

# Effect of Defocused CO<sub>2</sub> Laser on Equine Tissue Perfusion

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**A. Bergh<sup>1</sup>, G. Nyman<sup>2</sup>, T. Lundeberg<sup>3</sup> and S. Drevemo<sup>1</sup>: Effect of defocused CO<sub>2</sub> laser on Equine Tissue perfusion. Acta vet. scand. 2006, 47, 33-42.** – Treatment with defocused CO<sub>2</sub> laser can have a therapeutic effect on equine injuries, but the mechanisms involved are unclear. A recent study has shown that laser causes an increase in equine superficial tissue temperature, which may result in an increase in blood perfusion and a stimulating effect on tissue regeneration. However, no studies have described the effects on equine tissue perfusion. The aim of the present study was to investigate the effect of defocused CO<sub>2</sub> laser on blood perfusion and to correlate it with temperature in skin and underlying muscle in anaesthetized horses. Differences between clipped and unclipped haircoat were also assessed. Eight horses and two controls received CO<sub>2</sub> laser treatment (91 J/cm<sup>2</sup>) in a randomised order, on a clipped and unclipped area of the hamstring muscles, respectively. The significant increase in clipped skin perfusion and temperature was on average 146.3±33.4 perfusion units (334%) and 5.5±1.5 °C, respectively. The significant increase in perfusion and temperature in unclipped skin were 80.6±20.4 perfusion units (264%) and 4.8±1.4 °C. No significant changes were seen in muscle perfusion or temperature. In conclusion, treatment with defocused CO<sub>2</sub> laser causes a significant increase in skin perfusion, which is correlated to an increase in skin temperature.

*equine; CO<sub>2</sub> laser therapy; therapeutic heat; blood perfusion; laser Doppler flowmetry; temperature; rehabilitation.*

## Introduction

The goal of physical therapy is to promote healing of tissues through stimulation of normal physical processes, thereby restoring the function of the injured tissue (Stashak 1987). Therapeutic modalities in the form of hot packs, therapeutic ultrasound, and lasers have been advocated by numerous practitioners working with sports injuries, both in humans and horses (Lehmann 1990, Bromiley 1991). Simplified, laser therapy can be divided into surgical lasers (i.e. high effect laser) and lasers used for biomodulation, i.e. low level laser therapy (often treatment dosages of <1 to 4 J/cm<sup>2</sup> on treatment sites) (Basford 1995). However, lasers

originally made for surgery are used as biomodulating lasers; with a defocused beam and at a lower output effect, but with doses higher than in low laser therapy. A recent prospective study indicates that, defocused carbon dioxide (CO<sub>2</sub>) laser may be an applicable treatment for acute synovitis in horses (Lindholm *et al.* 2002). The effects of laser radiation on tissue structure and function are, however, unclear. Laser therapy is proposed to produce photochemical effects by excitation of electronic states in molecules and chromophores. Unlike low level laser, CO<sub>2</sub> laser is also proposed to have photothermal effects, by transformation of absorbed light en-

ergy to heat (Thomsen 1991). The light is absorbed at a depth of less than 0.1 mm (Bhatta 1994), which indicates a possible heating effect in superficial tissues.

Results from our own studies show that treatment with defocused CO<sub>2</sub> laser causes a significant increase in the temperature of skin and subcutis (Bergh et al. 2005). A rise in local temperature generally correlates with an increase in perfusion, and is believed to have a positive effect on pain and tissue regeneration (Lehmann 1990, Nannemann 1991, Oosterveld and Rasker 1994, Wright and Sluka 2001, Nadler et al. 2002). Vasodilatation increases blood flow to reduce ischemia of injured tissue, resulting in decreased activity of the pain receptors. A greater blood flow increases the supply of nutrients to the area for the repair process and removes by-products from the injured tissue. To the best of our knowledge, there are no studies published on the effect of defocused CO<sub>2</sub> laser on equine tissue perfusion.

Laser Doppler Flowmetry (LDF) technique is widely used for measurement of tissue perfusion (Norman et al. 1992, Adair et al. 1994, Raisia et al. 2000a, Berardesca et al. 2002, Edner et al. 2002, McGorum et al. 2002). This technique provides a continuous measure of relative perfusion, allowing detection of changes in blood flow over time on a single site (Raisia et al. 2000b, Raisia et al. 2000c, Humeau et al. 2004). In the present study, a hypothesis was formulated that treatment with defocused CO<sub>2</sub> laser increases temperature and perfusion in skin and underlying muscle. The objective was to measure temperature and perfusion in anaesthetized horses treated with active or sham laser. A further aim was to compare the effect of laser treatment on clipped and unclipped skin.

## Materials and Methods

### Horses

The study comprised ten, healthy Standardbred trotters (6 females and 4 geldings) with a mean weight of 497 kg (range 411-578 kg) and a mean age of nine years (range 4-19 years). Eight horses received laser treatment and two served as controls. All horses were pigmented at the irradiated area. The Ethical Committee on Animal Experiments in Uppsala, Sweden has approved the study.

### Anaesthetic protocol

Food was withheld for 12 hours prior to anaesthesia, but water was available until premedication. The horses were premedicated with detomidin (Domosedan® vet; Orion Pharma AB, Sollentuna, Sweden). Anaesthesia was induced intravenously with guaifenesin (Myolaxin® vet. diluted to 7.5%; Chassot & Cie AG, Berne, Switzerland) and tiopentone (Pentotal Natrium 12.5%; Abbott, Solna, Sweden). The horses were intubated, transported to the surgical table and placed in dorsal recumbency. Anaesthesia was maintained with isoflurane (Forene; Abbott, Solna, Sweden) in oxygen. Electrolytes (Ringer acetate; Pharmacia & Upjohn, Stockholm, Sweden) were infused through a catheter in the jugular vein. Spontaneous breathing was allowed from a semiclose, large-animal circle. To detect any changes in depth of anaesthesia, arterial blood pressure as well as heart rate was monitored throughout the research protocol.

### Peripheral perfusion and muscle temperature

Laser Doppler Flowmetry (LDF) was performed using a Periflux 4001 flowmeter (Perimed, Järfälla, Sweden). A treatment area of 6x7 cm on each semimembranosus muscle was prepared; one side clipped and the other with the coat intact. A small area for the measuring probes was prepared, in direct contact with each

Table 1. Laser parameters, dosage, and mode of application (KSV 25S Laser device)

Lasing media	CO <sub>2</sub>	HeNe
Wavelength	10 600 nm	633 nm
Continuous output power	16 W	0.0012 W
Mode of application	scanning	scanning
Diameter of beam at source	6 mm	0.61 mm
Divergence	1.5 mrad	2.0 mrad
Treatment distance	100 cm	100 cm
Irradiated area	42 cm <sup>2</sup>	42 cm <sup>2</sup>
Site of application	semimembranosus muscle	semimembranosus muscle
Treatment time	4 min	4 min
Dosage at skin surface	91 J/cm <sup>2</sup>	0.007 J/cm <sup>2</sup>

treated area. Skin perfusion was measured on the skin surface (Probe 407, Perimed, Järfälla, Sweden), 1 and 3 cm from the treated area.

For muscle perfusion, a straight microtip with slanted tip (MT A500-0.120 mm, 0.5 mm diameter, Perimed, Järfälla, Sweden) was placed in the semimembranosus muscle of the right and left hind limb, close to the skin perfusion probe at 1 cm from the treatment area. The microtip was inserted via a 0.7 mm cannula to a depth of 3 cm and connected to a probe (Master Probe; Probe 418-x, Perimed, Järfälla, Sweden), after which the cannula was retracted.

Skin and muscle temperatures were measured using thermistor probes (skin-440, muscle-442-PI, Perimed, Järfälla, Sweden) connected to a recording unit (PF 5020, Perimed, Järfälla, Sweden). The temperature probes were attached to the skin or inserted in the muscle approximately 1 cm from its corresponding perfusion probe and at the same distance from the irradiated area.

Flux, expressed in blood perfusion units, and temperature, were displayed and recorded continuously (Perisoft 1; 14, Perimed, Järfälla, Sweden). The total recording time was 50 min on average. To allow comparison of results, the LDF probes were calibrated in a standard motility solution provided by the manufacturer. The following skin and muscle blood flow and temperature features were analysed:

1. The average value before treatment, sampled for one minute (baseline).
2. The average value during treatment, sampled for one minute (treatment).
3. The peak value during treatment (peak).
4. The time from start of treatment to the peak value, in seconds (time to peak).

#### Laser protocol

A defocused CO<sub>2</sub> laser (10 600 nm, KSV 25S; EL.EN. SRL, Firenze, Italy) was used in the study, see Table 1. As guiding light, a HeNe source (633 nm) emitted continuously at 1.2 mW. The laser system was calibrated regularly, and an external detector (LaserMate Detector, COA-33-0191-000; Gamma Optronics AB, Uppsala, Sweden) was used to measure the intensity of the laser beam before and after each treatment. The laser system was set to give continuous output power of 16 W at a distance of 1 m, during a treatment period of 4 min. The treatment area was 42 cm<sup>2</sup> (6x7 cm) and the irradiation energy 91 J/cm<sup>2</sup>. Laser-treated and control horses, as well as the order of the treatment to the clipped and unclipped area, were randomized. There was an average of a 60-min pause between the irradiation of the clipped and unclipped area.

Table 2. Temperature response to laser treatment in clipped and unclipped skin, and in underlying muscle, measured 1 and 3 cm from the irradiated area

Area	Baseline °C	Treatment °C	Difference °C	Peak value °C	Time to peak s
Muscle					
clipped (n=8)	32.8±1.3	34.0±0.7	1.2±0.7	n.d.	n.d.
unclipped (n=8)	34.0±0.5	34.4±0.3	0.4±0.3	n.d.	n.d.
Skin 1 cm					
clipped (n=7)	30.4±1.0	35.9±0.8*	5.5±1.5	36.9±1.4	175.7±28.8
unclipped (n=8)	29.7±1.3	34.5±1.6*	4.8±1.4†	35.1±1.9	164.0±18.8†
Skin 3 cm					
clipped (n=8)	31.5±1.0	37.0±1.0*	5.5±1.5	37.0±1.3	169.6±16.4†
unclipped (n=7)	32.6±0.7	34.7±1.0*	2.1±0.4	35.6±1.1	230.1±21.9

Values are presented as means ±SE; measured as temperature (°C); time to peak (s), n.d.= not detected.\* Significantly different from baseline, † significantly different from unclipped skin 3 cm from irradiated area, p<0.05.

### Statistical analysis

Statistica 6.0 (Statsoft, 2001; Statsoft Scandinavia AB, Uppsala, Sweden) was used for data analysis, and results are presented as means and standard errors (SE). Microvascular perfusion and temperature were calculated as the average of one minute of stable recordings; immediately before start of treatment, at the end of treatment, and at 4 min after the end of treatment. The perfusion data are presented as relative changes in perfusion, using arbitrary perfusion units (PU). The data were individually corrected by setting the baseline before treatment to 100%. Biological zero (i.e. the laser Doppler signal from non-perfused tissue) was not subtracted; however, perfusion values under 3.5 PU were excluded from the analysis as the biological zero for equines is approximately 1.6 PU for skin and 3.5 PU for muscle (unpublished results).

Treatment data were compared to baseline data within each group. Statistical calculations comparing the time to maximum (peak) values and peak values for both skin temperature and perfusion were also made. Statistical calculations

were performed with Wilcoxon signed rank test and Mann-Whitney test, when appropriate. Statistical significance was accepted at p<0.05.

### Results

None or only minor differences in arterial blood pressure or heart rate were found within each protocol or between treatment and control.

The results are presented separately for treated and control horses. Figure 1 shows representative temperature and perfusion curves from one laser and control recording, respectively. As demonstrated by the laser recording, the increase in temperature is almost immediately followed by an increase in perfusion.

#### *The laser-treated group* *Temperature*

The temperature response to laser treatment is presented in Table 2. The results presented for temperatures response of clipped skin, 1 cm and unclipped skin, 3 cm are from seven horses, as measurements from one horse had to be excluded due to technical problems. 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. The temperatures did not return to the pre-treatment baseline at 30 min after treatment in: 2/7 of the horses in the skin 1 cm clipped group, 2/8 in the skin 1 cm unclipped group, 5/8 in the skin 3 cm clipped group, and 2/7 in the 3 cm unclipped group.

No significant difference was recorded in muscle temperature for either clipped or unclipped hair coat. The time of peak response to laser treatment is presented in Table 2. Peak temperature occurred earlier in clipped than in unclipped skin measured 3 cm from the irradiated area. In the unclipped groups, peak temperature occurred later in the 3 cm than in the 1 cm recording point.

### Perfusion

The perfusion response to laser treatment is presented in Table 3. The results presented for muscle perfusion are from seven (clipped skin) and six (unclipped skin) horses, since values less than 3.5 PU were excluded from statistical

analysis. There was a significant increase in perfusion in all skin recordings, i.e. 1 and 3 cm from the irradiated area for both clipped and unclipped hair coat. The perfusion did not return to the pre-treatment baseline 30 min after treatment in: 3/8 of the horses in the 1 cm clipped group, 1/7 in the 1 cm unclipped group, 2/8 in the 3 cm clipped group, and 2/7 in the 3 cm unclipped group. There was no significant difference in muscle perfusion.

The time for peak response to laser treatment is presented in Table 3. There was no significant difference in time to peak.

### The control group

Temperature and perfusion data are presented in Table 4. None or only minor changes were seen in tissue temperature or tissue perfusion in the two horses used as controls.

### Discussion

In the present study, there was an increase in temperature and perfusion in skin, but not in the underlying muscle. Two major findings were identified: (1) treatment with defocused CO<sub>2</sub>

Table 3. Perfusion response to laser treatment in clipped and unclipped skin, and in underlying muscle, measured 1 and 3 cm from the irradiated area

Area	Baseline %	Treatment %	Difference %	Peak value %	Time to peak s
Muscle					
clipped (n=7)	100	105.6±3.6	5.6±3.6	n.d.	n.d.
unclipped (n=6)	100	103.5±7.7	3.5±7.7	n.d.	n.d.
Skin 1 cm					
clipped (n=8)	100	434.0±170.5*	334.0±170.5	497.5±182.8	157.3±35.3
unclipped (n=8)	100	363.8±120.2*	263.8±120.2	428.2±139.3	167.2±29.3
Skin 3 cm					
clipped (n=8)	100	345.2±123.6*	245.2±123.6	407.9±142.4	184.0±28.4
unclipped (n=8)	100	216.5± 65.3*	116.5± 65.3	263.2± 80.9	208.2±47.1

Values are presented as means ±SE, perfusion (PU) with the baseline set to 100%; time to peak (s), n.d.= not detected. \* Significantly different from baseline,  $p < 0.05$ .

Table 4. Temperature and perfusion response in the control horses; temperature and perfusion in clipped and unclipped skin, and underlying muscle, measured 1 and 3 cm from the irradiated area

Area	Temperature		Perfusion	
	Baseline °C	Treatment °C	Baseline %	Treatment %
Muscle				
clipped	34.5 (34.4-34.6)	34.5 (34.4-34.6)	100	102.5 (99-106)
unclipped	34.4 (33.8-35.0)	34.4 (33.7-35.0)	100	98.0 (98)
Skin 1 cm				
clipped	29.1 (25.2-33.0)	28.9 (24.9-32.9)	100	93.0 (86-100)
unclipped	26.7 (26.5-26.9)	26.6 (26.2-27.0)	100	96.0 (96)
Skin 3 cm				
clipped	32.8 (32.8-32.9)	32.7 (32.5-32.9)	100	102.5 (94-111)
unclipped	32.9 (32.2-33.6)	32.8 (32.2-33.4)	100	79.0 (76-82)

Values are presented as means/ medians and ranges; measured as temperature (°C); perfusion (PU) with the baseline set to 100%, n=2.

laser causes an increase in temperature of the skin in clipped and unclipped haircoat, 1 and 3 cm from the irradiated area; (2) the increase in temperature was accompanied by an increased perfusion. To the best of our knowledge, no study has been performed on the effects on blood perfusion of defocused CO<sub>2</sub> laser treatment in horses. Therefore, comparisons can only be made with studies on other modalities with an effect on tissue temperature and/or blood perfusion. Studies on acupuncture, transcutaneous electric nerve stimulation, superficial heat and continuous therapeutic ultrasound have all shown increase in temperature and/or perfusion (Nannemann 1991, Oosterveld and Rasker 1994, Cramp *et al.* 2000, Levine *et al.* 2001, Wright and Sluka 2001, Kuo *et al.* 2004). Therapeutic application of heat plays a major role in rehabilitation programs. The rationale for using different heating modalities is based primarily on the fact that they produce peak temperatures in different locations. The goal is to achieve a "therapeutic" level of temperature elevation without causing adverse responses.

As one of the explanations for the mode of action of defocused CO<sub>2</sub> is its photothermal effect, it is important to identify the heating pattern of laser treatment. In the present study, the increases in temperature and perfusion were in superficial tissues, and not in muscle temperature and blood perfusion. As in other superficial heating modalities, the deeper tissues including muscles are usually not significantly heated. Heat transfer from the skin surface into deeper tissues is inhibited by the subcutaneous fat, which acts as a thermal insulator, and by the increased blood flow in more superficial tissues which cools the tissues by transporting away the heat (Lehmann 1990).

The physiological effect of the applied CO<sub>2</sub> laser irradiation is related to the activation of warmth and heat receptors and afferents; cutaneous thermosensitive A $\delta$  and the C-fibres (Arendt-Nielsen and Chen 2003). In the present study, it is likely that both A $\delta$  and C-fibres were stimulated, with a secondary influence on blood perfusion. The mechanism for vasodilatation is suggested to be activation of the axon/dorsal

root ganglion reflex from heat sensitive nociceptive afferents, which releases neurotransmitters that increase blood flow. These neurotransmitters may stimulate nitric oxide release causing further vasodilatation (Kellogg *et al.* 1999, Minson *et al.* 2001, Stephens *et al.* 2001). Thermally evoked vasodilatation has also been found following non-painful stimulation when using a slowly increasing heat stimulus (Magerl and Treede 1996, Minson *et al.* 2001). In the present study, the perfusion at 1 cm from the irradiated area increased with 146 PU on average, when the temperature had increased by about 6 °C, to a mean of approximately 36 °C. This is consistent with results from other studies that report on significant vasodilatation between local temperatures of 30-35 °C (Barcroft and Edholm 1943, Taylor *et al.* 1984, Johnson *et al.* 1986, Stephens *et al.* 2001). In humans, local warming of the skin to 42 °C has been reported to increase blood flow tenfold, at the end of a 20-min warming period (Saumet *et al.* 1998).

The increase in perfusion started directly after the first rise in temperature. This is in agreement with an earlier study showing a correlation between the first sensation of non-noxious heat and the onset of cutaneous vasodilatation, and that the vasodilatation correlates better with the sensation of heat compared to actual skin temperature (Stephens *et al.* 2001). Our findings, and the fact that vasodilatation was detected 3 cm from the irradiated site, support the suggestion that the observed vasodilatation was caused by an axon/dorsal root ganglion reflex of nociceptive afferents, probably in combination with a secondary release of nitric oxide (Kellogg *et al.* 1999, Minson *et al.* 2001).

There were no significant differences between the temperatures of clipped and unclipped skin. These results do not agree with earlier studies in which the skin temperature was higher in animals with long haircoat, compared to short or

clipped hair (Steiss and Adams 1999, Bergh *et al.* 2005). However, it is possible that the relatively thin haircoat at the actual experimental site had an influence on the results. As the irra-

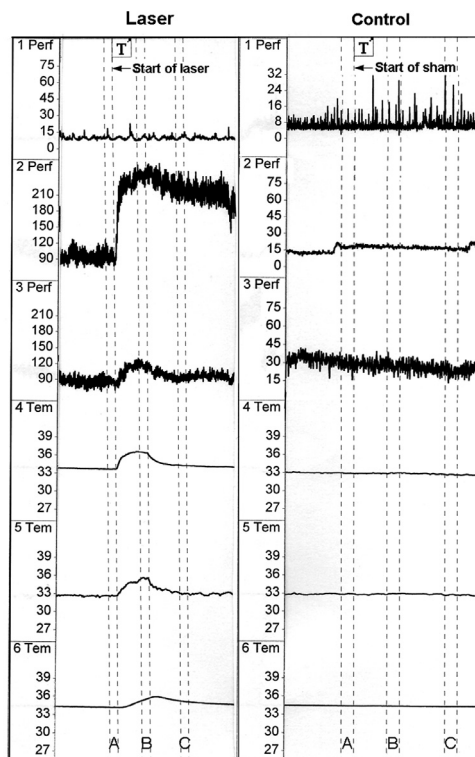


Fig. 1. Representative tracing from one laser treated and one control horse, displaying temperature and perfusion response. The perfusion is presented as arbitrary Perfusion Units (PU) and temperature as °C. Channel 1; perfusion in muscle. Channel 2; perfusion in skin at 1 cm from the irradiated area. Channel 3; perfusion in skin at 3 cm from the irradiated area. Channel 4; temperature in muscle. Channel 5; temperature in skin 1 cm from the irradiated area. Channel 6; temperature in skin at 3 cm from the irradiated area.

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 treatment, respectively.



diations of the clipped and unclipped areas were randomized, it is unlikely that a consensual effect of the irradiation would have a major influence on the results.

Movement artefacts are a common problem using Laser Doppler Flowmetry technique. This was reduced as the horses were anaesthetised during the study. It is possible that tissue perfusion was affected by the anaesthetic agent and to some extent, by the position of the limb. In order to minimize the negative effects on peripheral perfusion, the anaesthesia was maintained with isoflurane, since hind-limb blood flow has been found to be higher during isoflurane than halothane anaesthesia, due to a less cardiac depression and greater peripheral vascular dilatation (*Raisis et al.* 2000a). Blood flow to a region is influenced by its vertical position relative to the heart (*Hennig et al.* 1995). This positional effect was minimized in our study since the position of the probes was horizontal and approximately at the level of the heart. However, it is likely, due to the influence of general anaesthesia and positioning of the limb, that the registered increases in blood perfusion in the anaesthetized horses were similar or less pronounced than would be expected in non-anaesthetized animals.

It has been reported that the surface temperature of the distal limb differs between individuals (*Kameya and Yamaoka* 1968, *Webbon* 1978, *Palmer* 1983) and it is known that the ambient temperature has an influence on skin temperature (*Kameya and Yamaoka* 1968, *Webbon* 1978). This variation was greater at an ambient temperature of 5 °C than at higher temperatures (15–25 °C) (*Kameya and Yamaoka* 1968, *Palmer* 1983). In the present study, the ambient temperature varied between approximately 16 and 20 °C.

In our study, defocused CO<sub>2</sub> laser radiation increased temperature and tissue perfusion in the skin, but not in deeper tissues. However, the

question as to whether this has therapeutic significance remains to be investigated. The biophysical effects of similar temperature elevation in human body tissue include increased local blood flow and metabolism, elevated pain threshold, decreased muscle spindle firing rate, and increased extensibility of connective tissue. Heat can provide analgesia, promote relaxation, reduce muscle spasm, and enhance flexibility of muscles and periarticular structures (*Lehmann* 1990, *Nannemann* 1991, *Minor and Sanford* 1993, *Wright and Sluka* 2001). Heat also assists in resolution of inflammatory infiltrates, oedema and exudates (*Lehmann* 1990, *Nannemann* 1991). Consequently, the increase in temperature and perfusion in the present study may have had an effect on pain and tissue regeneration.

In conclusion, defocused CO<sub>2</sub> laser causes a significant increase in skin perfusion, which is correlated to the increase in skin temperature, both measured at 3 cm from the irradiated area. No differences were observed between clipped and unclipped haircoat, or in muscle. Further studies are needed to investigate if the increase in temperature and perfusion achieved by defocused CO<sub>2</sub> laser enhances tissue regeneration, decreases pain and restores impaired function.

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### Sammanfattning

Lokala blodflödesförändringar hos häst vid behandling med defokuserad CO<sub>2</sub> laser.

Laserbehandling sägs stimulera och påskynda ned-sänktsprocessen, men dess verkningmekanism är oklar. En nyligen publicerad studie visar att behandling med defokuserad CO<sub>2</sub> laser ger en ökning av temperaturen i ytliga vävnader hos häst. En ökning av vävnadstemperatur åtföljs ofta av en ökning av det lokala blodflödet, med en positiv inverkan på vävnaders läkning. Så vitt vi vet saknas publicerade studier om defokuserad CO<sub>2</sub> lasers effekt på blodflöde hos häst. Syftet med denna studie var att undersöka effekten av defokuserad CO<sub>2</sub> laser på lokalt blodflöde (med hjälp av Laser Doppler Flowmetry) och att korrelera blodflödet till temperaturen i rakad och orakad hud, samt i underliggande muskelvävnad. Tio hästar inkluderades i studien, varav åtta fick aktiv laser och två placebo. Den aktiva laserdosen var 91 J/cm<sup>2</sup> och gavs på ett 42 cm<sup>2</sup> stort område över semimembranosus muskulaturen. Den aktiva laserbehandlingen ökade signifikant blodflöde och temperatur, med i genomsnitt 146.3±33.4 perfusionsenheter (334%) och 5.5±1.5 °C i rakad hud, och 80.6±20.4 perfusionsenheter (264%) och 4.8±1.4 °C i orakad hud. Inga statistiskt signifikanta skillnader kunde noteras i blodflöde och temperatur i underliggande muskel, eller mellan rakad och orakad hud. Fortsatta studier får visa om denna temperatur- och blodflödesökning kan leda till smärtlindring och förbättrad läkning.

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