

**Defocused CO₂ Laser Irradiation
in
the Rehabilitation of Horses**

An Experimental and Clinical Study

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Abstract

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There is an increasing interest in the use of physical medicine in rehabilitation of animals. The main goal in rehabilitation is to regain best possible physical function after illness or injury, by use of different physical modalities. The aim of the present investigation was to study the photothermal effects of defocused CO₂ laser irradiation on equine tissue, one modality used in the rehabilitation of horses. The thesis comprises studies on the effect of irradiation on temperature, blood flow, morphology, concentration of anti-inflammatory and pain modulating mediators, and finally, lameness due to traumatic arthritis of the fetlock joint.

Three experimental studies revealed that defocused CO₂ irradiation (91 J/cm²) causes a moderate to vigorous heating effect (3-6 °C) in superficial tissues, with a concomitant increase in blood flow, detected by Laser Doppler Flowmetry. Mild to severe dose-dependent morphological changes were detected in the skin after irradiation with doses ranging from therapeutic to near-surgical (91-450 J/cm²). A clinical study demonstrated a decrease in the degree of lameness in both groups of lame horses after irradiation. No statistical difference was detected between lame horses treated with laser or placebo, evaluated by conventional lameness examination and accelerometer technique. Nor was there a difference in the concentration of inflammatory mediators such as substance P and PGE₂, or the opioid Met-enkephalin-Arg-Phe in synovia. A higher concentration of Met-enkephalin-Arg-Phe was measured in sound horses compared to horses with traumatic arthritis.

In conclusion, the present thesis reveals that irradiation with defocused CO₂ laser causes a moderate to vigorous heating effect in superficial tissue, and a marked increase in blood flow. The increase in temperature was of such intensity that there is a potential risk of thermal injuries to the skin. The results also suggest that treatment with defocused CO₂ laser is not statistically better than placebo at reducing the grade of lameness in horse with traumatic arthritis of the fetlock joint.

Key words: rehabilitation, laser therapy, horses, thermal effect, Laser Doppler Flowmetry, histopathology, Met-enkephalin-Arg-Phe, traumatic arthritis, accelerometer technique.

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Appendix

Papers I-IV

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

I: Anna Bergh, Görel Nyman, Thomas Lundeberg and Stig Drevemo. 2005. Effect of defocused CO₂ laser on equine skin, subcutis and fetlock joint temperature. *Equine and Comparative Exercise Physiology* 2(1):61-69.

II: Anna Bergh, Görel Nyman, Thomas Lundeberg and Stig Drevemo. 2006. Effect of defocused CO₂ laser on equine tissue perfusion. *Acta Veterinaria Scandinavia* 47(1):33-42.

III: Anna Bergh, Yvonne Ridderstråle and Stina Ekman. Defocused CO₂ laser on equine skin: A light microscopy study. (Manuscript).

IV: Anna Bergh, Görel Nyman, Mattias Hallberg, Qin Zhou, Lars Roepstorff, Stig Drevemo and Karin Roethlisberger-Holm. Defocused CO₂ laser therapy in traumatic arthritis of the fetlock joint: A randomised clinical study. (Manuscript).

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Abbreviations

CO ₂	Carbon dioxide
EIA	Enzyme immunoassay
HeNe	Helium neon
IR	Infrared
LDF	Laser Doppler Flowmetry
MEAP	Met-enkephalin-Arg-Phe
PGE ₂	Prostaglandin E ₂
RIA	Radioimmunoassay
SP	Substance P

Introduction

Physical medicine and rehabilitation

Interest in physical medicine as a method for rehabilitation of animals has grown during the last decades, and so has the need for well designed and validated rehabilitation programs. The primary aim in rehabilitation is to regain best possible physical function after illness or injury. Tissue healing is promoted by stimulation of normal physical processes, thereby restoring the function of injured tissue. To achieve this, the therapist may use different physical modalities.

Rehabilitation generally addresses the consequences of pathology rather than the pathology itself. A “functional diagnosis” is established, based on the impairments present in the animal, i.e. the alterations in anatomical, physiological structures or function as the result of some underlying pathology (International classifications on impairments, disabilities, and handicaps, WHO 2006). Decreased joint ranges of motion, increased muscle tonus and muscle weakness are examples of such impairments. Impairments may lead to a functional limitation, such as inability of the animal to perform.

The design of a rehabilitation protocol should be based on the functional diagnosis, as well as the understanding of the patho-physiological processes of the injured tissues involved. The primary aim of rehabilitation is to restore function and if possible improve healing. In order to facilitate this, different forms of physical treatments have been tried including therapeutic heating. However, there is limited knowledge of the effect of some of the methods and it is imperative to specify the physiological effects and possible side effects of any selected physical modality, since this not only determines the choice of modality - but also when and how to use it.

Rehabilitation using defocused carbon dioxide (CO₂) laser has recently attracted interest (Lindholm *et al.*, 2002) as it has been reported to restore function and improve healing in horses with traumatic arthritis of the fetlock joint. It has been hypothesised that the laser, besides having a specific photobiological effect, induces an increase in local tissue temperature, with a secondary increase in local blood flow. Further, the increase in temperature and blood flow has been suggested to influence pain perception and tissue regeneration (Lehmann, 1990).

Laser light as a rehabilitation tool

The first relatively high power lasers were developed in the 1960s and were used in surgery. During the 1970s, professor Mester in Budapest observed that low levels of laser energy had a “photobiostimulating” effect improving tissue healing in mice skin (Mester *et al.*, 1971). Today, lasers are used in the treatment of both humans and animals. Besides being used as a surgical tool, the main treatment indications are pain relief, wound healing, inflammation and musculoskeletal injuries.

What is a laser?

LASER is the acronym for Light Amplification by Stimulated Emission of Radiation. Laser light is created by devices that convert electromagnetic radiation into narrow-frequency wavelengths of ultraviolet, visible or infrared radiation. A laser device can be characterised according to 1) the medium; i.e. the material which emits excess energy as photons of light with specific wavelengths, 2) the output power that is surgical or high-power (W) or low-level laser (mW), and 3) the potential risk for skin or eye injury; arranged in class I-IV, where class IV involves a definite risk.

The laser device consists of a power source and a chamber with a lasing medium with molecules or atoms that can store and release energy, and with two mirrors at each end (Figure 1).

Laser light is created when energy from the power source excites the atoms or molecules from a ground state to a higher energy level. When returning to the ground state, the excess energy is released as photons. When there is a collision between two stimulated atoms/molecules, both release energy simultaneously in equal amounts, which creates a stimulated emission. Amplification of light is achieved when the photons are reflected back-and-forth between the mirrors. A coherent light is created, which is a highly synchronised light with the waves in phase over long distances. As one of the mirrors is partly reflecting, it transmits a small part of the photons, thus emitting the laser beam. A lasing medium creates light with one specific wavelength, monochromaticity. The infrared (IR) light is classified based on its wavelength into near IR: 760-3000 nm, middle IR: 3000-30000 nm and far IR light: 30000 nm -1mm (Schieke *et al.*, 2003). The classification partly corresponds with the light's absorption pattern, and for the IR light, the depth of absorption in the skin decreases with increasing wavelength (Figure 2). The light can be emitted continuously or pulsing and scanning devices may be attached to the laser.

Laser types and treatment parameters

When evaluating the effects of laser irradiation a number of physical properties have to be taken into account: wavelength, power, pulse rate, irradiated area, dose at the location of injury, exposure time and treatment intervals.

Laser therapy may be divided into the use of surgical lasers (high-power laser) and lasers used for biomodulation, so-called low-level laser. However, lasers originally made for surgery, such as the CO₂ laser used in the present investigation, are used as biomodulating lasers, with a defocused beam and low output effect.

In low-level laser therapy there is normally an output power ranging between 10^{-3} and 10^{-1} W, wavelengths between 300 and 10600 nm (Schindl *et al.*, 2000; Nussbaum *et al.*, 2003; Chow & Barnsley, 2005), an energy density between 10^{-2} and 1 W/cm², and a dose of 10^{-2} to 10^2 J/cm² (Schindl *et al.*, 2000). A high-power laser has a power range of 10^{-1} W to hundreds of W (Carruth & McKenzie, 1986). The laser beam is usually focused when used as a surgical laser, and the dose can be several hundreds to thousands J/cm² (see Table 1).

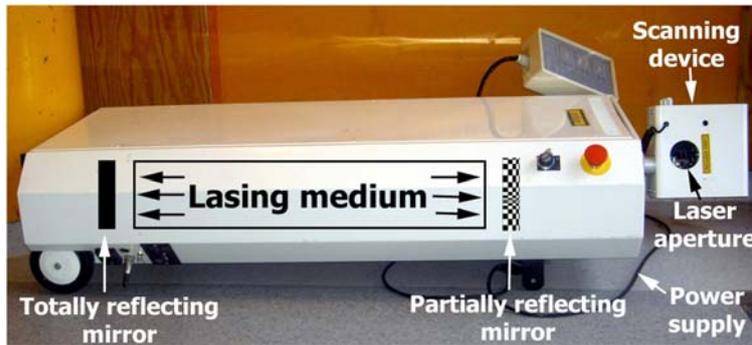


Fig. 1. Simplified design of a CO₂ laser.

The laser/tissue interaction

Laser light may be reflected, transmitted, absorbed or scattered (change of direction). The depth of penetration is defined as the depth where the intensity of the light is approximately 36% of the original intensity (Baxter, 1994). It is difficult to predict the exact amount of absorption and scattering because of the non-homogenous properties of tissue. In general, the degree of scattering decreases with increasing wavelength. Consequently, the tissue reaction is dependent on the wavelength, as the wavelength of a laser beam determines the absorption and the depth of penetration (Hecht, 1992).

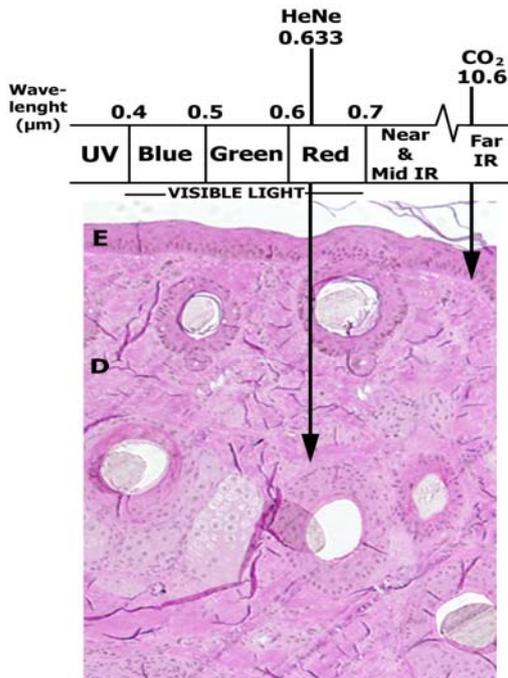


Fig.2. Wavelengths of two lasers and their relative absorption depth in skin.

UV = ultraviolet light,
IR = infrared light.

HeNe = helium-neon laser,
CO₂ = carbon dioxide laser.

If absorbed, light energy is converted into other forms of energy such as thermal or chemical energy. Simplified, visible and near ultraviolet light are absorbed by chromophores (light-sensitive molecular structures), such as haemoglobin and melanin. Infrared light is absorbed by water and can induce vibrational changes in biomolecules (Hecht, 1992). Thus, the transmission of light with 1200 nm and longer wavelengths through epidermis is dependent on the thickness and water content but not on the pigmentation of epidermis (Andersson, 1994).

Table 1. *Dosimetric parameters of laser irradiation (after Schindl, 2000)*

Parameter	Comments	Units	Low level	High-power
Radiant flux (Power output)	Power= energy/time (W=J/s)	W	10 ⁻³ -10 ⁻¹	>10 ⁻¹
Irradiance (Power density, Intensity)	Irradiance= power/area irradiated	W/cm ²	10 ⁻² -1	>1
Dose (Energy density, Radiant exposure)	Dose= power X irradiation time/area	J/cm ²	10 ⁻² -10 ²	> 10 ²
Pulse rate	Pulses/ second	Hz	0- 5000	
Pulse duration	Time when laser light is emitted	ms	1-500	
Pulse interval	Time when the laser is off between pulses	ms	1-500	
Treatment time	Total time when the laser light is emitted	s		

Possible mechanisms of action

Despite of the frequent use of laser therapy there is a lack of knowledge about its mechanisms. Effects such as local circulation enhancement (Kubota, 2002), anti-inflammatory effect (Campana *et al.*, 1999; Campana *et al.*, 2004; Bjordal *et al.*, 2006), enhancement of cartilage and bone healing (Chen & Zhou, 1989; Tsai *et al.*, 1997; Cho *et al.*, 2004), and peripheral nerve stimulation and analgesic effect (Wesselmann *et al.*, 1991; Baxter *et al.*, 1992) have been suggested. Although several biological mechanisms have been proposed to explain this wide range of effects, the data available is difficult to interpret owing to a wide diversity in experimental protocols and studies (Basford, 1995; Bjordal *et al.*, 2003; Chow & Barnsley, 2005).

The photobiological effects of laser irradiation have been attributed to photochemical, photothermal and photomechanical changes (Nussbaum *et al.*, 2003). The photochemical effect has been suggested to caused by excitation of light-sensitive molecules (photoacceptors, chromophores), some of which are

suggested to be cytochrome enzymes in mitochondria and cell membranes (Karu, 1989). Photothermal effects result from transformation of absorbed light energy to heat (Thomsen, 1991; Hecht, 1992). Photothermal effects are unlikely to apply to low-level laser therapy, as temperatures in skin are elevated by less than 1 °C (Stadler *et al.*, 2004). Photomechanical effects are secondary to rapid heating with ultrashort laser pulses, causing cell and tissue damage.

The defocused CO₂ laser

The defocused CO₂ laser is a high-power laser, emitting infrared light in the far IR spectrum, Table 2.

Table 2. *Lasing medium parameters (KSV 25S Laser device)*

	CO ₂	HeNe
Wavelength	10600 nm	633 nm
Continuous output power	0-25 W	1.2 mW
Mode of application	scanning	scanning
Diameter of beam at source	6 mm	0.61 mm
Divergence	1.5 mrad	2.0 mrad

In the present studies, light was continuously emitted through a scanning device causing a quasi-continuous delivery of radiation with a 6 mm diameter large laser spot scanning back-and-forth over the treatment area.

Since soft tissue consists of 70-80% water (human skin), it is assumed that the tissue absorbs infrared light as it would do in water (Hecht, 1992). The penetration depth of CO₂ laser is about 20 µm in water (Hecht, 1992). Absorption in tissue is approximately 90% within the first 100 µm (Carruth & McKenzie, 1986) and scattering is negligible (Thomsen, 1991). The long wavelength and absorption pattern of the CO₂ laser produce direct kinetic excitation rather than electronic excitation and resulting in a photothermal effect (Anderson, 1994). The CO₂ laser is a potent laser device, with a thermal effect ranging from very mild warming to coagulation and carbonation of tissues (Thomsen, 1991). Incorrect use of the CO₂ laser may therefore cause serious side effects.

Traumatic arthritis

Fetlock arthritis is a commonly registered diagnosis in Swedish insured riding and leisure horses (Penell *et al.*, 2005). The inflammation causes distress to the horse and limits its ability to normal function. Most lesions are induced by acute trauma, repetitive loading or overload (Howard & McIlwraith, 1996).

Symptoms

Traumatic arthritis is often accompanied by synovitis, with symptoms of synovial effusion, increased skin temperature over the joint area, a palpable thickening of the joint capsule, a decrease in joint range of motion and lameness. Cartilage damage may also be present.

There is a significant relationship between synovitis, capsulitis and articular cartilage damage. The local responses are influenced by inflammatory mediators such as prostaglandin E₂ (PGE₂) and substance P (SP), which can function as indicators of the severity of synovitis (Bertone, 2001). Besides causing vasodilatation, SP and PGE₂ influence the threshold in mechanoreceptors (Birrell *et al.*, 1991; Schaible, 2006) and pain may be elicited by mechanical stimuli such as palpation, movement within normal range of motion, and weight loading, which normally would not elicit pain (Schaible, 2006).

Neurogenic inflammation

Recent studies have demonstrated that the peripheral nerves contribute to the inflammatory response, so-called neurogenic inflammation, by the release of inflammatory substances (Löfgren *et al.*, 1997). One of these substances is substance P (SP), a member of the tachykinin family of neuropeptides. It is detected in small diameter unmyelinated nerves of periarticular periosteum, joint capsule, ligaments and subintimal synovial layers of horses (Nixon & Cummings, 1994). Down-regulation of the inflammatory response is mediated by the release of opioids from immune-cells in the inflammatory tissue. The opioid peptide Met-enkephalin-Arg-Phe (MEAP) is an important neuromodulator in anti-nociception and inflammation (Rosen *et al.*, 2000). According to Caron *et al.* (1996), the nociceptive articular nerves have two objectives - they transmit the pain signal to the CNS, and when activated they release neurotransmitters into the synovial membrane and fluid thus modulating inflammation.

Diagnosis and treatment

The diagnosis is traditionally made by conventional lameness examination: grade 0-5 (Åsheim & Lindblad, 1976), palpation, radiographs and analysis of blood and synovial fluid. Flexion tests and intra-articular analgesia may help to locate the site of pain.

The conventional treatment of traumatic arthritis focuses on pain relief and functional recovery by inhibiting cartilage deformity factors as much as possible. The treatment of choice is often intra-articular injections with corticosteroids and/or sodium hyaluronate, alone or together with systemic non-steroid anti-inflammatory drugs. Current treatment includes complementary methods, of which laser therapy is one modality used.

To my knowledge, only one study has described the clinical outcome of defocused CO₂ laser therapy in horses (Lindholm *et al.*, 2002). It reports a decrease in lameness after treatment of traumatic fetlock arthritis (10600 nm, 60 J/cm²) superior to the effect of intra-articular injection of betamethasone in combination with sodium hyaluronate.

A comparison of treatment protocols in humans is often difficult due to inadequate specifications of treatment parameters (Beckerman *et al.*, 1992; Basford, 1995; Bjordal *et al.*, 2003; Chow & Barnsley, 2005) and direct extrapolation of benefits from humans to horses is difficult to make (Ramey & Basford, 2000).

Assessment of treatment outcome

Accelerometer technique

The main measure of a successful treatment of musculoskeletal injuries is the reduction of pain, frequently evaluated as a reduction in lameness by conventional lameness examination. However, an accelerometer technique has been suggested to be a more objective method (Weishaupt *et al.*, 2001; Keegan *et al.*, 2002; Leleu *et al.*, 2004) and the technique allows detection of subtle changes in the movement pattern of horses.

Aims of the investigation

The overall hypothesis of this investigation was that treatment with defocused CO₂ laser causes a reduction in the degree of lameness. The principal aim was to evaluate the effects of defocused CO₂ laser on equine tissues, using objective methods also applicable to other rehabilitation modalities.

The specific aims of the investigation were to:

- measure the effect of defocused CO₂ laser on local temperature and blood flow in equine tissue by use of temperature probes and Laser Doppler Flowmetry technique
- investigate the effects of different dosages of defocused CO₂ laser on equine skin morphology by light microscopy
- determine the concentration of inflammatory mediators and opioids in synovia, i.e. substance P, PGE₂ and Met-enkephalin-Arg-Phe
- examine the effect of defocused CO₂ laser treatment on traumatic inflammation of the fetlock joint, evaluated by conventional lameness examination and accelerometer technique.

Materials and methods

Materials and methods are described in detail in the papers I-IV, whereas a more general description will be presented herein. The research plan and procedures involving the use of animals were reviewed and approved by the Ethical Committee on Animal Experiments in Uppsala, Sweden.

Horses

The horses in Study I-III were all healthy Standardbred trotters owned by the Department of Large Animal Sciences, SLU, Uppsala, Sweden. Study IV comprised 16 privately owned horses of various breeds, from a russ pony to Swedish Warmblood horses. They were all referred to the University equine clinic due to forelimb lameness. The horses were examined according to the research protocol and if the horse met all inclusion criteria, a written consent allowing the horse to be included in the study was obtained by the owner. Table 3 summarises data for the horses investigated in the respective studies.

Table 3. Summarised data for the horses investigated in the present studies

Group		No	Breed	Age (year)	Weight (kg)	Sex
I	Standing	10	Stb.Tr.	7 (3-13)	489 (403-580)	4 mares, 6 geldings
	Anaest.	12	Stb.Tr.	7 (2-20)	501 (375-580)	11 mares 1 gelding
	Anaest.	7	Stb.Tr.	9 (2-24)	464 (375-548)	4 mares 3 geldings
	Anaest.	6	Stb.Tr.	8 (5-13)	483 (460-507)	4 mares 2 geldings
II	Anaest.	10	Stb.Tr.	9 (4-19)	497 (411-578)	6 mares 4 geldings
III	Anaest.	13	Stb.Tr.	10 (2-24)	486 (375-578)	8 mares, 5 geldings
IV	Standing Traumatic arthritis	16	Various*	9 (4-18)	not registered	5 mares 11 geldings

Age and weight are given as the group median with the range within parenthesis.

No = numbers; Stb.Tr. = Standardbred trotter; Anaest = anaesthetised horses. *See study IV for a specification of the different breeds of horses.

Procedures

Local tissue temperature and blood flow, skin morphology, laboratory analysis of blood and synovia and lameness examination were used to assess possible physiological and clinical effects of defocused CO₂ laser. Blood flow measurements and skin biopsies were performed during anaesthesia, as well as some of the temperature recordings and blood and synovia samplings. Table 4 shows an overview of the different procedures used in each study. In depth details on each study design are given in paper I-IV.

Temperature measurements in Study I and II were performed on the skin and subcutis of the dorsal side of the fetlock, and in the fetlock joint. An area of 6 x 7 cm on the lateral, dorsal and medial sides of the fetlock was clipped. The lateral side was washed with antiseptic solutions (Hibiscrub, Hibitan; Zeneca, Göteborg, Sweden). Joint temperature: a flexible probe, 0.8-mm diameter (MAA-08500-A; ELLAB, Rødovre, Denmark), was inserted 3–4 cm into the joint, from the lateral aspect. Subcutis temperature: a needle probe measuring 0.8 mm in diameter

(MKA-08050-A; ELLAB, Rødovre, Denmark) was inserted approximately 15 mm under the skin, in the midline of the dorsal side of the fetlock joint and 2 cm proximal to the treatment area. Skin temperature: a skin probe (MHB-08025-A; ELLAB, Rødovre, Denmark) was attached with adhesive tape over the midline of the dorsal side of the fetlock, 2 cm proximal to the treatment area. After each treatment occasion, the probes were controlled in a water bath using a mercury thermometer.

In Study I, the effect of defocused CO₂ laser on the temperature of the skin, subcutis and fetlock joint was examined in standing and anaesthetised horses.

The experiment on the standing horses was a cross-over study with randomised laser and sham irradiation (fictitious irradiation, i.e. the laser beam directed on the non-reflecting floor). Temperature probes were attached to the skin and subcutis on the dorsal side of the fetlock, and inserted into the joint as illustrated by Figure 3. In two horses, the location of the temperature probe in the joint was confirmed by radiographs. Consecutive irradiations of 91 J/cm² were applied to the lateral, dorsal and medial aspects for 4 min, respectively. Temperature was measured every 30 s from 5 min before the start of the irradiation, during the irradiation, and 5 min after irradiation.



Fig. 3. Temperature probes adapted to a) subcutis, b) skin and c) fetlock joint of an equine forelimb.

Three studies were conducted on anaesthetised horses in order to avoid movements of the temperature probes and to obtain data over a longer period. The first study comprised 12 horses; eight received laser irradiation and four served as controls. The horses were anaesthetised, placed in left recumbency and irradiated with 137 J/cm² (16 W, 6 x 7 cm) on the dorsal aspect of the fetlock joint. Temperatures in skin, subcutis and fetlock joint were measured every 30 s from 5 min before the start of the irradiation, during the 6 min of irradiation and until 30 min after irradiation (recordings were carried out every min from 10 min after the end of treatment).

A second cross-over experiment was carried out on seven anaesthetised horses in order to examine changes in temperature between clipped and unclipped hair coat. A treatment area (6 x 7 cm) on each side of the croup was prepared; one side clipped and the other side with the hair coat intact. Skin and subcutis temperature probes were attached and irradiation of 171 J/cm² (20 W) was carried out randomly. The temperature recordings were done every 30 s from 5 min before the start of the irradiation, during the 6 min of irradiation, and 5 min after irradiation.

Table 4. An overview of different procedures of each study

Study	Group	Premed	Induction	Anaesthesia	Laser protocol	Irradiation area	Assessment
I.	Standing				91 J/cm ²	Fetlock	Temperature, blood and synovia
	Anaesthesia	Ace Meth	GG+Thio	Halothane	137 J/cm ² 171 J/cm ²	Fetlock Croup	Temperature, blood and synovia Temperature
	Anaesthesia	Det	GG+Thio	Isoflurane	91 J/cm ² 137 J/cm ² 171 J/cm ²	Fetlock Fetlock Croup	Temperature, blood and synovia Temperature, blood and synovia Temperature
II	Anaesthesia	Det	GG+Thio	Isoflurane	91 J/cm ²	Hamstring	Temperature, blood flow
	III Anaesthesia	Ace Meth	GG+Thio	Halothane	137 J/cm ² 450 J/cm ²	Fetlock Loin	Skin biopsy Skin biopsy
IV	Anaesthesia	Det	GG+Thio	Isoflurane	91 J/cm ²	Hamstring	Skin biopsy
	Standing				137 J/cm ² 450 J/cm ²	Fetlock Loin	Skin biopsy Skin biopsy
		Traumatic arthritis				91 J/cm ²	Fetlock

Abbreviations: Premed = premedication; Ace = acepromazine; GG = guaifenesin; Thio = thiopentone; Meth = methadone; Det = detomidine.

During the course of the previous experiments we detected that the joint temperature fluctuated before and during irradiation. Therefore, a third extended experiment was carried out on six anaesthetised horses; four received laser and two served as controls, in order to await a steady state in temperature before the start of laser irradiation. A temperature probe was inserted into the fetlock joint and after a steady state in temperature was reached, the dorsal side of the fetlock area was irradiated with 91 J/cm^2 (16 W, 4 min, 6 x 7 cm). Temperature was registered every minute until a new steady state was reached after irradiation.

In all studies, blood samples from the jugular vein and synovia samples from the fetlock joint were collected, before and after each irradiation of the joint area.

The aim of Study II was to examine if an increase of the temperature of equine superficial tissue during laser irradiation was accompanied by an increase in the local blood flow. Ten horses were anaesthetised and placed in dorsal recumbency. A treatment area of 6 x 7 cm was prepared on each semimembranosus muscle; one side clipped and the other with the hair coat intact. A small area for the measuring probes was prepared in direct contact with each irradiated area. Skin temperature and perfusion were measured on the skin surface (for further details on Laser Doppler Flowmetry, see page 18), 1 and 3 cm from the irradiated area. Muscle temperature and perfusion probes were inserted to a depth of 3 cm, approximately 1 cm from its corresponding 1 cm skin probe, and at the same distance from the irradiated area. The treatment areas were randomly irradiated with 91 J/cm^2 or with sham laser (with the beam directed away from the horse). The temperatures were displayed and recorded continuously (Perisoft 1; 14, Perimed, Järfälla, Sweden) for approximately 50 min. The average values during one minute before irradiation, during irradiation, the peak value during irradiation and the time to the peak value were analysed. Blood samples from the jugular vein and synovia fluid from the fetlock joint were taken before and after irradiation.

In Study III, the aim was to describe *in vivo* effects of three different doses of defocused CO_2 laser on equine skin morphology. Thirteen of the horses from Study I and II were irradiated on three sites. Treatment areas were clipped and irradiation over the proximal semimembranosus muscle (hamstring: 91 J/cm^2 ; 16 W, 6 x 7 cm, 4 min), on the dorsal fetlock (137 J/cm^2 ; 16 W, 6 x 7 cm, 6 min), and on the loin (450 J/cm^2 ; 20 W, 4 x 4 cm, 6 min) was conducted. Approximately 90 min after irradiation, skin biopsies were taken from irradiated and corresponding non-irradiated sites (for Skin biopsy technique, see page 19). The horses that received the 137 and 450 J/cm^2 doses were kept anaesthetised since they were designated to be used for surgical training and were subsequently euthanised with an overdose of pentobarbitone sodium (Avlivningsvätska för djur, 100 mg/ml, Apoteksbolaget AB, Umeå, Sweden).

In Study IV, the effect of defocused CO_2 laser on traumatic inflammation of the fetlock joint was evaluated by conventional lameness examination and an objective acceleromometer technique (for Accelerometer technique, see page 21), as well as analyses of substance P, PGE_2 and Met-enkephalin-Arg-Phe in synovia (for Blood and synovia analyses, see page 20). The inclusion criteria for the study were: the horse should be used for riding/trotting and be more than 3 years of age,

have an initial lameness graded between 0.5 and 2 degrees in a forelimb, demonstrate a decrease ($\geq 75\%$) in lameness after intra-articular anaesthesia of the fetlock joint, and have normal to minor findings on radiographs of the actual joint.

The horses stayed in the clinic for one week and were walked by hand for 2 x 10 min daily. Eight horses received laser treatment and eight received sham treatment in a random order. Blood samples from the jugular vein and synovia from the fetlock joint were collected 1 day before the treatment started. Consecutive irradiations of 91 J/cm^2 (16 W, 6 x 7 cm) were applied to the lateral, dorsal and medial aspects of the fetlock for 4 min, respectively, on five occasions during one week (once daily, with a non-treatment day after the first session). For the horses that received sham treatment, the laser beam was directed away from the horse. The horses were re-examined by the same blinded clinician using the same protocol at 7 and 21 days after the initial examination and blood and synovia samples were withdrawn.

Techniques

Laser Doppler Flowmetry

Laser Doppler Flowmetry (LDF) is a completely different use of laser light. It is a technique designed to provide continuous measurements of microvascular perfusion, in terms of relative changes of blood volume and velocity over time at a single site (Nilsson *et al.*, 1980; Öberg *et al.*, 1984). When the tissue is illuminated by the LDF laser light (780 nm) from a fibre-optic probe, the light photons hit both moving red blood cells and static structures. The light that hits moving structures changes its direction causing a shift of the Doppler frequency, which is directly related to the number and velocity of the blood cells. The scattered light is then re-emitted from the tissue and the wavelength shift is captured by a photodetector that produces a LDF signal. The instrument measures blood flow in a tissue volume of about $1\text{-}1.5 \text{ mm}^3$ and is integrated over the entire volume measured. Thus, the units are arbitrary and reflect relative changes in perfusion. The unknown orientation of the vessels leads to a variability in the perfusion measured in different subjects and in the same subject, if the probe is repositioned. To allow comparison of results, a calibration of the probes is made in a standard motility solution provided by the manufacturer.

In Study II, skin and muscle blood perfusion was measured by LDF, using a Periflux 4001 flowmeter with additional microtips and probes (Perimed, Järfälla, Sweden). A small area for the measuring probes was surgically prepared, in direct contact with the treatment area over the proximal semimembranosus muscle. Skin perfusion was measured on the surface (Probe 407), 1 and 3 cm from the irradiated area. For muscle perfusion, a straight microtip with slanted tip (MT A500-0.120 mm, 0.5 mm diameter) was inserted into the semimembranosus muscle of the right and left hind limb, close to the skin perfusion probe at 1 cm from each irradiated area (Figure 4).



Fig. 4. Laser Doppler Flowmetry probes attached to skin and muscle in the semimembranosus muscle area.

The microtip was inserted via a 0.7 mm cannula to a depth of 3 cm and the cannula was retracted. Thereafter, the microtip was connected to a probe (Master Probe; Probe 418-x, Perimed, Järfälla, Sweden). Blood flow (flux), expressed in blood perfusion units (PU), was displayed and recorded continuously. The data were transferred to a computer using a software program (Perisoft 1; 14, Perimed, Järfälla, Sweden). The average values during one minute before irradiation, during irradiation, the peak value during irradiation and the time to the peak value were analysed.

Skin biopsy technique

Skin samples in Study III were obtained from the dorsal aspect of the fetlock, the loin and the proximal area of semimembranosus muscle (hamstring) with an 8 mm disposable biopsy punch (Miltex Instrument Company, Inc.). The samples were taken from the centre of the laser-irradiated and corresponding non-irradiated control sites, approximately 90 minutes after the laser irradiation. The skin samples were divided into two parts along the longitudinal axis, and were immediately fixed in cacodylate-buffered 3% glutaraldehyde (pH 7.2). The specimens were rinsed in 0.067 M cacodylate buffer (pH 7.2), dehydrated in a graded series of ethanol, infiltrated and embedded in Histo-resin (Leica Microsystems Nussloch GmbH, Heidelberg, Germany). Sections of 2 μ m thickness were cut and stained with haematoxylin-eosin (H&E). The sections were examined with special reference to the appearance of epidermis, epidermis-dermis junction, adnexal structures, cell infiltrates and blood vessel appearance in the dermis. Furthermore, computer-assisted measurements of epidermal thickness (Easy Image Analysis System) were obtained on every fiftieth micrometer of all sections and the shortest distance between the dermal-epidermal junction and the innermost aspect of the stratum corneum were measured.

Blood and synovia analyses

Blood samples in Study I, II and IV were collected from the jugular vein and synovia samples (after surgical preparation) from the lateral aspect of the fetlock joint, before and after laser irradiation. Vials containing EDTA were used for measurement of white blood cell count and serum vials were used for determination of total plasma protein concentration according to the routine methods used at the Division of Clinical Chemistry, SLU. Additional synovia samples were centrifuged (2 x 20 min, 600 x g) and stored at -80 °C until being analysed.

Measurements of substance P (SP) and Met-enkephalin-Arg-Phe (MEAP)

The synovial fluid samples (230 µl) were acidified by adding 200 µl of 0.1 M HCl and homogenised by ultrasonication. The homogenates were diluted (1:10) with 0.1 M formic acid /0.018 M pyridine, pH 3.0 (buffer I) and subsequently centrifuged for 2 min at 3000 x g (4 °C). The supernatant fractions were purified by ion exchange chromatography. Small plastic columns were packed with SP-Sephadex C-25 gel (GE Healthcare, Uppsala, Sweden, packed gel volume = 1 ml) and washed with 20 ml buffer I before the samples were added. After additional washing with 10 ml buffer of I and 5 ml of 0.1 M formic acid /0.01 M pyridine (buffer II), the fraction containing SP and MEAP was eluted with 4 ml of 1.6 M formic acid /1.6 M pyridine, pH 4.4 (buffer V). All buffers (I, II, V) contained 0.01% mercaptoethanol. The eluates were evaporated in a Speed Vac centrifuge (Savant, Hicksville, NY, U.S.A.) and subsequently analysed by radioimmunoassay (RIA).

The radioimmunoassay for SP and MEAP were based on the charcoal adsorption technique and were conducted as described by Hallberg *et al.* (2000) and Johansson *et al.* (2000). For all RIAs the antibodies were raised in rabbits against the peptide-thyroglobulin conjugate and ¹²⁵I-labelled Met-enkephalin-Arg⁶-Phe⁷ or Tyr⁸-substance P were used as tracers. The cross-reactivity for the antiserum with SP fragments (3-11), (5-11) and (6-11) was 100, 60 and 20% respectively, with all SP N-terminal fragments and other SP related peptides less than 0.1% and the detection limit of the RIA was about 5 fmol/tube (Sharma *et al.*, 1990; Hallberg *et al.*, 2000). The cross-reactivity of the MEAP antiserum was 0.5% with Met-enkephalin-sulphoxide and less than 0.1% with Met-enkephalin, Met-enkephalin-Lys⁶, Leu-enkephalin-Arg⁶ and Leu-enkephalin (Johansson *et al.*, 2000). The intra-assay variance was 8.5% for SP and 40% for MEAP.

Measurements of PGE₂

The concentration of PGE₂ was determined by direct enzyme immunoassay (EIA) as previously described by Skarzynski *et al.* (2000). Cross-reactivity for the antiserum was: PGE₂ 100%; PGE₁ 18%; PGA₁ 10%; PGA₂ 4.6%; PGB₂ 6.7%; PGD₂ 0.13%; PGF₂α 2.8%; PGJ₂ 14% and 15-keto PGE₂ 0.05%. The PGE₂ standard curve ranged from 0.08 ng/ml to 20 ng/ml. The intra-assay coefficient of variation was 8.4%.

Accelerometer technique

Accelerometer technique is a method to use signal processing to analyse data from accelerometers. Two two-axis differential-capacitance accelerometers (ADXL250, Analog Devices, USA) were mounted perpendicular to each other on a board together with analogue components, memory, a/d-converters, battery and RF-equipment, which altogether were mounted in a box. The accelerometers measured acceleration, ± 50 g, with a resolution of 0.01 g and a sampling frequency of 1000 Hz in three directions; x, y and z, thus creating three-dimensional acceleration data. The accelerometers measure the instant change of velocity during a given interval that corresponds to the acceleration applied at the position of the accelerometer. The acquisition duration was 10 s. Data were sampled synchronously from all seven transducers with software developed by Rolab AB (Gluten, Uppsala, Sweden). Data sampling was triggered telemetrically and after the recordings a number of data recordings were downloaded from the transducers devices to a PC via a serial interface for further analysis.

The conventional lameness examination in Study IV was complemented with the accelerometer technique. The transducers were fixed to pockets in boots, girths and a neck piece. The seven transducers were applied to the four legs, wither, neck and croup (Figure 5), with approximately horizontal and vertical measuring axes, and an offset set to zero. Three separate accelerometer recordings of the initial lameness were made at slow trot by hand. Thereafter a flexion test of the phalanges was performed followed by new recordings. An intra-articular analgesia was performed to secure the diagnosis, and accelerometer recordings were carried out again before and after a second flexion test.

In order to get more detailed data for a calculation of the correlation between the subjective (conventional) and objective (accelerometer) lameness grading, a visual re-evaluation was done from the video-recordings by the same clinician (blinded). The re-evaluation was performed randomly on all horses and registrations, after completing the practical part of the study.

Accelerometer data analysis

Data from the dorso-ventral and longitudinal axis of the transducer on the withers and the dorso-ventral axis of the transducer on the neck were selected for further analysis. Power spectrum for the three-time series and subsequently the quotient between the first and second harmonic were calculated. Data from each recording of the initial lameness were averaged.

The second harmonic (at approximately 2.5 Hz) represents the step frequency, which in trot is half of the stride frequency. If the trot is symmetrical this will be the dominant harmonic. In case the horse is lame, i.e. asymmetrical, a harmonic (the first harmonic) equal to the stride frequency will start to increase. Consequently, the quotient between the first and second harmonic will constitute an objective measure of the gait asymmetry in trot. The three quotients were summed and used as an objective parameter of the gait asymmetry.

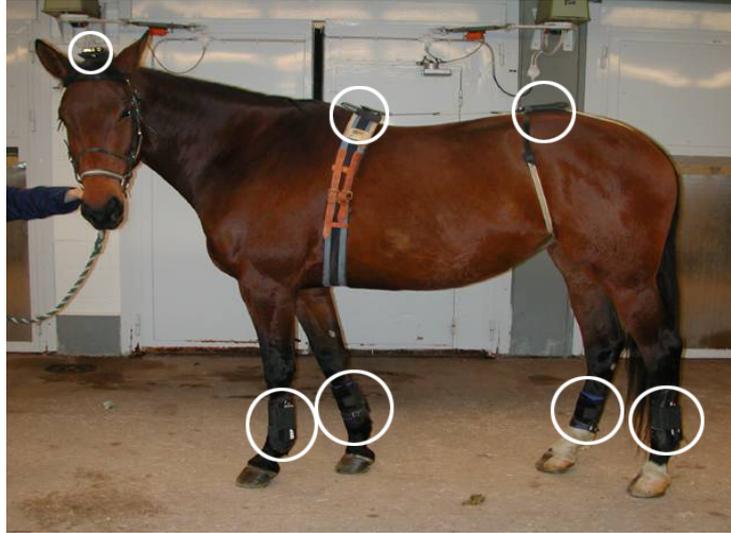


Fig. 5. Accelerometer devices adapted to the neck, withers, croup and legs.

Statistical analysis

Statistica software (Statsoft Inc., Tulsa, OK, USA) was used for statistical analyses in all articles.

Before the statistical calculations in Study I, the data were individually corrected by subtracting the mean for each individual pre-treatment period. The mean temperature was calculated from the recordings performed every 30 s during each time period: pre-treatment, treatment and post-treatment. Thereafter, the area under the curve was calculated in order to describe the total heating effect during a specific time period.

Before the statistical calculations in Study II, perfusion data were individually corrected by setting the baseline before treatment to 100% and treatment data were compared to baseline data within each group. Perfusion values less than 3.5 perfusion units (PU) were excluded from the analysis as the mean value for biological zero (i.e. the LDF signal from non-perfused tissue) for equines is approximately 1.6 PU for skin and 3.5 PU for muscle (Edner, 2005).

In Studies I and II, temperatures and perfusions were compared using non-parametric tests. A Wilcoxon test was applied to detect within-group differences and a Mann-Whitney U test for differences between groups.

In Study III, epidermal thickness and changes in skin morphology were compared by Student's paired t-test for within-group differences and un-paired t-test for differences between groups.

In Study IV the correlation between the accelerometer data and conventional lameness score was performed with a Spearman's rank correlation test. All changes in lameness and in blood and synovia parameters were determined by calculating the data before and after treatment for each horse, comparing the differences within each horse with a Wilcoxon test and between groups with a

Mann-Whitney U test. In all studies, statistical significance was accepted at $p < 0.05$.

Results

Temperature in skin, subcutis and the fetlock joint (Study I and II)

The temperature results are demonstrated by Figure 6 and summarised in Table 5.

The increase in the temperature of skin and subcutis in Study I was significantly higher during laser irradiation compared to the controls in both standing and anaesthetised horses. In standing horses, the average increase in skin measured during irradiation from the dorsal side was 5.3 ± 1.4 °C and 0.5 ± 0.5 °C for laser irradiated (91 J/cm^2) and control, respectively. The increase in subcutis was 5.7 ± 1.0 °C and 1.8 ± 0.7 °C. No significant difference in the increase in temperature was observed between the irradiated and control fetlock joints (1.8 ± 0.4 °C and 2.9 ± 0.7 °C, respectively).

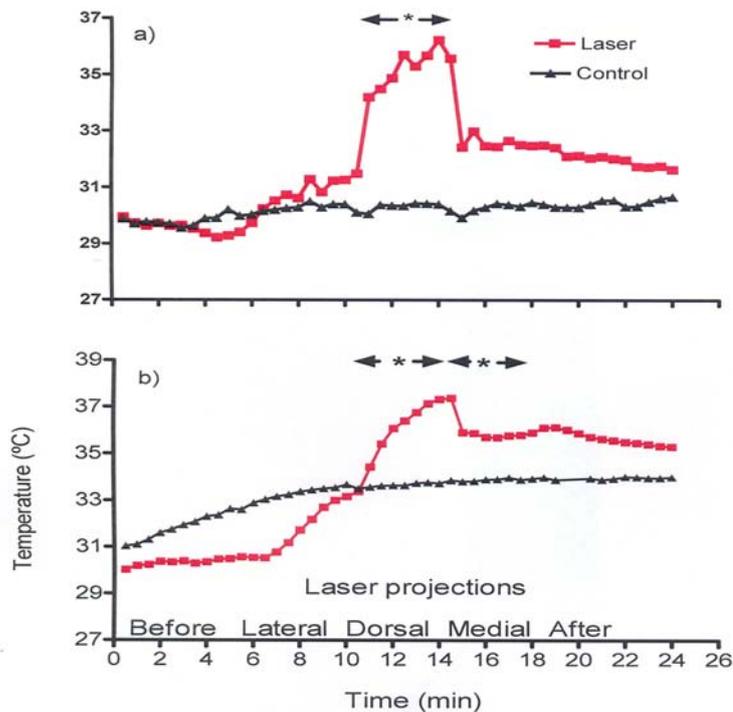


Fig. 6. Overview of temperature curves in a) skin and b) subcutis for standing horses.
*Significantly different from control group.

The results were similar in the anaesthetised horses. The average increase measured during irradiation from the dorsal side, was 3.2 ± 0.7 °C and 0.0 ± 0.2 °C in skin, and 5.5 ± 1.4 °C and -0.2 ± 0.4 °C in subcutis, for the laser irradiated (137 J/cm^2) and controls, respectively. A significant difference in skin temperature

was found during the 5 min period after laser irradiation compared to the controls (2.2 ± 0.5 °C and 0.3 ± 0.0 °C, respectively).

A significant difference in temperature of subcutis was seen both during the first 5 min period (2.9 ± 0.8 °C and 0.1 ± 0.3 °C, respectively) and the following 5 min period (1.7 ± 0.5 °C and 0.3 ± 0.1 °C, respectively) after laser irradiation compared to the controls.

The temperature in the fetlock joint was not altered by laser irradiation. A significant difference in skin temperature between clipped and unclipped areas (5.2 ± 1.4 °C and 11.3 ± 2.6 °C, respectively) was observed after laser irradiation with a dose of 171 J/cm², Figure 7.

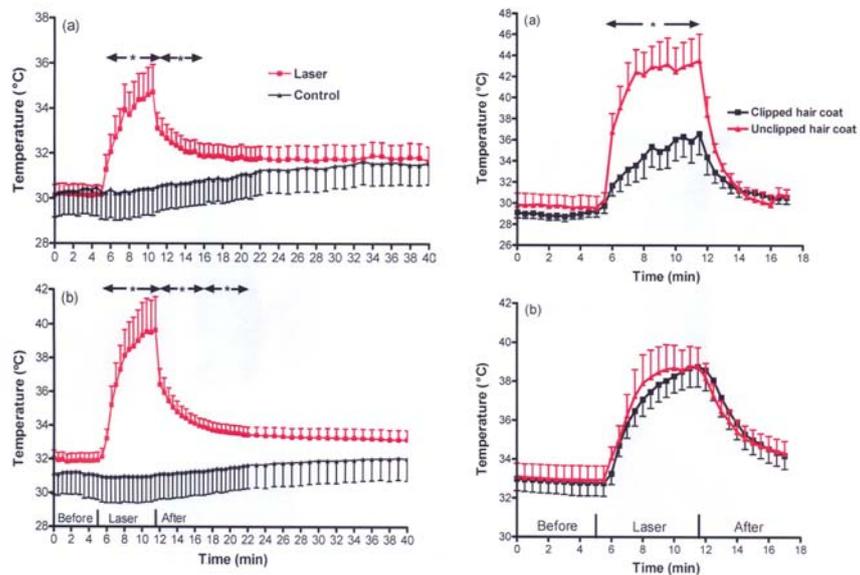


Fig. 7. Overview of temperature curves in a) skin and b) subcutis for anaesthetised horses. The curves to the right demonstrate the differences between unclipped and clipped hair coat, respectively. *Significantly different from control group.

In Study II, there was a significant increase in temperature in all skin recordings, i.e. 1 cm and 3 cm from the irradiated area, compared to the respective baseline recordings, for both clipped and unclipped hair coat (1 cm clipped 5.5 ± 1.5 °C and unclipped 4.8 ± 1.4 °C, 3 cm clipped 5.5 ± 1.5 °C and unclipped 2.1 ± 0.4 °C). No significant difference in muscle temperature measured 1 cm from the irradiated area and at the depth of 3 cm, was observed.

Table 5. An overview of temperature response to defocused CO₂ laser irradiation

	Dose (J/cm ²)	Pre-treatment (C)	Treatment (C)	Post-treatment (C)
Standing				
Skin (n=9)				
Laser	91	29.5±1.6	34.8±1.5*	31.9±1.1
Control	91	29.8±1.1	30.3±1.5	30.5±1.6
Subcutis (n=10)				
Laser	91	30.3±1.4	36.0±0.9*	35.6±0.1
Control	91	31.9±0.9	33.7±0.5	34.0±0.5
Fetlock joint (n=9)				
Laser	91	33.3±1.0	35.1±0.7	36.0±0.5
Control	91	29.9±1.4	32.8±0.8	34.3±0.5
Anaesthetised				
Skin (n=11)				
Laser	137	30.2±0.4	33.5±0.9*	32.3±0.5*
Control	137	30.3±1.1	30.3±1.2	30.7±1.1
Subcutis (n=12)				
Laser	137	31.9±0.4	37.4±1.4*	34.8±0.6*
Control	137	31.2±1.3	31.0±1.5	31.2±1.5
Fetlock joint (n=12)				
Laser	137	33.3±0.6	33.4±0.6	33.7±0.5
Control	137	34.1±0.7	34.2±0.8	34.4±0.8
Skin (n=5)				
Clipped	171	29.0±0.4	34.2±1.6#	31.6±0.7
Unclipped	171	29.7±1.1	41.0±2.0	32.1±0.5
Subcutis (n=6)				
Clipped	171	32.9±0.6	36.7±0.7	35.8±0.8
Unclipped	171	33.0±0.7	37.2±1.0	35.6±0.5
Extended study				
Fetlock joint (n=6)				
Laser	91	29.9±0.8	29.9±0.8	29.9±0.7
Control	91	29.4±0.4	29.4±0.4	29.4±0.4

Data is presented as mean±standard error (SE). The pre- and post-treatment recordings were performed during 5 min. *Significantly different from control group, # significantly different from unclipped group (p<0.05).

Blood flow in skin and muscle (Study II)

As demonstrated by the laser recording in Study II, the increase in temperature was immediately followed by an increase in perfusion (Figure 8). A significant increase in perfusion was found in all skin recordings, i.e. 1 and 3 cm from the irradiated area for both clipped and unclipped hair coat (1 cm clipped 334.0±170.5%, unclipped 263.8±120.2%; 3 cm clipped 245.2±123.6%, unclipped 116.5±65.3% compared to baseline measurements set to 100%). There was no significant difference in muscle perfusion in either laser irradiated or control horses. Nor was there a significant difference in the peak value or time to peak for skin.

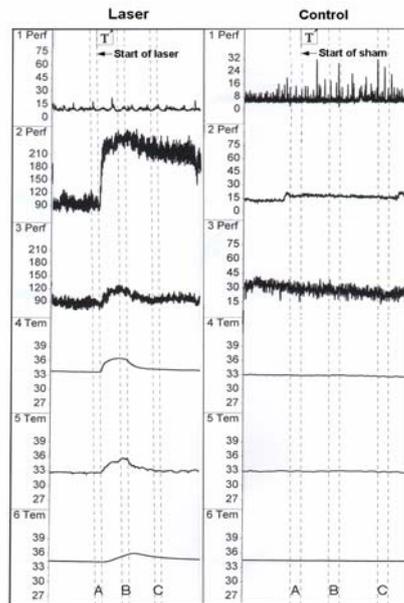


Fig. 8. Representative tracing from one laser treated and one control horse, displaying temperature and blood perfusion response in skin and semimembranosus muscle. The perfusion is presented as arbitrary Perfusion Units (PU) and temperature as °C.

Channel 1; perfusion in muscle.
 Channel 2; perfusion in skin at 1 cm.
 Channel 3; perfusion in skin at 3 cm.
 Channel 4; temperature in muscle.
 Channel 5; temperature in skin 1 cm.
 Channel 6; temperature in skin at 3 cm.

A = one minute tracing immediately before the start of the treatment.
 B = one minute tracing at the end of the treatment.
 C = one minute tracing at four minutes after end of the treatment.
 T = start of laser and sham laser treatment, respectively.

Skin morphology (Study III)

No macroscopic changes were detected, except erythema, observed at the loin approximately 5-10 min after irradiation.

Four different categories of morphological changes were observed (for illustrations, see Study III). The first category (0) showed no visible changes. The second (I) was characterized by multifocal spongiosis in the hair follicle epithelium and in the basal epidermis and with mild subepidermal cleft formations. The third (II) category had diffuse spongiosis of the epidermis and with intra- and subepidermal vesicles including eosinophilic material. The fourth (III) showed epidermal and dermal necrosis with destruction of adnexal structures. The relation between morphological changes and treatment doses is presented in Table 6.

Epidermis was significantly thinner after irradiation in the irradiated loin (450 J/cm²) compared to the non-irradiated loin (21.8±8.4 and 30.9±4.2 µm, respectively). Non-irradiated skin (controls) showed a significant variation in thickness of epidermis between fetlock (64.2± 13.8 µm), loin (30.9± 4.2 µm) and hamstring (25.3± 2.5 µm) areas (presented as mean and standard deviation).

Table 6. Number of animals with morphological changes after irradiation

Irradiation	Hamstring (n=4)		Fetlock joint (n=7)		Loin (n=7)	
	Laser	Control	Laser	Control	Laser	Control
Dose (J/cm ²)	91	0	137	0	450	0
Categories						
0	2	4	2	7		7
I	2					
II			5		3	
III					4	

Categories of morphological changes; 0 = no changes; I = multifocal, mild spongiosis in the basal epidermis with focal subepidermal cleft formations; II = diffuse spongiosis in the hair follicle epithelium and of the epidermis with intra- and subepidermal vesicles including eosinophilic material; III = epidermal necrosis with a thin epidermal layer, and coagulation necrosis of underlying dermis.

Clinical evaluation of traumatic arthritis of the fetlock joint (Study IV)

There was no significant difference in the degree of lameness between the laser treated (91 J/cm²) and sham treated group, evaluated by either conventional lameness examination (Table 7) or accelerometer technique. However, there was a weak correlation ($r^2=0.04$) in the degree of initial lameness between conventional lameness evaluation and accelerometer technique. Though not used to evaluate the effect of laser therapy the flexion test was used for diagnostic purposes. The flexion test was judged both subjectively and measured objectively. Here the correlation was considerably better, $r^2=0.66$, as demonstrated in Figure 9 and Table 8.

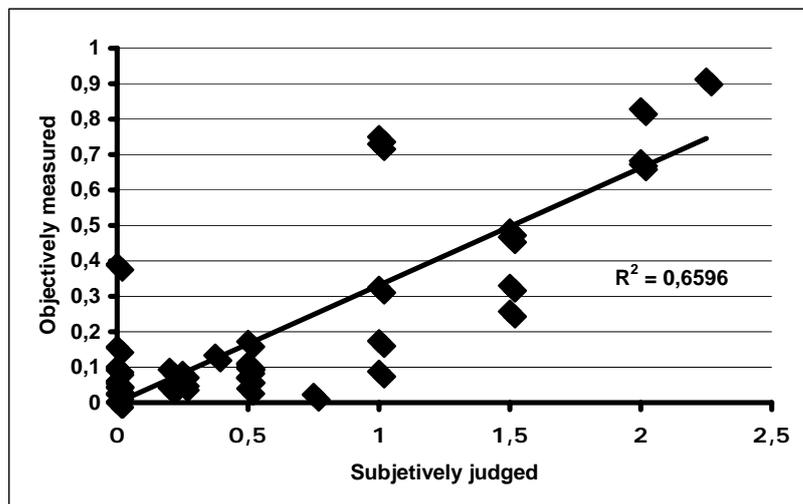


Fig. 9. Correlation between flexion test lameness grading assessed by conventional lameness examination (x-axis) and accelerometer technique (y-axis)

Table 7. Comparison of active laser and sham laser groups in respect to clinical outcomes at baseline, after therapy at weeks 1 and 3 (Laser group: 91 J/cm², sham group: 0 J/cm²)

Breed	Baseline		Week 1		Week 3		Lameness grading		Lameness grading	
	Lameness score initial	flexion	Lameness score initial	flexion	Lameness score initial	flexion	conv	accel	conv	accel
Active laser group										
Icelandic	0.5	1.5	0.5	1.0	n	0/-	n	0/-	n.c.	n.c.
W.b.	0.5	1.5	0.5	1.5	n	0/-	n	0/-	n.c.	n.c.
Icelandic	0.5	3.0	0.0	0.5	f	+	f	+	f	+
Connemara	0.5	2.5	0.5	2.0	n	0/-	n	0/-	n.c.	n.c.
Stb Tr.	0.5	2.5	0.0	0.5	f	0/-	f	0/-	p	+
Pony cross	0.5	3.0	0.0	0.5	f	n.c.	f	n.c.	f	n.c.
Russ	0.5	3.5	0.0	1.5	p	+	p	+	p	+
W.b.	0.5	2.0	0.5	0.5	n	+	n	+	n.c.	n.c.
Median	0.5 (0.5)	2.5 (1.5-3.5)	0.25 (0-0.5)	0.75 (0.5-2)					0 (0-0.5)	0.5 (0.5-1)
Sham laser group										
Stb Tr.	0.5	2.0	0.0	0.5	f	+	f	+	n	0/-
Pony cross	0.5	2.5	0.0	1.0	p	+	p	+	f	+
Pony cross	0.5	3.0	0.5	2.0	n	+	n	+	n.c.	n.c.
Pony cross	0.5	3.0	0.5	2.5	n	+	n	+	n.c.	n.c.
W.b.	0.5	2.5	0.0	0.5	f	+	f	+	p	0/-
Stb Tr.	0.5	1.0	0.5	0.5	n	0/-	n	0/-	n.c.	n.c.
Lipizzaner	1.5	2.5	0.0	2.0	p	+	p	+	n.c.	n.c.
Icelandic	0.5	2.0	0.0	0.0	f	+	f	+	n.c.	n.c.
Median	0.5 (0.5-1.5)	2.5 (1-3)	0.25 (0-0.5)	0.75 (0-2.5)					0 (0-0.5)	0.75 (0-2)

Stb Tr. = standardbred trotter; W.b. = warmblooded riding horse; Lameness score 0-5 (0 = no lameness, 5 = non weight-bearing); Lameness grading f = fully improved; p = partially improved; n = not improved; w = worse compared to the beginning of the study; 0/- = not improved; + = improved; conv = conventional lameness examination; accel = accelerometer technique; hy/cort = hyaluron acid and corticosteroids; ¹ = fully improved; ² = not improved at 21-27 days after injection; e = excluded from the study; n.c. = not classified.

As summarised in Table 9, together with own unpublished results from the healthy horses from Study I and II, there were no significant differences found in white blood cell counts and total protein in either blood or synovia. There was no significant difference in the concentration of SP, PGE₂ and MEAP in synovia between the laser group and the sham group.

Table 8. Comparison of the flexion test lameness grading by conventional lameness examination and accelerometer technique at initial examination, week 1 and 3 (Laser group 91 J/cm² and sham group 0 J/cm²)

Lameness grading according to flexion test						
	Initial examination	Week 1		Week 3		
		conv	conv	accel	conv	accel
Laser group						
Icelandic	1.5		+	+	e	e
W.b.	1.5		0/-	+	e	e
Icelandic	3.0		+	+	+	+
Connemara	2.5		+	n.c.	e	e
Stb Tr.	2.5		+	n.c.	+	n.c.
Pony cross	3.0		+	n.c.	+	n.c.
Russ	3.5		+	n.c.	+	n.c.
W.b.	2.0		+	+	e	e
Sham group						
Stb Tr.	2.0		+	+	0/-	n.c.
Pony cross	2.5		+	n.c.	+	n.c.
Pony cross	3.0		+	+	e	e
Pony cross	3.0		+	+	e	e
W.b.	2.5		+	+	+	n.c.
Stb Tr.	1.0		+	+	e	e
Lippizaner	2.5		+	0/-	e	e
Icelandic	2.0		+	+	+	+

Stb Tr. = standardbred trotter; W.b. = warmblooded riding horse; Lameness score 0-5 (0 = no lameness, 5 = non weight-bearing); Accelerometer grading 0/- = not improved compared to initial examination; + = improved compared to initial examination; conv = conventional lameness examination; accel = accelerometer technique; e = excluded; n.c. = not classified.

Table 9. Results from blood and synovia analyses in sound horses and horses with traumatic arthritis of the fetlock joint irradiated with active laser or sham laser (Laser group = 91 J/cm², sham group = 0 J/cm²)

	Sound horses, one irradiation Laser (n=10) (n=12)		Sham (n=10) (n=8)		Injured horses, five irradiations Laser (n=7)		Sham (n=8)	
	Before	After	Before	After	Before	After	Before	After
Blood								
B-LPK 10x9/L	7 (4-10)	7 (5-10)	7 (6-10)	6 (5-11)	7 (5-10)	7 (6-9)	8 (6-8)	6 (5-9)
S-Protein g/L	61 (2-73)	62 (3-71)	63 (51-72)	62 (55-69)	60 (55-69)	62 (57-71)	60 (55-64)	62 (55-66)
Synovia								
Leuk 10x6/L	156 (46-605) 182 (20-449)	275 (4-3310) 127 (8-326)	206 (45-840) 178 (7-300)	270 (55-1760) 136 (1-222)	142 (43-296)	256 (170-1140)	278 (80-820)	322 (226-940)
Protein g/L	8 (5-11) 7 (4-19)	18 (5-36) 9 (5-19)	8 (6-60) 8 (4-15)	15 (2-36) 9 (5-15)	8.5 (4-17)	5 (3-7)	8.5 (5-15)	5 (4-30)
Substance P fmol/ml	28 (12-69) 24 (9-84)	32 (5-87) 25 (7-89)	53 (12-112) 34 (16-45)	41 (14-93) 26 (18-40)	25 (18-38)	23 (8-40)	25 (15-51)	19 (11-28)
MEAP fmol/ml	104 (75-203) 101 (67-146)	122 (94-191) 106 (38-193)	91 (42-146) 108 (58-161)	94 (80-173) 101 (82-168)	44 (34-70)	67 (36-93)	72 (47-104)	47 (28-65)
PGE ₂ pg/ml	746 (15-9935) 1342 (15-6005)	576 (15-3336) 846 (15-3524)	1544 (200-2541) 1421 (15-1840)	770 (15-1532) 668 (15-946)	1489 (15-4447)	1490 (330-33822)	3113 (280-42464)	1654 (561-38904)

B-LPK = Blood leukocytes; S-Protein = Serum protein; Leuk = Synovia leukocytes; Protein = Protein in synovia; MEAP = Met-enkephalin-Arg-Phe; PGE₂ = prostaglandin E₂. Data is presented as medians and range. Two groups of sound horses: standing (91 J/cm²) and anaesthetised (91-137 J/cm²).

Discussion

The general aim of the present investigation was to evaluate the thermal effects of defocused CO₂ laser on equine tissues by use of objective methods also applicable to other rehabilitation modalities. The reasons for focusing on the thermal effects were twofold. Firstly, the kinetic excitation of the defocused CO₂ laser results in a photothermal effect (Hecht, 1992; Andersson, 1994). Secondly, many of the postulated effects of high-power laser therapy, such as increased blood perfusion and modulation of pain, have an increase in temperature as the proposed mode of action. The clinical effects were studied on horses with traumatic arthritis of the fetlock joint, a diagnosis chosen because of its patho-physiological manifestation with pain and inflammation, for which laser therapy is postulated by some authors to have a positive effect (Baxter, 1994; Campana *et al.*, 2004; Bjordal *et al.*, 2006). Besides being a suitable injury to treat, the fetlock arthritis was also registered as the most common condition in Swedish riding and leisure horses (Penell *et al.*, 2005), consequently the choice of condition also had both an animal welfare and an economic aspect.

Physiological effects of defocused CO₂ laser irradiation on temperature, blood flow and skin morphology

The application of therapeutic heat is often part of a rehabilitation program. The therapeutic goal is to achieve a sufficient rise in temperature at the site of injury, without causing any negative side effects such as thermal injuries. The heat can be administered superficially or to deeper situated tissues as in therapeutic ultrasound. Due to its absorption pattern, the defocused CO₂ laser can be classified as superficial heat.

It is generally accepted that heat modulates pain perception and stimulates regeneration of injured tissue. A proposed mechanism of action is an increased tissue temperature with secondary increase in blood flow. This increase in blood flow (Löfgren *et al.*, 1997) may help removing inflammatory agents from the injured location, thus reducing the sensitisation of mechanoreceptors. Furthermore, a high tissue temperature directly influences nerve transmission (Klumpp & Zimmermann, 1980; Lehmann, 1990; Wesselmann *et al.*, 1991) and the sensory stimulation may influence the pain modulation by activation of pain inhibitory systems (Melzack & Wall, 1965; Sluka *et al.*, 1999; le Bars, 2002).

In the present study, the temperature increase of at least 3-6 °C in skin and subcutis could be classified as a moderate to vigorous heating effect according to Lehmann (1990).

No significant change in the temperature was observed in the fetlock joint. Previous studies have shown both an increase (Weinberger *et al.*, 1988; Osterweld *et al.*, 1994a; Osterweld *et al.*, 1994b) and a decrease (Hollander & Horwath, 1949) in joint temperature in response to therapeutic heat. Use of heat in acute injuries is not recommended, as a higher temperature may result in an increase in blood flow, causing an oedema in soft tissues (Lehmann, 1990) and a possible activation of cartilage-degenerative enzymes (Castor & Yaron, 1976;

Weinberger *et al.*, 1989). The unaffected joint temperature in the present study indicates a possibility to use defocused CO₂ laser irradiation without increasing the risk to activate cartilage-degenerative enzymes.

We found a rise in skin blood flow, but not in the temperature or blood flow in muscle. This could be due to either the limited penetration depth of the laser light (Carruth & McKenzie, 1986) and/or a sufficient temperature regulation due to vascular heat dissipation in the skin. This is in accordance with other studies on superficial heat where both the temperature and blood flow in muscle remains relatively unaffected (Johnson *et al.*, 1976).

Studies on laser therapy with “near” infrared light has reported a non-photothermally mediated vasodilatation in the skin of rats (830 nm, 185 J/cm², Kubota, 2002) and humans (780 nm, 5 J/cm², Schaffer *et al.*, 2000). In the present study, the increase in skin blood flow was observed shortly after the onset of irradiation and immediately after the first rise in temperature, thus suggesting that the increase in flow could be caused by neuronal reflexes. It is likely that an axon reflex was activated since the increase in blood flow appeared fast and was detected almost immediately at a distance of 3 cm from the irradiated. There are studies showing that the axon reflexes can be activated by non-painful thermal stimulation (Minson *et al.*, 2001; Stephens *et al.*, 2001) in the interval of 30-42 °C (Barcroft & Edholm, 1943; Taylor *et al.*, 1984; Johnson *et al.*, 1986; Magerl & Treede, 1996). One can assume that both warmth receptors and heat-sensitive nociceptors, with a threshold of approximately 40 °C (Torebjörk *et al.*, 1984; Tillman *et al.*, 1995), were activated during our investigation. The activation of heat-sensitive nociceptors has two important consequences. It can contribute to the inflammatory response through the release of inflammatory substances and with a secondary release of opioids (Sprengler *et al.*, 2006) it may down-regulate the inflammatory response. A painful stimulus activates the withdrawal reflex, protecting the tissue from thermal injuries. It is therefore important that the horse can perceive the pain signal (i.e. that it is not heavily sedated) to be able to react on too high doses. In our studies, we observed a high increase in skin temperature in unclipped long hair coat compared to clipped skin after irradiation with 171 J/cm², indicating a higher risk for thermal injuries when irradiating horses with long hair coats. This is in accordance with a study on surface temperature in dogs receiving therapeutic ultrasound (Steiss & Adams, 1999). However, there was no significant difference in temperature increase between horses with short hair coat and clipped coat, receiving a lower irradiation dose (91 J/cm²). This might be due to the lack of insulating properties of the short or clipped hair coat.

The temperatures recorded in our study were measured at 1 and 3 cm from the irradiated area. It is most likely that the temperature at the irradiated site was higher, thus activating more heat-sensitive nociceptors. This assumption is based on studies using pulsed surgical laser irradiation (Fried *et al.*, 1999; Osmond *et al.*, 2000) in which the temperature was measured directly at the irradiated area and stepwise at distances of 6 and 7 mm from that area. A difference up to 30 °C was noted between the irradiated area and about 1 cm from there. The assumption is also supported by the morphological changes observed in the present study. The threshold for tissue protein denaturation is a function of temperature and heating time (Moritz & Henriques, 1947), and some of the observed morphological changes indicate tissue temperatures well above 45 °C. It is most likely that the

morphological changes are different degrees of thermal injury, since similar changes are reported from other studies (Laor *et al.*, 1969; Zweig *et al.*, 1990).

Thermal injury may also cause an inflammatory reaction, which activates a complicated cascade of reactions in the immune and vascular systems, inducing a vasodilatation (Löfgren *et al.*, 1997). The purpose of the inflammatory reaction is to initiate healing of damaged tissue. It has been suggested that the tissue injury acts as a counter-irritant, which activates the central diffuse noxious inhibitory system and thereby reduces pain (Le Bars, 2002). The laser beam in the present studies was scanned over the irradiation area, which creates a quasi-continuous delivery of radiation with pulse duration of approximately 0.07 s. With a pulse duration longer than the tissue temperature relaxation time, there is a risk for thermal injury due to heat conduction (Walsh *et al.*, 1988; Zweig *et al.*, 1990). Our results showed dose-dependent changes in skin morphology after defocused CO₂ laser irradiation with all three different doses, ranging from therapeutic to near-surgical.

Some changes were observed below estimated laser absorption depth, which indicates heat conduction in the tissue. This was especially relevant for the differences in morphological changes observed after the 91 and 137 J/cm² doses (with more changes and at a greater depth after the 137 J/cm² dose), since the only factor that distinguished the doses from each other was the irradiation time, i.e. the number of scanning repetitions. Notably, the morphologic changes after the 137 J/cm² dose were not correlated to any macroscopic changes observed within 90 min after irradiation.

The doses that caused the moderate to severe changes (137 and 450 J/cm² doses) are, to our knowledge, not normally used in bio-modulating CO₂ laser therapy, but can be attained by accidentally reducing the irradiation area.

In the present study the temperature in the centre of the irradiation area was not measured. It would have been desirable to have temperature probes designed for direct measurement. We can assume that the temperature at the irradiation area was at least higher than those recorded at 1 cm from the irradiated area. It would also have been desirable to measure changes in blood flow on standing horses, but experience shows that the movement artefacts would have limited the possibilities to draw any conclusions from the recordings (Edner, 2005).

The present physiological effects caused by defocused CO₂ laser irradiation may be compared to other therapeutic heat modalities: warm water (42-45 °C) from a water hose increased the skin temperature by approximately 10 °C (Kaneps, 2002). A gel wrap heated to 40 °C and applied to the metacarpal region for 30 min raised skin temperature by 5 °C, and therapeutic ultrasound has been shown to cause a difference of less than 1 °C (1.5 W/cm²) between treated and untreated limbs in horses (Turner & Wolfsdorf, 1991).

Due to the limitations of the LDF technique caused by different experimental setups, it is difficult to compare the effect on blood flow with other reports. However, studies on transcutaneous electric nerve stimulation and acupuncture in humans show a two to threefold increase in the microcirculation in the skin (Cramp *et al.*, 2002; Kuo, 2004) and tenfold in muscles during muscle activity (Sjaastad *et al.*, 2003).

Effects of defocused CO₂ laser irradiation on traumatic arthritis of the fetlock joint

The clinical study did not reveal any significant difference in the reduction of lameness between horses treated with defocused CO₂ laser (91 J/cm²) for traumatic arthritis of the fetlock joint compared to a sham group horses. Nor was there a significant difference between the groups in the results from the blood and synovia analyses. However, considering the physiological effects of temperature, blood flow and morphology observed in our experimental studies it is important to consider possibilities why the treatment did not result in a difference in detectable clinical improvement.

Laser studies on osteoarthritis in humans have shown relief of pain (Nivbrant & Friberg, 1992; Bertolucci & Grey, 1995; Basford *et al.*, 1999; Gur *et al.*, 2003; Bjordal *et al.*, 2003), or no differences between treated and controls (Basford, 2000; Brosseau *et al.*, 2004; Tascioglu *et al.*, 2004; Brosseau *et al.*, 2005). The lack of confirmed mechanisms of action and the diversity of clinical outcome generate uncertainties about proper dosage and treatment indications. When planning the present study, no recommended standard therapy programs regarding dose and duration of the laser treatment were available for horses. This is also the case with laser therapy in humans, where no consensus has been reached on optimal treatment parameters (Basford, 1995; Tuner & Hode, 1998; Basford *et al.*, 2000; Bjordal *et al.*, 2003; Nussbaum *et al.*, 2003) or conclusive evidence of dose-dependency has been shown (Bjordal *et al.*, 2003; Gur *et al.*, 2003; Naeser, 2006). The dose used in the present study mimics clinical practice and was promoted by the manufacturer of the laser system and calculated in order to achieve a possible therapeutic dose in the fetlock joint. Based on the results from the studies on temperature, blood flow and skin morphology, a decrease in dose would likely limit the physiological effects leading to a reduced clinical effect, while an increase in dose could hazard the condition of the superficial tissues. Only one joint in each horse was treated, although some of the horses had bilateral traumatic arthritis of the fetlock joint. It has been proposed that laser treatment unilaterally may induce similar changes in the contra-lateral site (Rochkind *et al.*, 1989). It is unlikely that there was a major systemic effect of the laser irradiation, since there was no difference in the blood parameters analysed.

Relatively few horses met the inclusion criteria, but the study was randomised, blinded and controlled. The horses were examined by the same experienced clinician using the same protocol. Based on preliminary power analysis calculations, our aim was to include 30 horses in the study. In an early stage of the trial, after experience difficulties in recruiting suitable horses, a discussion on changing inclusion criteria was held, and it was decided to widen the criteria to include bilateral forelimb lameness. Other changes in the protocol, such as exclusion of the control group or allowing other lameness's, were rejected since that would have negatively influenced the scientific quality. The study was then re-started. In total, 45 horses were examined and due to strict inclusion criteria, 16 horses were accepted. The partly inconclusive results may be caused by the sample size.

In our material, the improvement rates in both groups were approximately 50%.

These results may be compared to an earlier prospective study on defocused CO₂ laser therapy on traumatic fetlock arthritis in horses (Lindholm *et al.*, 2002) which reports an improvement rate of 80% after laser treatment, compared to 68% after intra-articular injection of bethametasone together with sodium hyaluronate. In the observer-blind part of the referred study, improvement was confirmed in 3/5 horses (60%) in both groups after 3-5 weeks. To our knowledge, no studies have been published on the clinical effects of other types of therapeutic heat in horses, and there is a lack of scientific studies on different rehabilitation modalities. However, studies on other conventional treatments of traumatic or induced arthritis show an improvement or return to health at approximately 3 weeks that differs between 45% (10/22 horses) receiving intra-articular injection of 0.9% NaCl (Gaustad *et al.*, 1999), 52% (21/41) of horses treated with sodium hyaluronate (Verschooten & Desmet, 1997), 69% (38/55) for polysulphated glucosaminoglycan or sodium hyaluronate (Gaustad & Larsen, 1995), 12 (1/8) to 80% (49/61) for corticosteroids (Rydell *et al.*, 1970; Vernimb *et al.*, 1977) and “excellent” for 83% (10/12) of the horses receiving the combination sodium hyaluronate and corticosteroids (Rydell *et al.*, 1970). In a study by Gaustad *et al.* (1999) the improvement rate for rest alone was 56% (9/16 horses). In the present study, it is possible that an effect from the laser therapy have not been able to be separated from a self-healing effect. It would be interesting to extend the follow-up period in order to see if there are any delayed effects not detected 3 weeks after the initial examination

The previously mentioned studies comprised different assessment tools and the horses exhibited different degrees of lameness. Since traumatic arthritis has both a pain and an inflammatory aspect, and assessment of pain is often done by examining the degree of lameness, the evaluation in the present study was complemented by an objective accelerometer technique, as well as analyses of SP, PGE₂ and MEAP in synovia. The lameness evaluation was based on comparison of changes in initial lameness. Contrary to some of the previously mentioned studies, the flexion test was used only as a diagnostic tool and not primarily in the evaluation of the degree of lameness. This choice was based on studies showing that the response of a forelimb flexion test may vary with time, with the clinician performing the test and from day to day, and may not be well correlated with radiological findings or future occurrence of forelimb lameness (Ramey, 1997; Verchooten & Verbeeck., 1997; Busschers & van Weeren, 2001). However, if the evaluation had instead been done on the outcome of the flexion test (improvement defined as a reduction in the degree of lameness) all horses but two would have been classified as improved, however without any significant difference between laser and sham treatment.

An accelerometer technique is a method earlier described and proven as an objective method for lameness evaluation (Weishaupt *et al.*, 2001; Keegan *et al.*, 2002; Leleu *et al.*, 2004). However, in the present study the correlation between the grading of the clinician and the accelerometer data was weak. These results are contrary to previous studies in which a symmetrical vertical head movement was lost at forelimb lameness (Barrey & Desbrosse, 1996; Vortenbosch *et al.*, 1997) and the degree of asymmetry detected by accelerometer technique was related to the degree of lameness confirmed by conventional lameness examination

(Weishaupt *et al.*, 2001) The reason may be the low variation in the conventionally assessed degree of initial lameness in the horses entering the study. On the other hand, there was good correlation between the grading of the clinician and the accelerometer data of the flexion test when the variation in the grading was higher. Our conclusion is that the accelerometer technique was a valuable complement to get a more complete evaluation of low-grade initial lameness and that it enhance the validity of the conclusions.

It is reasonable to assume that pain in traumatic arthritis may have synovitis as a major origin (Howard & McIlwraight, 1996). As we have measured effects on superficial tissue temperature and blood flow indicating a sensory stimulation, which possibly has a pain reducing, and an anti-inflammatory effect, analyses of the inflammatory mediator SP, PGE₂ and the opioid MEAP in synovia were performed. The opioid MEAP is a neuromodulator, important in anti-nociception and inflammation (Kiser *et al.*, 1983; Rosen *et al.*, 2000) and a recently published study reports on an opioidergic activation in the medial pain system after heat stimulation (Sprengler *et al.*, 2006). Contradictory results have been reported in studies on human cerebrospinal fluid, with a lower concentration of Met-enkephalin in patients with chronic pain compared to those without pain (Simmonet *et al.*, 1986) and higher levels of MEAP in patients with fibromyalgia (Baraniuk *et al.*, 2004). The concentration of met-enkephalin has been detected in pituitary effluent blood, peripheral blood and cerebrospinal fluid in horses (Luna *et al.*, 1998). Another study on horses report on opioid receptors in the synovial membranes and that opioids can decrease inflammatory-induced pain through inhibition of the release of SP from peptidergic neurons (Sheehy *et al.*, 2001). However to our knowledge; no studies have been published on MEAP data in horses. Our unpublished results show a significant difference in the concentration of MEAP between sound horses (one group of standing and one group of anaesthetised horses) and horses with traumatic arthritis of the fetlock joint, with a significantly lower concentration in the synovia of the lame horses ($p < 0.001$). Although, the intra-assay variance was relatively high, it is still likely that our results are accurate as all samples were randomly ordered, assayed the same day and tested in the same RIA. However not significant ($p < 0.14$), the concentration of MEAP was increased in 5 out of 7 horses in the laser group compared to 2 out of 5 horses in the sham group. In the present study, the tendency towards increased MEAP may suggest a possible pain modulating effect, correlated to the clinical status of the horses in the laser group.

An anti-inflammatory effect has earlier been shown after laser irradiation (Amano *et al.*, 1994; Ulugöl *et al.*, 1997; Campana *et al.*, 1999; Bjordal *et al.*, 2006). No significant difference in the concentration of SP or PGE₂ was observed between groups, or between sound horses and horses with traumatic arthritis. However, other studies have shown a higher concentration of the pro-inflammatory SP and PGE₂ in synovia from horses with joint disease compared to normal joints (Caron *et al.*, 1992; May *et al.*, 1994; Gibson *et al.*, 1996; Owens *et al.*, 1996; Hawkins *et al.*, 1993; Hardy *et al.*, 1997; Kirker-Head *et al.*, 1999; Bertone *et al.*, 2001). It is likely that relatively low concentrations measured in the lame horses reflect the severity of the traumatic arthritis since the horses exhibited a low grade lameness.

Summary and conclusions

Objective outcome measurements such as Laser Doppler Flowmetry and temperature recordings together with accelerometer technique to examine lameness proved to be valuable when evaluating a rehabilitation modality such as the defocused CO₂ laser. It is likely that these methods can be of equal value when assessing the effect of other physical rehabilitation modalities used in veterinary medicine. The hypothesis that defocused CO₂ laser irradiation reduces the degree of lameness, due to traumatic arthritis of the fetlock joint, could not be verified.

Irradiation with defocused CO₂ laser resulted in changes of the following parameters:

Local temperature

- an increased temperature in skin and subcutis
- an accumulation of heat in the subcutis
- a greater increase in temperature in unclipped compared to clipped skin
- no changes in the temperature of the fetlock joint or in muscle

Local blood flow

- an increased blood flow in skin
- no changes in the blood flow in muscle

Skin morphology

- no to mild morphological changes for the 91 J/cm² dose
- mild to moderate morphological changes for the 137 J/cm² dose
- moderate to severe morphological changes for the 450 J/cm² dose

Blood and synovia

- a higher concentration of MEAP in synovia in healthy horses compared to horses with traumatic arthritis of the fetlock joint
- no difference in the concentration of SP, PGE₂ and MEAP in synovia between laser and sham treated lame horses

Clinical effects

- no difference in the degree of lameness between the active laser treated (91 J/cm²) and sham group horses, evaluated by either conventional lameness examination or accelerometer technique

Clinical implications

The present investigation was undertaken to study physiological effects of defocused CO₂ laser irradiation and assess the therapeutic value in horses with traumatic arthritis of the fetlock joint.

The results showed that irradiation with defocused CO₂ laser with our specified doses had a photothermal effect, causing an increase in temperature in superficial tissues. The increase in skin temperature was greater in unclipped horses compared to clipped, indicating a greater risk for thermal injuries when irradiating horses with a long hair coat. The increase in skin and subcutis was of such intensity that restrictions are suggested on the use of irradiation on dermal injuries in the acute stage, in order to avoid the risk for oedema. There was no increase in the temperature in the fetlock joint, which indicates that it may be possible to irradiate traumatic arthritis without activating cartilage-degenerative enzymes.

The photothermal effect also involves a risk of thermal injuries. The blood flow in muscle may be stimulated by a higher dose than 91 J/cm², but the risk of thermal injuries in skin is obvious. Whenever possible, more efficient methods, such as active muscle work, is preferable to increase blood flow in muscles.

The risk of negative side effects increases with an accumulation of heat when the laser is applied with insufficient relaxation time between irradiations or between each scanning repetition. A power density that normally does not induce morphological changes may do so when repeated. Consequently, there were more morphological changes in the skin after irradiation with 137 J/cm² (16 W, 6 x 7 cm, 6 min) compared to 91 J/cm² (16 W, 6 x 7 cm, 4 min). Therefore, the use of an average dose higher than 91 J/cm² on equine skin should be considered carefully. It is important to be aware of the risk of significant microscopic damage without simultaneous macroscopic changes after laser irradiation. Since the increase in temperature is correlated to the degree of thermal injury, it is essential that the treatment set-up permits the horse to withdraw the irradiated limb if the treatment becomes painful. Therefore heavy sedation of the horse before treatment should be avoided.

Finally, it is essential to handle the CO₂ laser device correctly. The effect of the laser should be monitored regularly by use of an external output power detector as the mirrors reflecting the laser beam easily becomes dusty, and consequently give a lower dose than expected. Measurement of the irradiation area on the target should also be closely monitored, as an accidentally reduced area of treatment increases the irradiation dose and endangers the welfare of the horse.

Future research

Further research should focus on validating rehabilitation assessment tools. Most important, further studies on pain assessment are necessary in order to evaluate rehabilitation modalities, as a majority of the modalities in one way or another focuses on pain relief.

One main outcome effect of a successful treatment on musculoskeletal injuries is the reduction of pain, evaluated as a reduction in lameness. It is therefore important to refine and develop the accelerometer technique, in order to objectively detect and confirm subtle changes in movement at both training and clinical settings.

Many of the modalities used in rehabilitation have an explanatory model based on increased blood flow. Thus it would be of great interest to refine a non-invasive Laser Doppler Flowmetry technique for intramuscular blood flow measurements on standing animals.

Further research on physical rehabilitation modalities should focus on defining modes of action and validating treatment protocols. Before applying defocused CO₂ laser treatment in clinical practices, it is important to continue studies on morphology with alternative doses and treatment locations, as well as extending the present clinical study on more horses and to evaluate clinical effects on other types of injuries.

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Populärvetenskaplig sammanfattning

Intresset för rehabilitering har ökat markant inom veterinärmedicinen under de senaste åren. Målet med rehabilitering är att återställa bästa möjliga funktion efter skada eller sjukdom. Vid rehabiliteringen används olika fysikaliska behandlingsmetoder, däribland behandling med defokuserad CO₂ laser. Vid sådan behandling omvandlas laserljusets energi till värme i vävnaden. En ökad temperatur anses kunna lindra smärta samt påskynda läkning av olika vävnader, och värmebehandling har därför använts sedan urminnes tider.

I denna avhandling har de fysiologiska effekterna av behandling med defokuserad CO₂ laser undersökts i fyra olika studier: tre experimentella och en klinisk. Syftet har varit att studera om behandlingen resulterar i en värmeökning, och – om så är fallet – om ökningen åtföljs av ett förhöjt blodflöde, om en ökning av värme och blodflöde leder till smärtlindring och stegrad läkningstendens samt om värmeökning kan påverka hudens mikroskopiska bild.

Temperatur, blodflöde och morfologiska förändringar i huden har studerats på friska vakna och sövda hästar. Resultaten visar att laserljuset gav upphov till en värmeökning i huden och underhuden, medan temperaturen i muskler och kotleder föreblev opåverkad. Temperaturökningen i huden orsakade beroende på dos laserljus (energi/kroppsyta) milda till kraftiga mikroskopiska förändringar, som liknar dem man kan se vid brännskador. Temperaturökningen kvarstod längre i underhuden än i huden och den blev också större hos hästar med lång hårrem än hos dem med klippt päls. Laserbehandlingen ökade blodflödet i huden, men inte i underliggande muskulatur.

I den kliniska undersökningen studerades laserbehandlingens smärtlindrande och antiinflammatoriska effekter genom att hästar med traumatisk ledinflammation i kotleden undersöktes. Som kontrollgrupp användes också hästar som fått placebobehandling. Smärtlindring mäts i detta sammanhang ofta genom att man undersöker om det skett en minskning i graden av hälta, eftersom hästens subjektiva smärtupplevelse inte kan fastställas. I studien användes både konventionell håltbedömning utförd av en erfaren kliniker och objektiv mätning av rörelsen med ett accelerometersystem. Dessutom mättes förändringar i koncentrationen av markörer för smärta och inflammation i ledvätska före och efter behandling. Resultaten visar att hästarna blev bättre efter behandlingsperioden, men att det inte var någon skillnad mellan de hästar som fått laserbehandling och de som fått placebo. Det var heller ingen skillnad i koncentrationen av markörer mellan grupperna.

Sammanfattningsvis visar resultaten att behandling med defokuserad CO₂ laser resulterar i en markant värmeökning i huden och underhuden, med åtföljande ökning av det lokala blodflödet. Temperaturökningen kan medföra en risk för skador i huden. Studien kunde inte påvisa någon signifikant skillnad i resultatet av behandling av hästar med traumatisk kotledsinflammation mätt genom graden av hälta och koncentrationen av markörer för smärta och inflammation. Innan man utvidgar behandling med defokuserad CO₂ laser bör ytterligare studier göras på ett urval behandlingsregimer och skador.