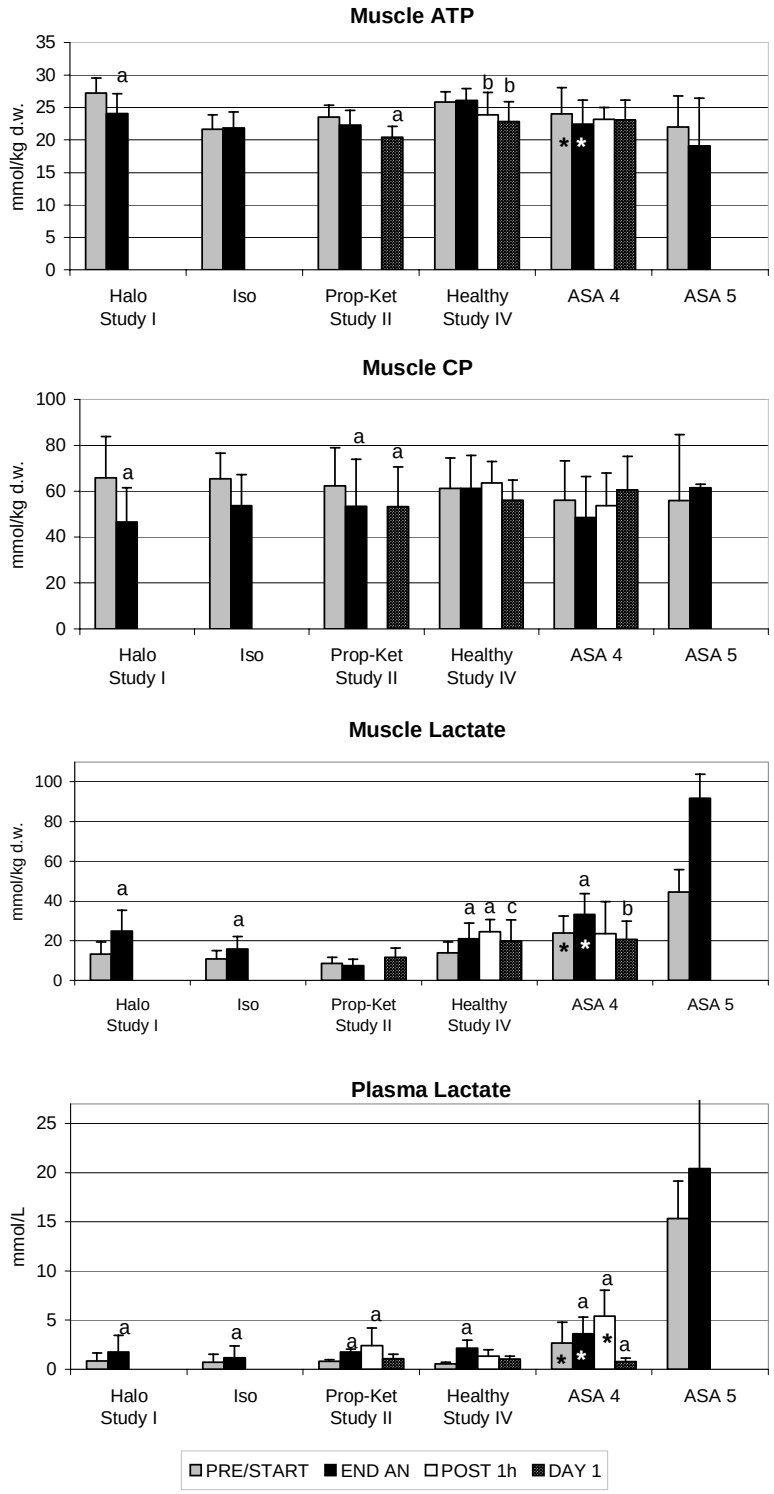


Figure 14. Concentrations of lactate and glucose in dialysate in the dependent (D) and non-dependent (ND) gluteal muscle and in plasma in one laterally recumbent horse in Study I (isoflurane), from before anaesthesia (Pre) to the end of anaesthesia.
60 AN=60 minutes of isoflurane anaesthesia; 30' Dob=at the end of a 30-minute infusion of dobutamine; 30' and 60' aDob, Det or Ace= 30 and 60 minutes after administration of dobutamine, detomidine or acepromazine respectively; AN <7, 8, 9 etc=total hours from induction of anaesthesia. Student surgical training began at AN <8h.

Figure 15. (Opposite page). Mean concentrations of muscle ATP, CP and lactate in mmol/kg d.w. before (PRE) or immediately after induction (START) of anaesthesia, at the end of anaesthesia (END AN), and one hour (POST 1h) and one day after recovery to standing (DAY 1) in horses from Studies I, II and IV.
Halo= halothane anaesthesia; Iso= isoflurane anaesthesia; Prop-Ket= propofol-ketamine anaesthesia; ASA 4 or 5= colic horses with American Society of Anesthesiologists physical status 4 or 5 (scale 1-5). a=Significantly different from PRE/START; b= significantly different from END AN; c=significantly different from POST 1h; *= significant group difference between healthy horses and ASA 4 horses in Study IV.


Muscle ATP
mmol/kg d.w.
Halo Study I
Iso
Prop-Ket Study II
Healthy Study IV
ASA 4
ASA 5
Muscle CP
mmol/kg d.w.
Muscle Lactate
mmol/kg d.w.
Plasma Lactate
mmol/L
PRE/START
END AN
POST 1h
DAY 1

Muscle metabolic changes induced by anaesthesia in healthy horses

Anaerobic metabolism occurred during anaesthesia with both halothane (Study I) and propofol-ketamine (Study II), as indicated by the decreased content of ATP and/or CP at the end of anaesthesia (Figure 15). In Study I these changes were accompanied by an increased content of lactate in muscle and plasma at the end of anaesthesia. Similar increases in lactate were also found in Study IV (Figure 15). In Study II, anaesthesia induced a lactate increase in plasma but not in muscle. In all instances the increases in plasma were small but significant.

The increased lactate concentrations found in the blood during anaesthesia in the healthy horses were in good agreement with previous reports (Robertson, 1987; Taylor, 1991; Luna & Taylor, 1995; Luna et al., 1996). An increase in the concentration of lactate after induction of anaesthesia was also seen in the dialysate in Study I (Figure 14). No dialysate samples were obtained before induction of anaesthesia in Study V, but the lactate levels in the dialysate during anaesthesia in the healthy horses (Figure 16) were similar to those in Study I (Study I: 2-4 mmol/L; Study IV: 1.5-5.5 mmol/L), and it is therefore likely that an increase compared to the pre-induction value also occurred in Study IV. The lactate level in the dialysate in the healthy horses was on all occasions higher than in plasma, which is in agreement with studies in humans using similar flow rates for dialysis and confirms that the muscle is a producer of lactate (Hagström-Toft et al., 1997; Rosdahl et al., 1998). Continued changes in dialysate during anaesthesia were not always accompanied by similar changes in plasma, and the magnitude of the increase in lactate after induction of anaesthesia in Study I was also greater in dialysate than in plasma (Figure 14). When lactate increases in dialysate but not in plasma, the possibility of impaired venous drainage from the tissue must be considered (Müller et al., 1995). In such cases lactate concentrations measured only in venous plasma may lead to an underestimation of the actual lactate production. This was shown, in fact, in a human study using microdialysis (Müller et al., 1995). In an earlier study (Serteyn et al., 1987b), when anaesthetised horses were turned into dorsal recumbency after a period in lateral recumbency, the plasma lactate level increased abruptly and a significantly greater increase was detected in horses that developed PAM than in normal horses. The increase was also more pronounced in venous blood from the former dependent limb (in lateral recumbency) as compared to the non-dependent limb. This indicated that there was a washout of accumulated lactate from the former dependent muscles. Up to now, however, no one has actually measured the lactate content within the muscle of the horse. The present results show that increases in plasma lactate concentrations during anaesthesia do result from an increased muscle lactate production and as seen from the lactate level in the dialysate the increase occurs early in the anaesthetic period. Interestingly and for comparison, in a study on healthy human patients undergoing general anaesthesia for cholecystectomy the muscle content of lactate increased during anaesthesia (Stjernström et al., 1981a), and in a similar study there was also an increased release of lactate into the circulation (Stjernström et al., 1981b).

For anaerobic metabolism to occur, the supply of oxygen to the tissues must be inadequate (Enger et al., 1978; Newsholme & Leech, 1983; Harris et al., 1986; Heppenstall et al., 1986; Jansson et al., 1987; Harris et al., 1991; Schuback & Essén-Gustavsson, 1998). When evaluating effects of anaesthesia on adenine nucleotides, CP, lactate, for example, it is important to bear in mind that during anaesthesia the horse does not perform any work and that the resting energy and oxygen requirements should be low. In all studies the body temperature, measured rectally, in the blood or in the muscle, decreased during the course of anaesthesia, indicating that the energy consumption was presumably lower than in the awake, standing horse. As discussed above, both the central circulation and muscle perfusion have been shown to be decreased during inhalation anaesthesia (Weaver & Lunn, 1984; Manohar et al., 1987b), and this may lead to diminished oxygen availability in the tissues. If this is combined with an increased resistance to flow through the tissues caused by external compression of the muscle (Lindsay et al., 1980; Lindsay et al., 1985; Serteyn et al., 1991), decreased venous outflow (Serteyn et al., 1987b; Taylor & Young, 1990) or increased transmural pressure (Kamm & Pedley, 1989; Nielsen, 1991; Raisis et al., 2000c), the tissue will become hypoxic or ischaemic and will convert to anaerobic metabolism with lactate production. The slow increases in plasma hypoxanthine and xanthine concentrations that were observed during anaesthesia in Study II and together with an increase in uric acid reached significance after recovery to standing, support the theory of anaerobic metabolism during anaesthesia. These metabolites are often analysed during exercise research, since they are the end point of adenine nucleotide breakdown (Harris et al., 1991; Schuback & Essén-Gustavsson, 1998). Similar increases in uric acid were found during reperfusion in previous studies on anaesthetised horses and are in line with the present results (Serteyn et al., 1990; Serteyn et al., 1994). In conclusion, there is good evidence that hypoxia or ischaemia does occur in muscle during anaesthesia in the horse (Enger et al., 1978; Heppenstall et al., 1986).

During anaesthesia in Study IV, apart from increased concentrations of lactate, there were increases in FFA and glycerol as well as in cortisol and glucose levels, indicating an increased sympathetic output and lipolysis (Snow, 1979; Lager, 1991; Wolfe & Martini, 2000) (Figures 20 and 21). These findings are in accordance with a previous observation in healthy human subjects that fat metabolism dominated during anaesthesia (Stjernström et al., 1981a; Stjernström et al., 1981b). In the present investigation, even if the sympathetic output and lipolysis were increased in the healthy horses during anaesthesia, the decreases in the muscle content of ATP and CP support the conclusions that anaerobic metabolism did occur.

Muscle metabolic changes after anaesthesia in healthy horses

If the energy consumption is low during anaesthesia as is presumed from our results, a different situation could arise when the horse has to regain the standing position during recovery from anaesthesia. In some horses, recovery to standing is smooth and they stand up calmly and steadily, seemingly without any major effort. In others, however, regaining the standing position is more difficult. Repeated

attempts may have to be made before they remain on their feet, and some horses are very weak and unstable and have to put great effort into remaining standing, while others again paddle around in the recovery stall before finally standing up successfully.

Muscle biopsy samples were taken one hour after standing in seven healthy horses in Study IV, and plasma samples were obtained within one hour after standing in all horses in both Studies II and IV. In addition, dialysate was sampled from ten healthy horses in Study V during and immediately after standing. The results show that the muscle content of ATP was decreased (significantly) in five out of seven horses in the biopsy sample taken one hour after standing in Study V, but the content of muscle lactate was not different at that time from the value at the end of anaesthesia (Figure 15). There were large variations in plasma lactate in the first sample obtained after standing in both Studies II and IV, with concentrations of up to 5 and 7 mmol/L in some horses, but decreased concentrations in others. At least with the highest values there was some association with the quality of recovery. The two horses that did not show any decrease in ATP content after standing in Study IV actually had very smooth recoveries. Another horse, however, with a seemingly easy recovery, had a marked decrease in the muscle content of ATP and increase in muscle lactate. In plasma and dialysate in Study IV, one hour after standing the lactate values had decreased to levels significantly lower than during anaesthesia (Figure 16). Lactate can be rapidly removed from the circulation or consumed by the muscle cell when the circulation is adequate (Enger et al., 1978; Larsson & Hultman, 1979; Gladden, 2000) and this may explain why the lactate level in plasma and dialysate one hour after standing in Studies IV and V had decreased compared to the anaesthetic levels, while muscle lactate in some horses still remained at the anaesthetic level.

It is possible that the decrease in the muscle content of ATP and increase in plasma or dialysate lactate levels that occurred during and after the horses' attempts to stand in Studies II and IV were exercise-like responses. However, an increased content of muscle lactate and decreased content of muscle ATP are usually only observed after intense exercise, when there is a high demand for rapid energy production, forcing the muscle cell to convert to anaerobic metabolism (Harris et al., 1991; Schuback & Essén-Gustavsson, 1998). Standing up is not comparable with maximal exercise on the racetrack or treadmill and therefore one would not expect to find a decrease in ATP or CP in the sample obtained one hour after standing. Under normal circumstances the regeneration of ATP is rapid and ATP has been reported to return to the baseline level within 15 minutes of a maximal treadmill exercise in horses (Schuback & Essén-Gustavsson, 1998; Schuback et al., 2000). By one hour and definitely one day after recovery to standing, ATP should have been regenerated, given there was no obvious limitation of either oxygen or substrate. At least in the healthy horses, there was no reason to believe that the availability of either oxygen or substrate was limited post anaesthesia. The decreases in muscle CP and ATP on the day after anaesthesia in Studies II and IV were therefore unexpected, especially since the muscle lactate was low. Other reasons for these findings than anaerobic metabolism seem more likely and one may speculate that instead of an increased demand for ATP during this period, there was a decreased capacity for regeneration of ATP and CP.

The present results are comparable with those in a human study on incomplete ischaemia, where the total creatine pool (creatine+CP) and ATP had not been regenerated 16 h after declamping of the aorta (Sjöström et al., 1982). The suggested explanation for the post-clamping decrease in high-energy phosphagens was an inadequate or slow synthesis of adenine nucleotides due to functional disturbances of the mitochondrial membranes. The fall in total creatine was thought to be due to loss through disrupted cellular membranes. It is possible that similar mechanisms were operative in the present investigation.

A decreased capacity for regeneration of ATP after anaesthesia may therefore be a likely explanation for the low phosphagen levels seen after recovery from anaesthesia in the present investigation.

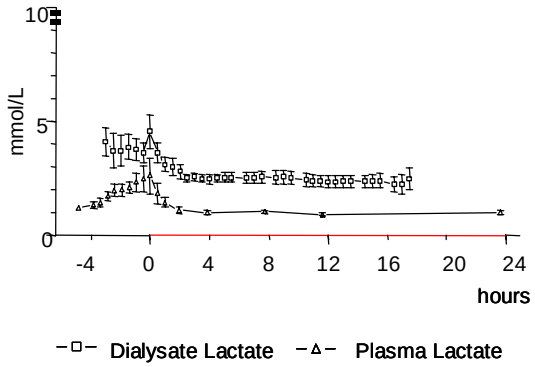


Figure 16: Lactate (mmol/L) in dialysate and plasma (mean and SEM) in 10 healthy horses in Study V, during anaesthesia and up to 24 hours after standing. For dialysate samples, n decreases with time. The graphs are synchronised to the moment of regaining the standing position and this point was set to time 0. Note that in some horses some samples obtained before 0 may represent samples during recovery, while in others they may still be anaesthetic samples, since the horses spent different lengths of time lying down in recovery.

There are indications that post-anaesthetic myopathy in horses develops largely as a result of reperfusion injuries (Serteyn et al., 1991; Serteyn et al., 1994) occurring after termination of anaesthesia, and several authors have found parallels between PAM in horses and the compartment syndrome in humans (Lindsay et al., 1980; Steffey et al., 1980b; Norman et al., 1989; Taylor & Young, 1990). There is evidence that reperfusion in association with the compartment syndrome causes more damage to the muscle than does reperfusion after tourniquet application as shown in study in dogs (Heppenstall et al., 1986). The energy charge potential has been reported to be normalised within five to fifteen minutes of tourniquet removal in dogs or humans (Enger et al., 1978; Larsson & Hultman, 1979; Heppenstall et al., 1986), while CP, ATP and pH remained depressed even two hours after restoration of blood flow through fasciotomy and epimysiotomy in the compartment syndrome (Heppenstall et al., 1986). However, morphological changes and signs of membrane disturbances, such as fibre oedema and swelling of mitochondria after restoration of blood flow, have been seen in tourniquet studies in both humans and mice (Sjöström et al., 1982; Chan et al., 2004a). Type II fibres

are reported to be especially vulnerable to reperfusion injury (Sjöström et al., 1982; Chan et al., 2004a; Chan et al., 2004b). Interestingly, investigative electron microscopy of affected muscles in equine PAM has shown degeneration of predominantly type II fibres (White, 1982), with the same pattern of muscle degeneration changes as seen in human studies and in cases of equine acute exertional rhabdomyolysis (Lindholm et al., 1974). Further studies on similarities between reperfusion injury in humans and equine PAM are warranted.

The effect of type of anaesthesia

The observed differences in the contents of ATP and CP at the end of anaesthesia with the different anaesthetic protocols in this investigation may have several causes, and time may be one influencing factor. The halothane-anaesthetised horses in Study I were anaesthetised for the longest duration (approximately 7 h) and also showed the greatest changes at the end of anaesthesia. The anaesthetic protocol itself may have influenced the results, since various anaesthetic agents may affect the metabolic processes differently, malignant hyperthermia probably being the most well-known anaesthetic-triggered metabolic disturbance (Waldron-Mease, 1978; Byrd, 1983; Manley et al., 1983; Kirmayer et al., 1984; Allen, 1993). Several studies in different species have shown that halothane has a more pronounced negative effect on muscle than, for example, isoflurane, ketamine and propofol (McLoughlin et al., 1987; Fiacchino et al., 1989; Whitehair et al., 1996; Aktas et al., 1997). There are also reports that halothane may inhibit phosphorylation through uncoupling of NADH-reductase (Miller & Hunter, 1971; Miller, 1972) and this could possibly have been a factor in Study I (halothane).

Hypotension has been associated with an increased frequency of PAM (Grandy et al., 1987; Lindsay et al., 1989), but since cardiovascular function was not monitored as closely in the halothane-anaesthetised horses as in the other studies, no conclusions regarding the effects of central circulation on ATP and CP can be drawn. In addition, the horses anaesthetised with halothane were not allowed to recover and therefore we do not know whether any of these horses, which displayed the most severe muscle-metabolic changes of all studies at the end of anaesthesia, would have developed PAM.

Propofol-ketamine anaesthesia (Study II) in the present investigation was associated with changes in the phosphagen stores, despite the minor reported effects of these agents on muscle metabolism in earlier studies in rats and dogs (McLoughlin et al., 1987; Aktas et al., 1997) and despite the fact that these horses were anaesthetised for the shortest duration. There may be a species difference in the response to ketamine and propofol or a difference between the studied muscle groups (Jennische et al., 1979). The different metabolic response to the anaesthetic agents in Study II as compared to that in other species may also be attributed to the fact that, for the horse, lying down for a prolonged period of time is largely non-physiological.

Irrespective of the anaesthetic method, the horses in the present investigation showed signs of anaerobic metabolism, and decreases in muscle high-energy phosphagens were still evident the day after anaesthesia.

At the end of anaesthesia in Study IV, the plasma cortisol level was increased (Figure 21) compared to the pre-anaesthesia level, while in Study II it was decreased. The results in the healthy horses correspond to findings in earlier studies where inhalation anaesthesia with halothane or isoflurane induced an adrenocortical response (Taylor, 1991), while only small or no increases in cortisol were seen during total intravenous anaesthesia with propofol (Taylor, 1989a), thiopentone (Taylor, 1990) or a combination of detomidine, ketamine and guaifenesin (Taylor et al., 1995; Luna et al., 1996). The increase in plasma glucose concentrations during anaesthesia in the healthy horses in Study IV (Figure 20) was probably an effect of the anti-insulin properties of cortisol (Clerc et al., 1986; Lager, 1991).

Metabolism before anaesthesia in colic horses

Before anaesthesia high concentrations of plasma glucose, lactate, glycerol, FFA and cortisol were seen in the colic horses (Figures 20 and 21). Similar changes indicating parallel activation of the lipid and carbohydrate metabolic pathways may be observed in the critically ill human patient and are consequences of a greatly increased sympathetic output (Spitzer et al., 1989; Wolfe & Martini, 2000). In the healthy horse, adrenergic stimulation results in similar increases (Snow, 1979). The biochemical findings in Study IV are in accordance with previous reports on colic horses (Moore et al., 1976; Svendsen et al., 1979; Parry et al., 1983a; Milne et al., 1990; Stegmann & Jones, 1998; Protopapas, 2000). Under normal conditions there is a reciprocal relation between the availability of FFAs and glucose in plasma, depending, for example, on the time interval from feed intake or exercise (Lawrence, 1990; Witham & Stull, 1998; Wolfe & Martini, 2000; Spriet & Watt, 2003). Insulin is a key regulatory hormone in the maintenance of this balance. In the very ill individual, sympathetic outflow increases and both the lipid and carbohydrate metabolic pathways are activated, while a relative inefficiency of insulin develops (Clerc et al., 1986; Wolfe & Martini, 2000), leading to simultaneously high concentrations of glucose and breakdown products from lipolysis. Measurement of insulin in the colic horses before, during and after anaesthesia is an interesting future research area, since in human studies, insulin treatment of the critically ill patient has been shown to improve morbidity and reduce mortality (Krinsley, 2004; Pittas et al., 2004; Thorell et al., 2004).

Muscle metabolic changes induced by anaesthesia in colic horses

The colic horses in Study IV entered anaesthesia with a significantly lower muscle content of ATP and higher content of Cr and of both muscle and plasma lactate than the healthy horses of the same study, and these differences persisted throughout the anaesthetic procedure (Figures 15 and 20). The ATP content varied considerably in the colic horses and both the lowest (16.4 mmol/kg d.w.) and the highest (32.9 mmol/kg d.w.) values were found in this group. Whereas muscle lactate increased in all colic horses from the sample taken immediately after induction of anaesthesia until the end of anaesthesia, the same increase was not seen in plasma. Since the microdialysis probes (Study V) were inserted after induction of anaesthesia and the duration of anaesthesia varied considerably

between horses, it is questionable whether any group conclusions can be drawn from the dialysate results during this period. Statistical analysis was therefore only performed on the last sample during anaesthesia. At this time point there was no significant difference in lactate levels in the dialysate between colic and healthy horses (Figures 16 and 17). The individual variation during anaesthesia was large among both colic and healthy horses regarding the lactate concentrations (Study V) in plasma (1.5-6.9 and 1.6-3.8 mmol/L in colic and healthy horses respectively) as well as in dialysate (3.1-15.6 and 1.6-6.8 mmol/L in colic and healthy horses). Two severely ill colic horses not included in the statistical analyses in Study V had plasma lactate values of 15.4-28.5 mmol/L and dialysate values of 16.2-42.8 mmol/L. There was great individual variation as to how well lactate in plasma mirrored the changes and the levels in dialysate. The discrepancy between the concentrations of lactate in dialysate and plasma was larger in the colic than healthy horses. This finding emphasises the risk of underestimating the tissue concentration of lactate when lactate is measured in plasma, especially when venous drainage may be impaired (Müller et al., 1995).

In one colic horse the lactate concentration in the dialysate was 15 mmol/L, while in plasma a lactate concentration of 2.5 mmol/L was measured. In addition, during anaesthesia, the muscle content of CP decreased and muscle lactate doubled. In this horse, muscle oxygen supply must have been severely reduced and venous drainage impaired to allow these changes to occur. During the three-hour anaesthesia procedure, MAP varied from 52-63 mmHg and HR was stable around 32 beats/min, both parameters were below the mean for the colic horses and indicate depressed cardiovascular function. An interesting finding is that this colic horse had the most violent recovery of all colic horses, although finally successful. It is likely that the derangements in the muscle metabolic state that occurred during anaesthesia affected this horse's capacity to stand up smoothly. Post-operatively it showed slight hindlimb lameness and low CK values.

In the critically ill human patient β-adrenergic stimulation of glycogenolysis may contribute greatky to lactate production (Gore et al., 1996; Wolfe & Martini, 2000). In these patients hypermetabolism may be present and under these conditions, excess lactate production results from an increased formation of pyruvate that will not only enter the Krebs cycle but also form lactate, keeping the lactate-to-pyruvate ratio (La/Py ratio) constant (Gore et al., 1996; James et al., 1999; Jansson et al., 2003). Pyruvate may be measured in dialysate and the La/Py ratio is routinely used by many researchers to differentiate between lactate formed in ischaemia and that produced during hypermetabolism (Gore et al., 1996; James et al., 1999; Jansson et al., 2003; Setälä et al., 2004). Pyruvate was measured in dialysate in five colic horses and a "complete" set of samples was obtained in four horses (Figure 18), hence no statistical analysis was performed. The La/Py ratio was initially high (range 38-110) during anaesthesia, but decreased rapidly towards the end of anaesthesia in all but one horse (this horse is not included in the figure), indicating that lactate production initially derived mainly from anaerobic glycolysis but that this source decreased with time.

No changes in the muscle content of glycogen were seen in healthy horses at the end of or the day after anaesthesia, as determined by histological evaluation of

muscle biopsy samples obtained from five of the healthy and seven of the colic horses from Study IV (Essén-Gustavsson et al., 2003; Cedervind, 2004). In contrast, some colic horses entered anaesthesia with a low or no glycogen content, primarily in Type I but also in Type IIB fibres. Horses in the poorest physical condition before anaesthesia also had the largest number of glycogen depleted muscle fibres. Similar patterns were still evident at the end of anaesthesia and on the day after recovery. In the healthy horses in Study I (isoflurane anaesthesia) and Study II and in 13 healthy horses in Study IV, the muscle glycogen content was assayed biochemically and as in the histochemical assays of glycogen, no significant changes were detected at the end of anaesthesia. These results emphasise the extreme metabolic stress to which the colic horse may be subjected pre-operatively, since depletion of muscle fibres of glycogen is usually only observed after intensive exercise or anoxia (Gottlieb, 1989; Gottlieb et al., 1989; Hultman, 1995). Interestingly, in humans the muscle glycogen content has been found to decline after abdominal surgery, with the maximum decrease on the third to the seventh day (Bergström et al., 1965). In the light of the above results, an extended study on the muscle glycogen content should involve sampling daily for up to one week post anaesthesia in both healthy and ill horses.

The muscle contents of ATP, CP and lactate in the colic horses in the present investigation would be affected by several factors, such as the degree of illness and circulatory compromise (Bergström et al., 1976), endotoxaemia (Myrvold et al., 1975; Spitzer et al., 1989), acid-base and electrolyte balance (Relman, 1972; Stjernström et al., 1981a; Lovén et al., 1983b; Del Canale et al., 1986) and the exertion associated with colic behaviour. In a human study on critically ill patients (Bergström et al., 1976) a low adenine nucleotide level in muscle tissue was thought to result from increased deamination of AMP and/or a decreased rate of purine synthesis. These changes may be due to electrolyte and pH abnormalities causing alterations of enzyme functions and membrane properties (Stjernström et al., 1981a; Lovén et al., 1983b). Among the six colic horses from which samples were taken before anaesthesia for blood gas and acid base determination, the venous pH and base excess (BE) were below normal in one horse (pH=7.20, BE=-20). This horse was the one with the lowest muscle content of ATP. Before anaesthesia, the colic horses displayed various electrolyte disorders (decreased calcium, inorganic phosphate and potassium concentrations) that may have affected the mitochondrial function. In the present investigation, the individual colic "behaviour" may also have influenced the muscle contents of ATP, CP and lactate in some horses, since violent colic signs with kicking, rolling and thrashing around could be comparable to other exercise that consumes ATP. In a previous study, increased (sic) contents of ATP, CP and lactate were seen in muscle after administration of endotoxin in dogs (Myrvold et al., 1975). The increased content of high-energy phosphagens was thought to be caused by sympathetic-adrenal stimulation, possibly together with a direct effect of endotoxin at the cell membrane level. Endotoxaemia is common in colic horses that need surgery and they have a high sympathetic output (Svendsen et al., 1979; Stegmann & Jones, 1998), and the mechanism described above could possibly partly account for the variable content of muscle ATP in the present study.

Since the colic group of horses was heterogeneous regarding physiological parameters, apart from the fact that they all needed surgery, the factors influencing the content of high-energy phosphagens most likely differed between horses. The finding that their disease actually had influenced the muscle content of these compunds deserves further attention.

Muscle metabolic changes after anaesthesia in colic horses

In the colic horses, compared to the concentrations at the end of anaesthesia the lactate concentration in dialysate was significantly increased for two hours and in plasma for one hour after the standing position had been regained (Figures 17 and 20). Significantly higher concentrations of lactate in both dialysate and plasma were observed in the colic than in the healthy horses during the first hours after the standing position was regained. This shows that regaining the standing position after anesthesia induces a greater muscle metabolic response in colic than healthy horses.

The La/Py ratio continued to decrease after the end of anaesthesia in the four horses that were still alive and sampled (Figure 18). Immediately after recovery to standing there was a small increase in the ratio, resulting from a large increase in lactate and a slightly smaller relative increase in pyruvate. Thereafter, the La/Py ratio continued to decrease. In healthy individuals of other species the La/Py ratio has been reported to vary between 10 and 20 (Sjöström et al., 1982; Suistomaa et al., 2000; Ungerstedt et al., 2002; Jansson et al., 2003; Setälä et al., 2004). A ratio of 25 or below was reached in four of five animals 1-20 hours after standing, while in one horse it remained above 30 until the end of sampling 20 hours after recovery. Since pyruvate was only measured in a few horses, the results should be interpreted with care but deserve attention.

In the colic horses, in contrast to the healthy horses, the glucose concentration in the dialysate increased during and immediately after recovery to standing (Figure 19). In addition in the colic horses, the glucose level in the dialysate was found to exceed that in plasma by several mmol/L, especially during this period. This finding is puzzling, since free glucose is transported from the liver and taken up by the muscle (Newsholme & Leech, 1983; Wolfe & Martini, 2000), and is usually lower in the intercellular fluid than in plasma (Fuchi et al., 1994; Rosdahl et al., 1998). The observed increase in dialysate could possibly be an effect of increased blood flow (Hickner et al., 1992; Rosdahl et al., 1993; Rosdahl, 1998; Rosdahl et al., 1998), since at the flow-rate used, complete equilibration was not expected (Rosdahl et al., 1998). That an increased blood flow may have influenced the results is supported by similar increases in dialysate urea concentrations during attempts to stand (Rosdahl et al., 1998; Lundberg et al., 2002). However, glucose concentrations in dialysate that were 5-8 mmol/L greater than in plasma would still not be expected. Since no healthy horse showed similarly higher glucose concentrations in dialysate compared to plasma, this phenomenon must relate to some factor unique for the systemically ill horses. A breakdown of muscle glycogen to release free glucose may have occurred in the colic horses (Newsholme & Leech, 1983). Increased levels of free glucose have been found in

humans after intense exercise (Essén & Kaijser, 1978). Some of the increase observed in dialysate lactate after recovery to standing may partly have been due to an increased availability of glucose (Newsholme & Leech, 1983; Del Canale et al., 1986; Kerckhoffs et al., 1998).

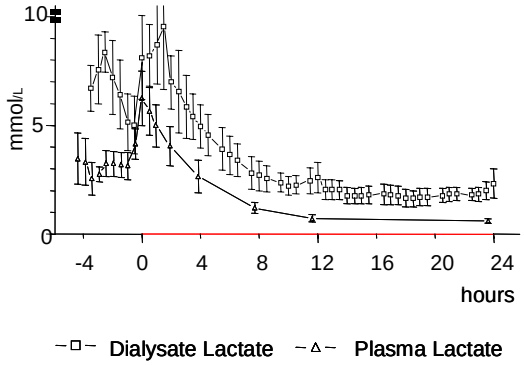


Figure 17: Lactate in dialysate and plasma (mean and SEM) in 8 colic horses in Study V during anaesthesia and up to 24 hours after standing. For dialysate samples, n decreases with time.
The graphs are synchronised to the moment of regaining the standing position and this time point was set to 0. Note that in some horses the samples obtained before 0 may represent samples in recovery, while in others they may still be anaesthetic samples, since the horses spent different lengths of time lying down in recovery.

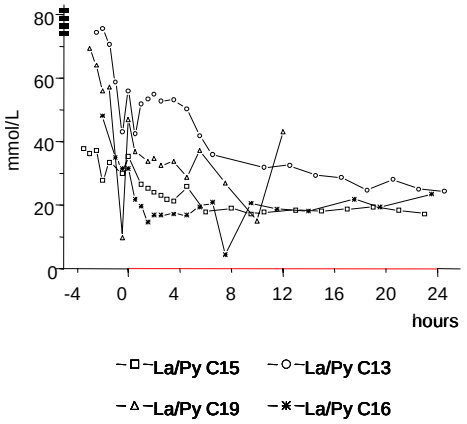


Figure 18: The La/Py ratio in four individual colic horses (C13, C15, C16 and C19) in Study V during anaesthesia and up to 24 hours after standing.
The graphs are synchronised to the moment of regaining the standing position (time 0).

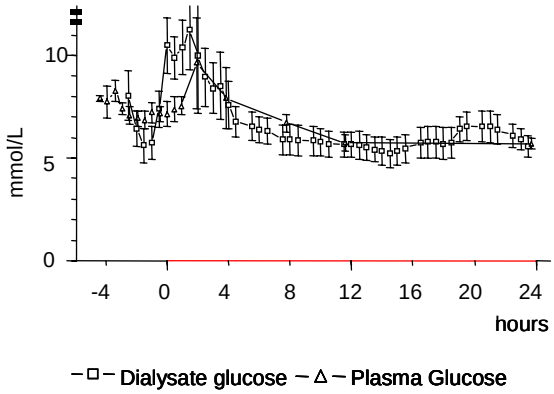


Figure 19: Glucose in dialysate and plasma (mean and SEM) in 8 colic horses in Study V during anaesthesia and up to 24 hours after standing. For dialysate samples, n decreases with time.
The graphs are synchronised to the moment of regaining the standing position and this time point was set to 0. Note that in some horses the samples obtained before 0 may represent samples in recovery, while in others they may still be anaesthetic samples, since the horses spent different lengths of time lying down in recovery.

In the colic horses in Study IV, lipolysis and high glucose concentrations continued in the early post-anaesthetic period, indicating continuation of high sympathetic stimulation and stress. Lipid metabolism had increased further by eight hours post-operatively, as seen from the suddenly elevated FFA and the simultaneously smaller increase in glycerol, while the glucose and lactate concentrations were approaching normal (Figure 20). The rate at which FFA and glycerol reached stable, low levels varied greatly between individual colic horses, but it was not until four days after anaesthesia that the concentration of FFA in the group of colic horses was similar to that in the healthy horses. This sudden increase in FFA after recovery from anaesthesia differed significantly from the metabolic events in the healthy horses, where the metabolism had normalised approximately one or two days after anaesthesia. These differences between groups may reflect the continued starvation in the colic horses, while the healthy horses had begun eating their normal feed ration only a few hours after recovery from anaesthesia. An increased lipid metabolism one day after abdominal surgery was also evident in an earlier study (Protopapas, 2000) and may be seen during starvation in horses (Naylor et al., 1980; Rose & Sampson, 1982; Lopes & White, 2002). The differences probably also related to the gastrointestinal disease in the colic group and the tissue trauma of colic surgery to which these horses were subjected. The average maximal weight loss of 8% observed in the colic horses of the present investigation compares well with that reported from other studies (Protopapas, 2000; Sellon et al., 2004), and in conjunction with the increased concentrations of FFA, further indicate that the colic horses were in a negative energy balance. Although the healthy horses returned to their normal daily routines and feed intake, there was a trend towards a decreased body mass in this group also.

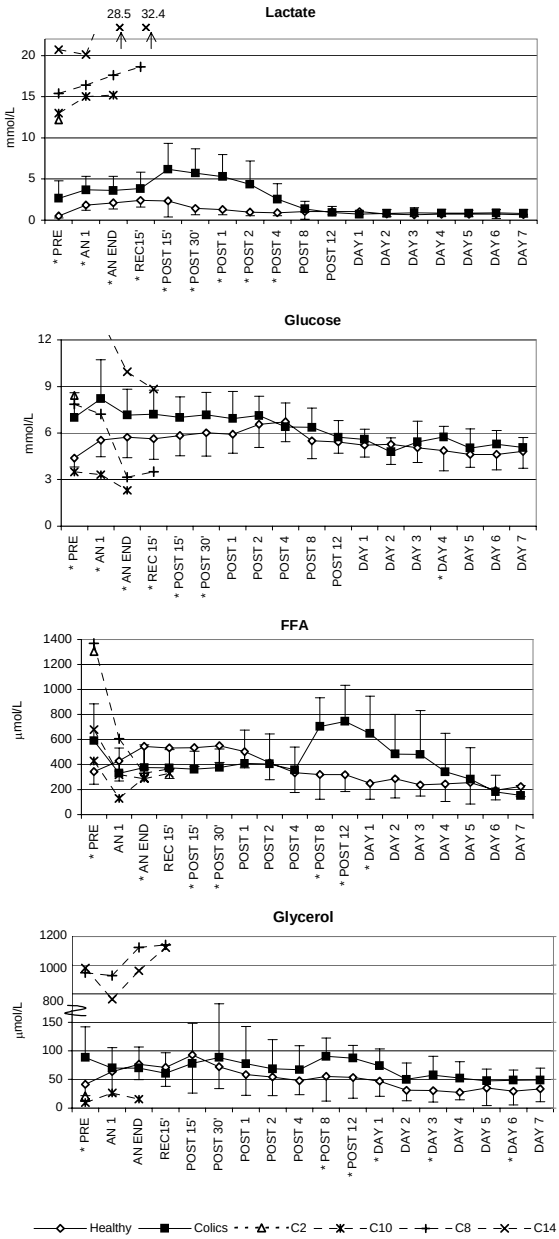


Figure 20: Concentrations (mean values and SD) of plasma lactate, glucose, free fatty acids (FFA) and glycerol, from before anaesthesia (PRE) to 7 days post anaesthesia in healthy (n=9-20) and colic horses (n=8-16) with the four ASA 5 colic horses shown individually (C2, C10, C8 and C14). Note that the time scale is not linear and that the Y axis is broken for glycerol. ASA 5= American Society of Anesthesiologists physical status 5/5. AN 1 and AN END= anaesthesia after 1 hour and at the end; REC 15'= 15 minutes after termination of inhalation anaesthesia; POST 15' and 30'= 15 and 30 minutes after regaining the standing position; POST 1, 2, 4, 8 and 12= hours after standing; DAY 1-7= days after standing.

Changes in electrolytes and creatine kinase before, during and after anaesthesia in healthy and in colic horses

A large proportion of the colic horses showed decreased serum concentrations of calcium, phosphate and potassium before anaesthesia (Figure 21), in agreement with findings in a recent study (Protopapas, 2000). In the human emergency ward low potassium levels are common, and low levels have been associated with a higher rate of mortality (Vanek et al., 1994; James et al., 1999). This hypokalaemia is due to an increased uptake of potassium into muscle through the effect of epinephrine on the shuttle Na^+, K^+-ATPase (Brown et al., 1983; James et al., 1999), which consumes ATP. The material in Study IV was too small to draw any conclusion regarding the relation between probability of death and potassium levels, but in a Danish study no association between death and potassium, phosphate or calcium was found in colic horses (Thoefner et al., 2000).

During anaesthesia in Study IV, the serum concentrations of phosphate increased and calcium decreased in both healthy and colic horses, while serum potassium decreased in the healthy horses but remained at pre-anaesthetic, low levels in the colic horses. A similar but not significant decrease in serum potassium was seen in the healthy horses in Study II. Increased phosphate and decreased calcium concentrations during equine anaesthesia have been reported (Johnson et al., 1978; Lindsay et al., 1989; Watson et al., 2002), but there is no definite explanation for these changes. Suggested causes are a breakdown of high-energy phosphagens (CP and ATP) (Johnson et al., 1978) or an alteration in renal function (Watson et al., 2002). The muscle content of ATP and CP was not decreased at the end of anaesthesia in Study IV and the only measure of renal function, the plasma urea concentration, remained at awake values in both groups of horses. A weak but significant correlation was found in Study IV between the degree of increase in phosphate and length of anaesthesia, a finding that supports earlier observations (Johnson et al., 1978). There was a similar increase in both groups, contradicting the idea of metabolic acidosis as a cause of the increase in the concentration of serum phosphate this study (Knochel, 1981; Shiber & Mattu, 2002), since only the colic horses exhibited metabolic acidosis. The exact cause of the anaesthetic hyperphosphataemia thus remains unclear.

Post anaesthesia, both healthy and colic horses (Study IV) went through a period of hypophosphataemia, which was similar in both groups (Figure 21), suggesting that anaesthesia per se was the cause. In humans rhabdomyolysis is one of the most common consequences of severe hypophosphataemia (Knochel, 1981; Knochel, 1992; Visweswaran & Guntupalli, 1999). This is interesting, since in a case report of post-anaesthetic myonecrosis in two horses, marked hypophosphataemia (0.19-0.29 mmol/L) was present postoperatively in both cases (Friend, 1981). A post-anaesthetic decline in inorganic phosphate has been observed in earlier studies, but little attention has been paid to this phenomenon (Steffey et al., 1980a; Lindsay et al., 1989). Where then, does the phosphate escape to and why? Rapid changes in the phosphate pool are largely governed by changes between intra- and extracellular compartments, while more long-term changes are regulated through the kidneys (Laroche, 2001). Protopapas (Protopapas, 2000) measured the urinary

excretion of phosphate after abdominal surgery and found that it was increased. Since phosphate is important in phosphorylation processes in the body (Lovén et al., 1983a; Lovén et al., 1983b) and hypophosphataemia apparently is involved in rhabdomyolysis, future attention should be paid to this potential factor in the development of PAM. It is interesting to note that the lowest values for serum phosphate in Study IV coincided with the decreased contents of muscle ATP (Studies II and IV) and CP (Study II).

After a short-lasting increase in the serum concentration of potassium seen immediately after standing in Studies II and IV, there was a 4-hour decrease in the healthy horses in Study IV but a slower and more long-lasting decline in the colic horses. This decrease might have been related to the absence of feed intake (Rose, 1990), but may, as discussed above, be an effect of β2-receptor stimulation by epinephrine (Brown et al., 1983).

Interesting to note in the post-anaesthetic period were the similar increases in plasma CK concentrations in the colic and healthy horses of Study IV even though the colic horses entered anaesthesia with higher concentrations (Figure 21). The peak values were seen between two and eight hours after recovery to standing. There was great interindividual variation in both groups of horses, however. While in the healthy horses some kind of association was found between the quality of recovery and post-anaesthetic signs of gait disturbances and the increase in CK concentrations, this association was not seen as clearly in the colic horses. In the colic horses it seemed as if the post-anaesthetic CK level was influenced more by the pre-anaesthetic values than by how the horses recovered to standing. The rises in CK were relatively small even in horses that displayed typical signs of PAM, as compared to earlier reported values for horses with PAM (Waldron-Mease, 1977; Lindsay et al., 1980; Friend, 1981; Lindsay et al., 1989). Does the overall increase in CK after anaesthesia in both groups in Study IV indicate general membrane damage caused by anaesthesia and prolonged recumbency per se?

The changes in muscle high-energy phosphagens, in serum electrolytes and in CK support the theory of altered membrane function post anaesthesia both in healthy horses and in those with colic.

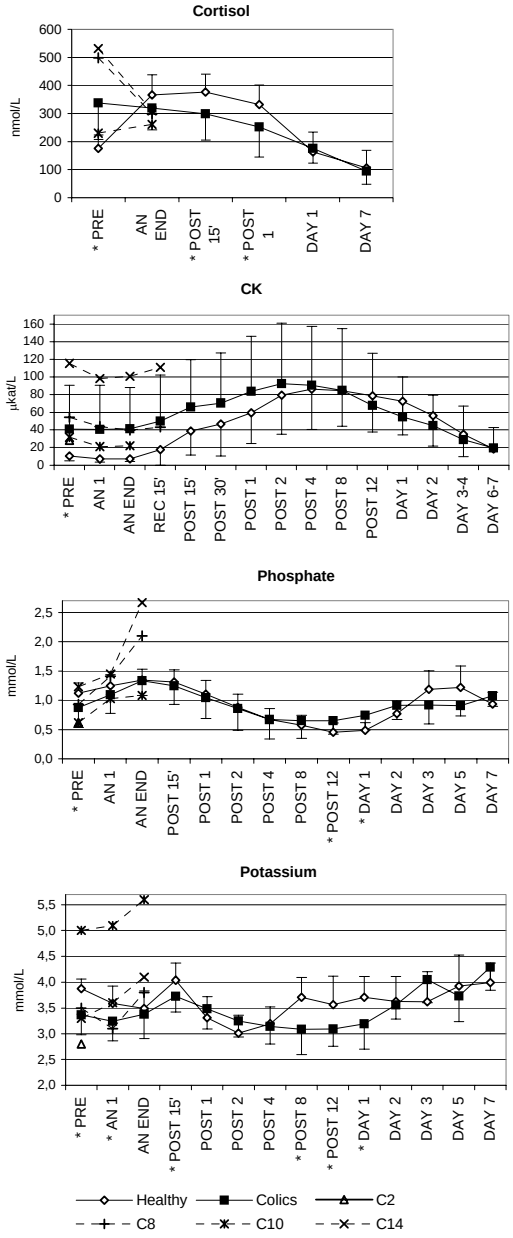


Figure 21: Concentrations (mean values and SD) of plasma cortisol and creatine kinase (CK), serum inorganic phosphate and potassium from before anaesthesia (PRE) to 7 days post anaesthesia in healthy horses (n=8-20) and colic horses (n=10-16) in Study IV, with the four ASA 5 colic horses shown individually (C2, C8, C10 and C14). Note that the time scale is not linear. For key see Figure 20.

Lactate as a predictor of outcome

In previous studies the pre-anaesthetic concentrations of several analytes have been investigated in colic horses in attempts to find markers that may be used as prognostic indicators (Kalsbeek, 1975; Moore et al., 1976; Svendsen et al., 1979; Parry et al., 1983a; Parry et al., 1983b; Puotunen-Reinert, 1986; Reeves et al., 1989; Thoefner et al., 2000; Ihler et al., 2004). Since colic surgery in horses is associated with high mortality and morbidity (Johnston et al., 1995; Mee et al., 1998b) and high costs, and is staff consuming, an important issue is to avoid abdominal surgery in those horses that have a very poor chance of survival. Although most studies have been carried out differently, with different caseloads and analysed parameters and use of different statistical methods, it is generally agreed that parameters that assess circulatory function are the best predictors of survival. In addition to the heart rate and the colour and capillary refill time of the mucous membranes, the concentration of plasma lactate is one of the most useful predictors (Kalsbeek, 1975; Moore et al., 1976; Parry et al., 1983a; Parry et al., 1983b). In one study the probability of survival was found to be 0.03 if the lactate concentration increased above 8 mmol/L (Parry et al., 1983a), and in another study there was a chance of survival of 93% if the lactate level was below 8.3 mmol/L. The present study supports these findings. In Study IV the highest value on admission in a surviving horse (Shetland pony) was 8.3 mmol/L but before induction of anaesthesia, the plasma lactate level had decreased to 6.4 mmol/L. In one of the two horses that were euthanised at an early stage after recovery to standing, plasma lactate increased rapidly from 5.1 to 10.9 mmol/L in the last two hours before euthanasia. In the other horse, no plasma sample was available for analysis immediately before euthanasia, but the lactate concentration in dialysate increased from 2.7 to 6.6 mmol/L during the last two hours. Since the last sample was obtained during a two-hour sequence, it is most likely that the actual interstitial concentration at the time of euthanasia was significantly higher than 6.6 mmol/L. These results show that lactate either in plasma or in dialysate may also be a useful parameter to follow in the postoperative period in the colic horse.

Clinical implications

The present investigation was mainly undertaken to study the muscle perfusion and local muscle metabolic events in horses in order to deepen the understanding of muscle function during and after anaesthesia and possibly in the development of PAM.

The results showed that the gluteal muscle metabolism was affected by the different anaesthetic protocols that were used and that the muscle had not recovered even 24 hours after anaesthesia. This implies that anaesthesia is a process that challenges the muscle and reduces its capacity to maintain its energy charge. The exact causes of the metabolic derangements are still unclear, but hypoperfusion during anaesthesia plays a central role. Different TIVA protocols are gaining increasing attention in equine anaesthesia for their many good clinical properties and capacity to maintain central circulation. Since the muscle metabolism was affected by TIVA as well as by the other anaesthetic protocols in

the present study, this suggests other TIVA protocols should also be submitted to muscle metabolic and muscle perfusion research.

Hypotension during anaesthesia is one of the most important and repeatable factors in the development of PAM and probably relates to a concomitant hypoperfusion of the muscle. Much effort is made to prevent hypotension from developing during anaesthesia, and treatment with fluid therapy or cardiotropic and vasoactive drugs is administered routinely. The perfusion studies, in agreement with previous reports, showed that mean arterial pressure may give a false impression of the quality of peripheral perfusion. Although adequate blood pressure and PaO_2 are desirable, this is not a guarantee for adequate tissue perfusion and oxygenation. This investigation, like previous studies, has shown that for example a low dose of dobutamine may result in an increased blood pressure while there is no increase in cardiac output or perfusion.

From what is known about PAM and reperfusion injuries, it is of great importance that muscle perfusion is maintained during anaesthesia. Until a technique for direct muscle perfusion measurements in the clinical setting is available, the anaesthesiologist has to use his or her skills and recent research results to optimise the conditions for adequate perfusion. This means keeping the anaesthetic procedure as short as possible and avoiding both hypotension and decreased blood flow. When pharmacological substances are used to raise the blood pressure, this should also aim at increasing cardiac output. Also, when mechanical ventilation is applied, it becomes even more important to ensure an adequate circulation, since IPPV, for example, both depresses cardiovascular function and affects peripheral perfusion.

To minimise intramuscular pressure and assure venous outflow, the positioning of the horse is important, as well as the properties of the surface on which the horse rests. The clinical impression at the horse clinic at SLU is that PAM has developed less frequently since the introduction of medical foam mattresses (Tempur®,Tempur AB, DanFoam, Denmark) that are specifically designed to reduce local pressure by distributing the pressure over a larger area of the body.

Plasma lactate has been shown in several studies to be a good predictor of survival in colic horses and should therefore be measured routinely in clinical practice. Repeated samples judged in parallel with the clinical status of the horse offer the clinician a valuable instrument as a basis to advocate euthanasia instead of surgery in difficult cases. Glucose can also be monitored, especially in cases where glucose therapy is instituted. It is possible that in the future, insulin will be part of the routine treatment in the critically ill horse, just as it is now used to treat critically ill human patients.

Conclusions

- Microdialysis and muscle biopsy sampling techniques
  - These methods proved to be excellent complements to analysis of blood samples in the study of metabolic events associated with anaesthesia and recovery in experimental as well as in clinical research.
  - They offer a possibility for repeated sampling over time, and microdialysis allows continuous monitoring of the metabolic events.

- Laser Doppler flowmetry
  - This technique is used to advantage for studying relative changes in perfusion in muscle and skin over time and in response to different provocations during equine anaesthesia.
  - LDF could not be used with the present investigation design for comparison of the level of perfusion between sampling sites or different anaesthesia occasions.
  - LDF not only allows quantitative but also qualitative measures of perfusion (e.g. flow-motion) in the tissue.

- Muscle metabolism
  - No differences between the dependent and non-dependent gluteus with the horse placed in lateral recumbency were detected on analysis of metabolites in muscle dialysate and muscle biopsy samples.
  - Irrespective of the anaesthetic method used, muscle lactate production increased immediately after induction of anaesthesia, and anaerobic muscle metabolism was evident at the end of or after anaesthesia.
  - Muscle phosphagen stores were still low one day after recovery from anaesthesia.

- Muscle perfusion
  - Intermittent positive-pressure ventilation negatively affected the central circulation, arterial blood pressure and peripheral perfusion compared to spontaneous breathing.
  - Decreases in central circulation and peripheral perfusion and increases in arterial blood pressure occurred after provocation with an alpha-2 receptor agonist (detomidine).

- Metabolism pre- and post-anaesthesia differed between healthy horses and horses subjected to emergency abdominal surgery:
  - Colic horses entered anaesthesia with activated carbohydrate and lipid metabolic pathways and consumption of high-energy phosphates.
  - Metabolism and electrolyte balance were affected in both healthy and colic horses during and after anaesthesia, but to a greater extent in the colic horses. It took approximately one week before most measured parameters had normalised, especially in the colic horses.

- Following abdominal surgery the horses lost body weight, and changes in plasma free fatty acids, glycerol and glucose concentrations suggested that they were in a hypermetabolic state.
- Changes in muscle lactate production as assayed in dialysate and biopsy samples were reflected by similar changes in plasma lactate, but plasma samples did not always truly reflect the extent of the changes.

Future research

Future research on muscle metabolic changes during and after anaesthesia in horses should continue to be focused on the local metabolic processes by analysis of muscle biopsy samples and by using microdialysis to sample muscle interstitial fluid. With these techniques valuable information may be gained that would otherwise be overlooked.

Future research projects should include:

- Microdialysis and muscle biopsy sampling of the triceps muscle during and after anaesthesia in lateral recumbency, since the triceps is the muscle possibly most often affected by PAM.
- Muscle metabolic studies during and after different TIVA protocols.
- Studies using, for example, immunohistochemistry to evaluate possible post-ischaemic injuries to try to find out whether changes occur in Type II muscle fibres in particular, similar to those found in humans.
- Studies of phosphate metabolism in an attempt to determine what factors underlie the phosphate concentration changes during and after anaesthesia and to investigate their clinical significance.
- Further studies on colic horses,
  - to investigate protein metabolism in comparison with that in healthy horses,
  - to study the effects of early post-operative nutrition and of different feeding regimens on metabolism, and
  - to evaluate the effects of specific therapeutic interventions such as insulin on metabolism.
- Metabolic studies of horses undergoing surgery for ovarian tumour removal, a case group that is known to be overrepresented regarding anaesthetic-related complications (PAM/fractures). These horses have often experienced prolonged starvation.
- Investigations in healthy horses of the effects of different schedules for pre-operative starvation on metabolism and gas exchange.
- Further studies on peripheral perfusion using laser Doppler flowmetry with multiple single fibres,
  - to compare inhalation anaesthesia with TIVA, and
  - to evaluate the effects on perfusion of elevating or lowering of a limb.

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On one of my numerous train trips between Falun and Uppsala I met a 9-year-old boy, busy drawing fantastic cartoon strips. He became interested in the problems regarding horses and anaesthesia. After a moment's thinking he came up with the question: "Why don't you anaesthetise these horses in weightlessness as in a spaceship, then there would be no problems with gravitational forces".
Why don't we? This will, however, be a challenge to another PhD student.