Thermographic analysis of the photochemical effect, a result of Multiwave Locked System Laser irradiation

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Abstract. Laser therapy induces an active hyperemia in the irradiated tissues (increases blood vessel diameter and permeability). The initial assumption was that the magnitude of the vasodilator effect of laser therapy depends simultaneously on the irradiation dose (power, frequency), on the wavelength of radiation, as well as on the physiological characteristics of the irradiated tissues. It was thus assumed that the treatment of well-vascularized tissues would produce a vasodilator effect, expressed by higher local temperature than in poorly-vascularized tissues. In order to test this assumption, skin temperature variations were measured by means of digital thermography. A group consisting of 20 healthy volunteers was analyzed and temperature of relevant areas was measured before and after exposure to laser radiation at time 0 as well as at 30, 60 and 90 minutes of irradiation. The temperature gradient between the area concerned and the contralateral non-exposed area was calculated in each of these moments. Temperature variations could be observed in all the areas studied after exposure to radiation, both in the treated and in the contralateral areas. These temperature variations have the same trend (either increasing or decreasing) for the same anatomical area, treated or not, with thermal variations of different magnitudes (higher for the exposed area). The maximum value of temperature variations can be reached after 30 - 60 minutes, not at time 0, as it would be considered normal. Therefore, even though the energy transfer is considerable (the peak power for the pulsed emission of 3x25 W), the findings suggest that by irradiating living tissues with MLS laser is not achieved the photothermal effect, but photochemical effect is the stimulus that determines the formation of mediators with vasodilatory effects.
Key words*: laser therapy, MLS therapy, thermography, photochemical effect*.

Introduction

Laser therapy has been used in clinical practice since the early 70s. The power of the devices used for biostimulation has increased significantly in time, from 100 mW to 3 KW. Once with the increased power of emission, pulses of light of extremely short duration began to be used, creating the premises for a significant energy transfer, with no danger of thermal burns.

All forms of light influence living organisms. It has been demonstrated, for example, that white light influences seasonal depressions and stimulates collagen production in the skin, being effective in treating wrinkles. There have also been researchers over time who argued that therapeutic effects of laser cannot occur due to the fact that they are contrary to the laws of physics. In terms of biological effects, King (1) and Greguss (2-4) claimed that laser therapy could be replaced by a source of “ordinary light” having the same optical characteristics, as laser radiation loses its coherence when crossing tissues, due to the phenomenon of dispersion. They have taken the matter even further, stating that laser radiation is incapable of having medical effects due to its minimal penetration into tissues.

These statements have been later contradicted: a large number of studies has been carried out on laboratory animals and on humans (5-14), comparing the effects of laser with those of light from other sources (LED). The significant effects that have been observed after using laser could not be evidenced after using other sources of light. The laser radiation coherence is not lost in tissues due to the phenomenon of dispersion (light can be coherent or not, but it cannot be *more* or *less* coherent). Moreover, no "ordinary light" is able to share the same properties with laser radiation, but if there were, it would certainly produce the same effects. Finally, laser penetration into tissues is not minimal.

The penetration depth of red light has been studied in association with the technique known as photodynamic therapy, by means of which hematoporphyrin derivatives are injected into a tumor area, which is then irradiated by laser at a wavelength of 630 nm. It has been established that the depth at which biological effects (tumor necrosis) are obtained is up to 10 mm (15, 16). Practical experience and clinical studies have shown that the biological effects of laser occur up to a depth of 1-4 cm, depending on laser type and power.
The action mechanisms of laser radiation can be divided into primary (interaction between photons and tissue molecules) and secondary mechanisms (chemical reactions induced by primary mechanisms). The dependence of biostimulative effects on doses indicates the existence of threshold intensities and their involvement in various mechanisms (a certain density of photons is necessary). There are many possible reasons which could explain this issue, one of them being the multiple action of photons. If the effects were only due to electron excitation and ionization, threshold intensities would not exist - the effects would also be obtained by the action of a single photon.
The biological effect of laser is based on various primary photochemical reactions: 1. photoreactivation with Cu-Zn-superoxide dismutase inactivated by the low pH from the hypoxic sites (17); 2.the photodynamic action of endogenous sensitizers ("the singlet oxygen hypothesis" - some photoabsorbing molecules, such as porphyrins and flavins, but especially hematoporphyrin, which can be detected in healthy people as well and the level of which can be increased in pathological conditions, can be temporarily converted into photosensitizers) (18); 3. the photolysis of metal-protein complexes with NO leading to the release of NO and to the reactivation of cellular respiration ("NO hypothesis") (19); 3. changes of the redox potential and acceleration of the electron transfer ("redox potential changes hypothesis"– the photoexcitation of some chromophores, such as cytochrome c oxidase, a and a3 heme molecules, influences the redox potential of these molecules and consequently the rate of electron transfer) (20, 21); 4. changes in the local biochemical activity induced by the local heating of chromophores ("transient local heating hypothesis" – when the electrons are excited by photons, a notable fraction of their energy is transformed into heat, which determines a transient increase in the temperature of chromophores (22).
The therapeutic effects of laser therapy (antalgic, anti-inflammatory and biostimulating) are obtained trough biological effects (photochemical, photothermal and photomechanical). Among the latter category, the photomechanical effect is present only in high-power lasers used in surgery. When it comes to the lasers used in physiotherapy, their effects are photochemical and photothermal. Both effects, directly or indirectly (by vasodilation and increased local metabolism), determine a rise in local temperature, which can be evidenced by means of infrared thermography.
To demonstrate these aspects, skin temperature is determined before, immediately after laser irradiation, within 30, 60, 90 minute interval of irradiation. This study was based on the premise that the photothermal effect is achieved immediately after irradiation, while the photochemical effect requires a latency time.
MLS therapy combines laser emission with two wavelengths (808 and 905 nm) in continuous (808 nm, with a maximum power of 1W) and pulsed systems (905 nm, with a maximum power of 25 W). The advantage of this combination consists in a better capacity of penetration, as well as in the possibility to increase energy output. MLS therapy uses laser emission stemming from semiconductor laser diodes, thus: InGa(Al)As for the continuous emission with a wavelength of 808 nm and InGaAs/GaAs for the pulsed emission of 905 nm. The peak power for the pulsed emission is 3x25W and for the continuous one 1W. In such conditions, MLS therapy comes under class IV, of high-power lasers (lasers with continuous emission in visible or infrared spectra, with a power exceeding 0.5W), according to the classification of lasers.
MLS emission is coherent – the therapeutic effect is greater if the emission is coherent, a fact which is explained by generation at the interaction between light and biological membranes of a coherent bio laser emission due to the existence of conductive tapes in the subcellular structures containing free charges. The wavelength of the MLS pulsed emission determines the deepest action – the interval between 600 and 1200 nm is known as "the therapeutic window", due to the fact that there are no chromophores to absorb these wavelengths. The wavelengths of MLS pulsed emissions come under this interval, entailing the fact that they can reach the deepest anatomical structures within it. Continuous emissions determine an intense anti-inflammatory and anti-edematous effect, being capable of stimulating lymphatic circulation and drainage and of interacting with the inflammation mediator synthesis and degradation. The pulsed emission acts on pain transmission both at the nociceptor and at related nerve fiber levels (due to its depth of action). The resting potential is restored quicker after the onset of the action potential, determining a higher threshold of cell and nerve fiber stimulation and a decreased pain sensation.

Moreover, the simultaneous activation of proprioceptive channels (through A alpha and A beta fibers) blocks the transmission of pain to the substantia gelatinosa of Rolando, being replaced by the kinesthetic sense. The analgesic effect immediately obtained is long-term. The synchronization of emissions forming the MLS impulse produces an intense and immediate therapeutic effect due to the fact that the anti-inflammatory and anti-edematous effects of the continuous emission and the analgesic effects of the pulsed emission are mutually-reinforced. MLS therapy makes the achievement of a large number of therapeutic effects with anti-inflammatory, anti-edematous and analgesic action possible, leading to rapid improvements. The advantages of MLS therapy are thus the following: very short-term treatment, rapid pain reduction or disappearance, including neuropathic pain, powerful anti-inflammatory effect, rapid healing of sprains and muscle contractures, rapid restoration of the structural integrity of injured muscles, rapid retrocession of edema, immediate improvement of local circulation, rapid healing of superficial wounds. For these reasons, MLS therapy is a useful therapeutic instrument when anti-inflammatory and analgesic effects are aimed at, as in the case of traumas or injuries occurring as a result of overstress in professional athletes. Given that thermal effect is absent in LLLT therapy, the question arises as to whether or not this also holds true for high-power MLS therapy (average power 3.3W, peak power 3x25W). This is a question that this study seeks to answer.
Laser therapy induces an active hyperemia at the level of the irradiated tissues (increases blood vessel diameter and permeability). In order to test this hypothesis, skin temperature variations were measured by means of digital thermography. A group consisting of 20 healthy volunteers was analyzed and temperature of relevant areas was measured before and after exposure to laser radiation at time 0 as well as at 30, 60 and 90 minutes of irradiation. In each of these moments, the temperature gradient between the area concerned and the contralateral healthy one was measured, as well as the temperature variation for the two areas (the difference between momentary and initial temperature) and the difference between the two.

Material and Method

The group comprises 20 people, of which 12 women and 8 men, with an average age of 29.8 years. The volunteers underwent laser therapy using Multiwave Locked System with an average power of 3300 mW, which combines two laser emissions with two wavelengths (808 and 905 nm) in continuous (808 nm, with a maximum power of 1W) and pulsed systems (905 nm, with a maximum power of 3x25 W). The irradiation parameters (power, time, frequency) complied with the programs corresponding to the respective periarticular soft tissue injuries (shoulder, elbow, fist, knee, insertion of Achilles tendon /plantar fascia). For the shoulder the energy of 5J/cm2 was used with an impulse frequency of 900 Hz, for 10 minutes. For the elbow, fist, knee and calcaneal region, the energy of 3J/cm2 was used with an impulse frequency of 700 Hz, for 7 minutes. Skin temperature was measured before and after laser therapy, at 30, 60 and 90 minutes from irradiation, both of the affected area and of the healthy contralateral area. Furthermore, was calculated the temperature variations in these areas.

Results and Discussions

The exposure to laser radiation led to a higher skin temperature of the exposed area, but also of the contralateral one. This rise in temperature was initially lower in the shoulder, elbow, fist and knee, reaching its peak value after 30 and 60 minutes, after which it started to drop. At shoulder-level, the temperature value had an identical evolution immediately after exposure to laser radiation, both at the level of the irradiated and the contralateral areas. However, within 30, 60 and 90 minute intervals, the rise in temperature was higher in the irradiated area. Moreover, the highest temperature was reached after 30 minutes, after which the temperature began to drop, without, however, going below the initial values, not even after 90 minutes.
If we analyze the time evolution of the difference between the rise in temperature at the level of the shoulder treated with MLS M6 laser and the rise in temperature in the contralateral shoulder, we notice a progressive increase between time 0 (immediately after ending treatment and the time 90 minutes, namely 90 minutes after ending treatment). Therefore, although a rise in temperature occurred in both areas, this rise is more significant in the irradiated area.

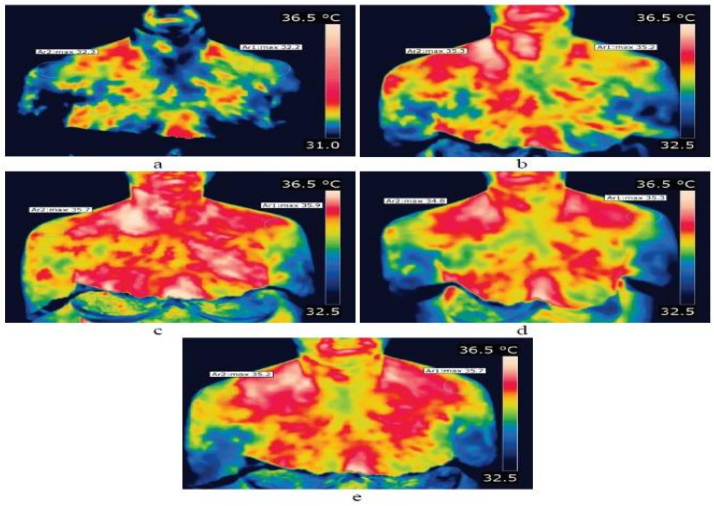


Figure 1. Skin temperature variations in shoulder after exposure to laser radiation (a-initial, b-at the end of exposure, c-after 30 minutes, d-after 60 minutes, e-after 90 minutes). A1 is the laser irradiated area and A2 is the contralateral area acting as a control area.

At elbow-level, the value of local temperature rose slightly after performing laser therapy, the rise being similar both in the irradiated and the contralateral areas. A similar drop in temperature is likewise observed in both areas after 30 minutes. The temperature rose in both regions after 60 minutes, but this rise was most significant at the level of the treated area. The temperature dropped in both areas after 90 minutes, reaching approximately equal values.

Figure 3 shows an example of skin temperature variation at the place of insertion of the common extensor tendon of fist and fingers (lateral epicondyle). The measurements were carried out both before and after laser exposure, as well as within 30, 60 and 90 minute intervals. In this case, the maximum was reached after 60 minutes of irradiation, then the temperature began to drop. The rise in temperature was higher in the exposed area.

Figure 2. Skin temperature variations in the shoulder

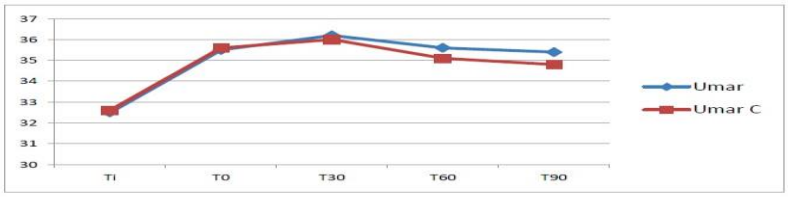


Figure 3. Skin temperature variations in elbow after exposure to laser radiation (a-initial, b-at the end of exposure, c-after 30 minutes, d-after 60 minutes, e-after 90 minutes). A1 is the laser irradiated area and A2 is the contralateral area acting as a control area.

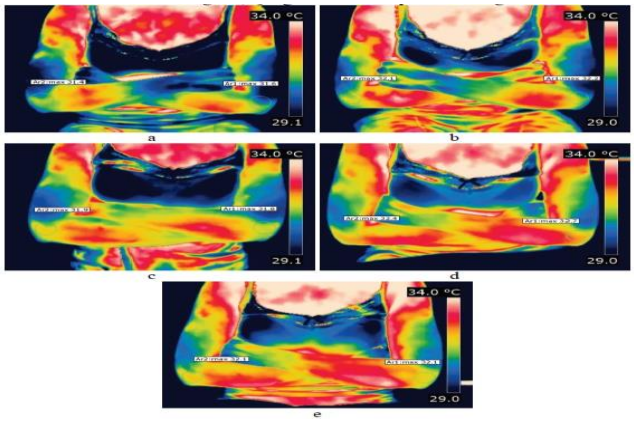
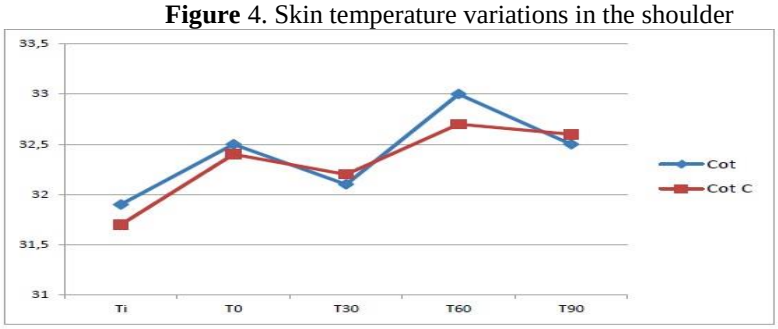


Figure 4. Skin temperature variations in the shoulder



A relatively equal rise in temperature could be observed in the hand, both in the irradiated area and in the contralateral areas, immediately after treatment and 30 minutes later. The temperature began to drop after 60 minutes, the fall being more significant in the non-irradiated area. The highest difference between temperature variations was recorded after 90 minutes.
Figure 5 illustrates an example of skin temperature variation in the hand (anatomical snuff box surface). The measurements were carried out both before and after laser exposure, as well as 30, 60 and 90 minutes later. The maximum value was reached immediately after irradiation, was maintained in plateau phase after 30 minutes, and then followed a downward trend. The rise in temperature was higher in the exposed area, and the cooling was slower.

Figure 5. Skin temperature variations in hand after exposure to laser radiation (a-initial, b-at the end of exposure, c-after 30 minutes, d-after 60 minutes, e-after 90 minutes). A1 is the laser irradiated area and A2 is the contralateral area acting as a control area

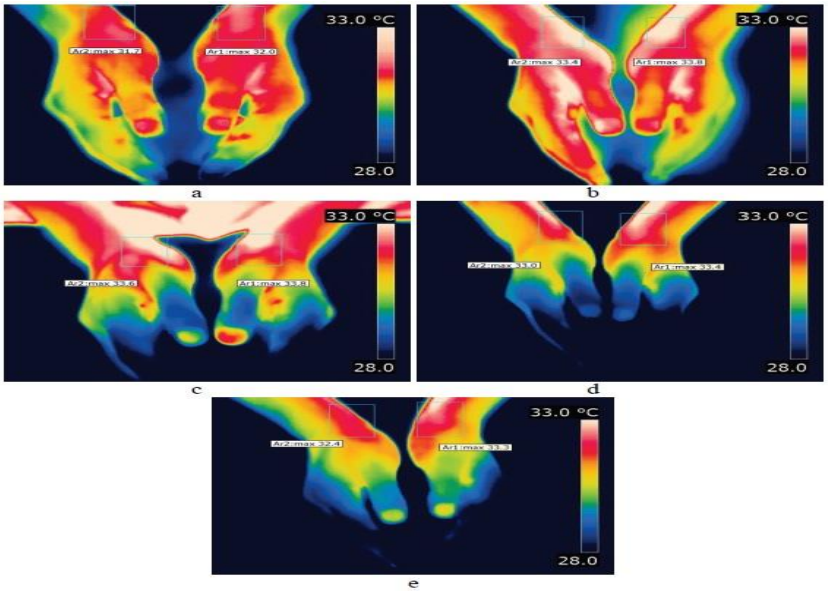
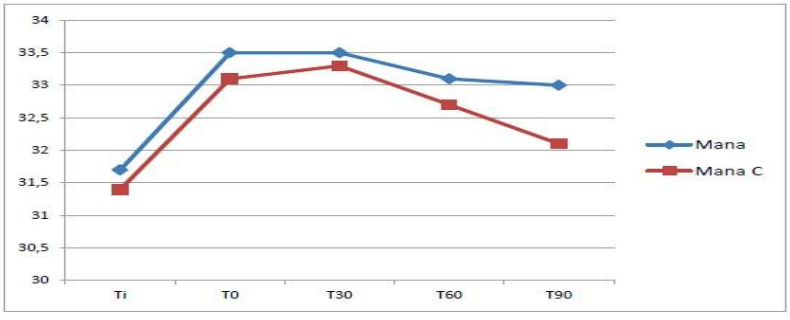


Figure 6. Skin temperature variations in hand (anatomical snuff box surface)



A relatively constant temperature value was maintained in the knee immediately after administering the treatment. A rise was observed after 30 minutes, both in the treated and the contralateral areas, the rise being more significant in the former. The temperature began to drop after 60 minutes, reaching values similar to those prior to the treatment. 90 minutes later it even dropped below the initial values.
Figure 7 shows an example of skin temperature variation in the knee. The measurements were carried out both before and after laser exposure, as well as 30, 60 and 90 minutes later. The maximum value was reached 30 minutes of irradiation, after which the temperature followed a downward trend. The rise in temperature was more significant in the exposed area and the cooling was slower.

Figure 7. Skin temperature variations in knee after exposure to laser radiation (a-initial, b-at the end of exposure, c-after 30 minutes, d-after 60 minutes, e-after 90 minutes). A1 is the laser irradiated area and A2 is the contralateral area acting as a control area.

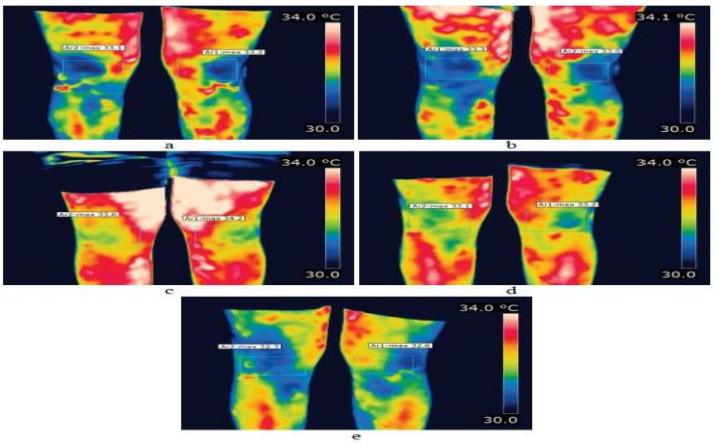
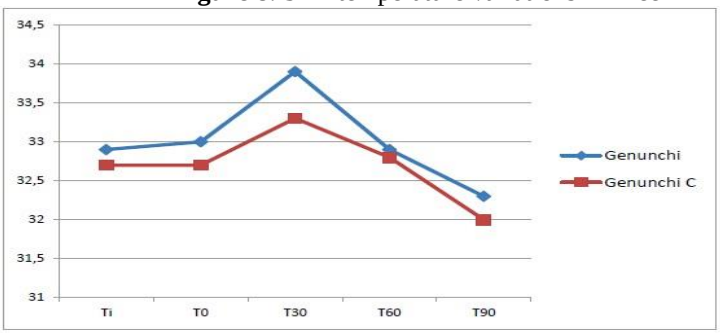


Figure 8. Skin temperature variations in knee



A paradoxical effect could be noticed in the calcaneal region (both in the insertion of the Achilles tendon and in the insertion of the plantar fascia), where temperature values dropped after laser irradiation. The drop was progressive in the insertion of Achilles tendon up to 60 minutes of therapy, when the minimum temperature was reached, and it was only slightly more significant in the region undergoing laser irradiation. The beginning of "reheating" was observed after 90 minutes, the temperature remaining lower in the irradiated area compared with the contralateral one.

Figure 9. Skin temperature variations at the level of the insertion of Achilles tendon after exposure to laser radiation (a-initial, b-at the end of exposure, c-after 30 minutes, d-after 60 minutes, e-after 90 minutes). A1 is the laser irradiated area and A2 is the contralateral area acting as a control area.

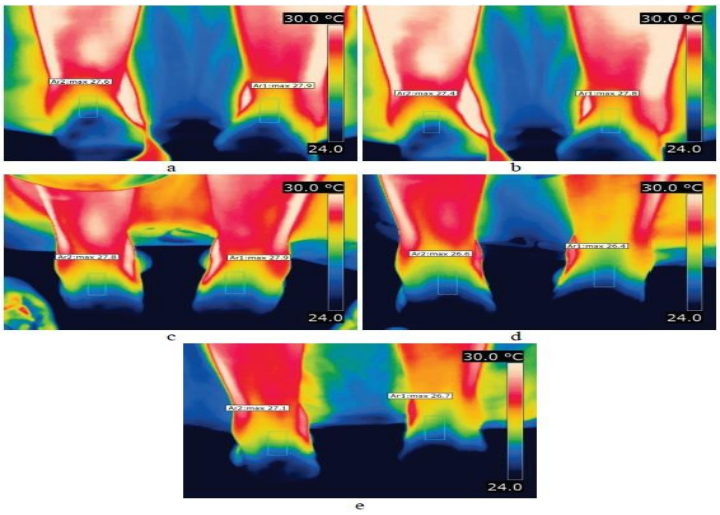
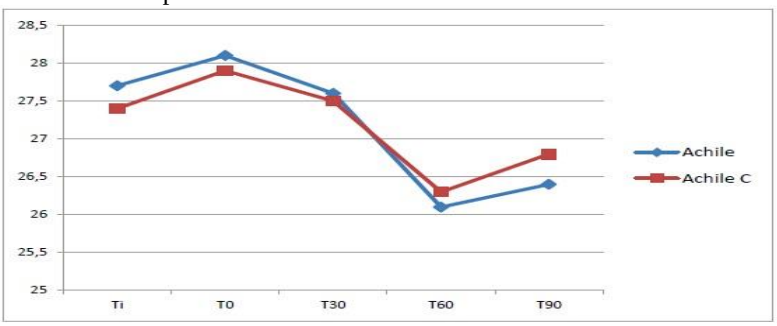


Figure 10. Skin temperature variations at the level of the insertion of Achilles tendon



A drop in temperature was recorded at the insertion of the plantar fascia up to 30 minutes of exposure, both in the treated and the contralateral areas, after which the temperature began to rise in both areas. However, reheating was quicker in the area that did not undergo laser irradiation.
Figure 11 presents an example of skin temperature variation at the level of the insertion of the plantar fascia. The measurements were carried out both before and after laser exposure, as well as 30, 60 and 90 minutes later. The minimum value was reached 30 minutes of irradiation, and the same level was maintained up to 60 minutes of the therapy, then the temperature began to slightly rise. The drop in temperature was more significant in the exposed area, and reheating was slower.

Figure 11. Skin temperature variations at the level of insertion of plantar fascia after exposure to laser radiation (a-initial, b-at the end of exposure, c-after 30 minutes, d-after 60 minutes, e-after 90 minutes). A1 is the laser irradiated area and A2 is the contralateral area acting as a control area.

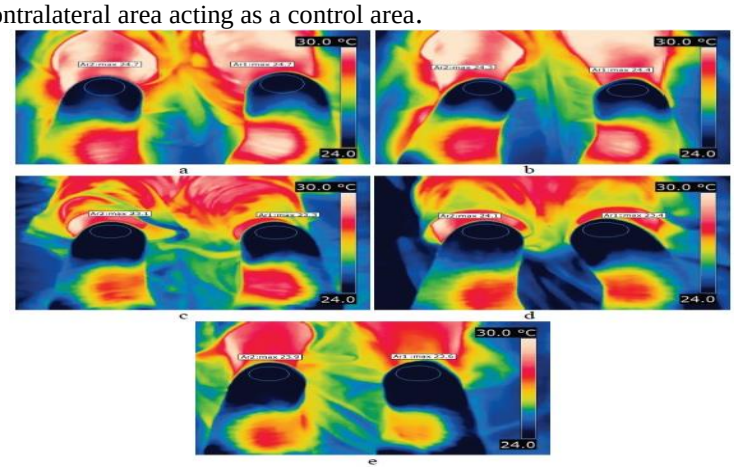
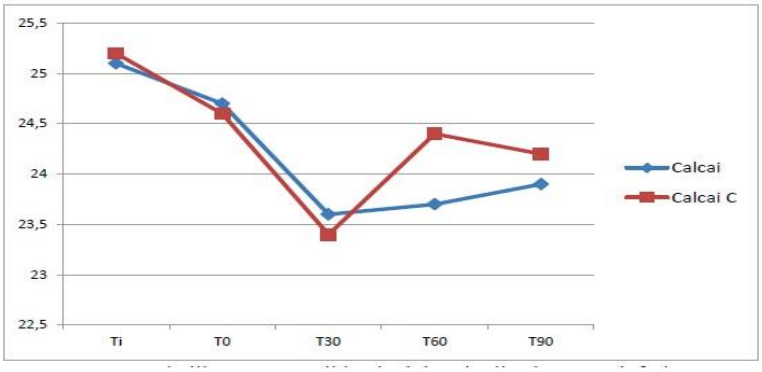


Figure 12. Skin temperature variations at the level of the insertion of plantar fascia



At the level of the palmar surface of the fingers, a slight rise in temperature was recorded up to 30 minutes of exposure, both in the treated and the contralateral areas, after which the temperature began to drop in both areas. Figure 13 presents an example of skin temperature variation at the level of the palmar surface of the fingers. The measurements were carried out both before and after exposure to laser, as well as 30, 60 and 90 minutes later. The maximum value was reached 30 minutes of irradiation, after which the temperature dropped significantly. 60 and 90 minutes of irradiation, the values were inferior to the initial ones.

Figure 13. Skin temperature variations of the palmar surface of the fingers after exposure to laser radiation (a-initial, b-at the end of exposure, c-after 30 minutes, d-after 60 minutes, e-after 90 minutes). A1 is the laser irradiated area and A2 is the contralateral area acting as a control area.

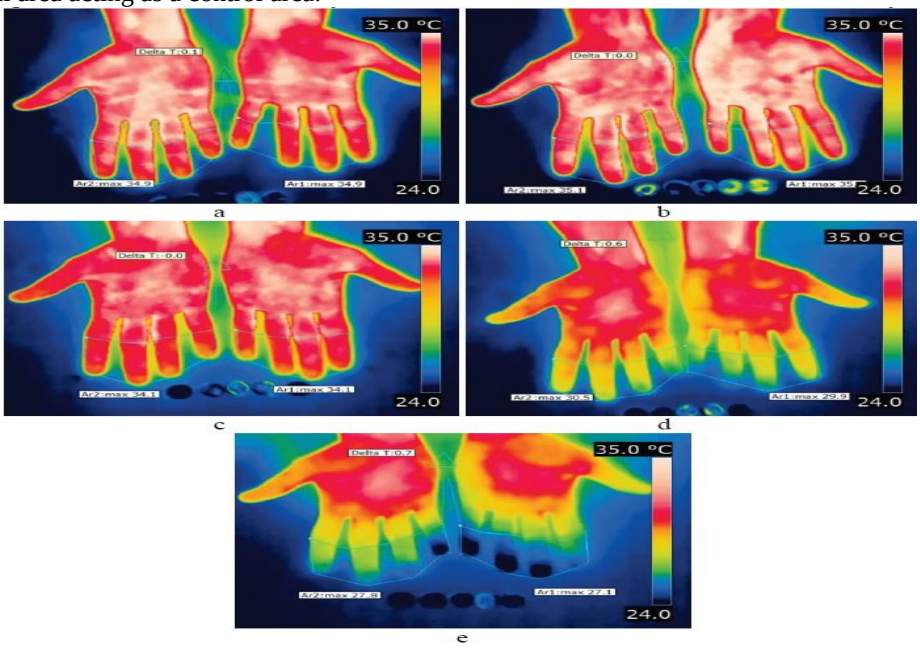
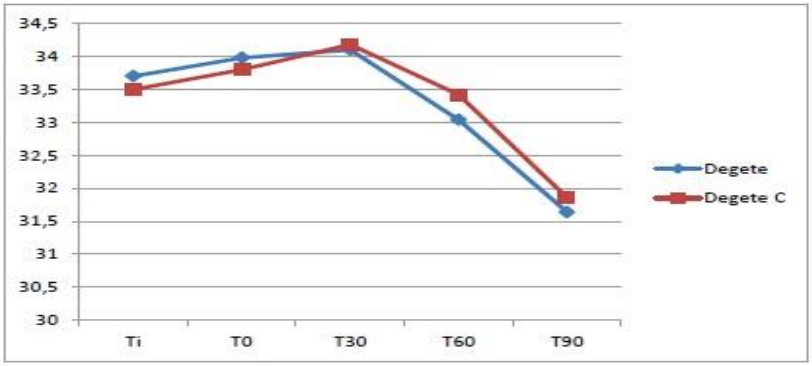


Figure 14. Skin temperature variations at the level of the palmar surface of fingers



For all the areas studied, temperature variations could be observed after exposure to radiation both in the treated and the contralateral areas. These variations have the same trend (either increasing or decreasing) for the same anatomical area, treated or not, with different magnitudes of variation (higher for the exposed areas). The maximum value of temperature variations was reached 30-60 minutes after the therapy was performed, suggesting the fact that even though energy transfer is important in the effects of MLS laser therapy (the peak power for the pulsed emission of 3x25W), it is not the photothermal effect of laser that is involved in the process (in which case the highest temperature variations would have been recorded immediately after the ending exposure), but the photochemical effect, which determines the formation of mediators with vasodilatory effects (possibly NO obtained by dissociation from cytochrome oxidase and by the photolysis of the nitrosyl complex of hemoglobin, myoglobin). The formation of these vasodilator mediators can explain both the emergence of maximum thermal variations within 30-60 minutes of exposure, as well as the occurrence of thermal variations in non-irradiated areas as well.

One of the primary mechanisms of action of laser radiation is the photolysis of metal-protein complexes with NO. Soon after this hypothesis was formulated, the photosensitivity of complexes formed by NO with hemoglobin and cytochrome c was demonstrated. EPR and spectrophotometric methods were used. It was found that the EPR signals of NO-hemoglobin complexes decrease under the action of laser radiation. NO is released simultaneously, a fact which was determined by the increase of EPR signals of NO. Under the influence of laser light, the amplitude of EPR signals decreases, at a directly proportional rate to the intensity of the radiation. As it has been demonstrated experimentally, NO is also released. Thus, the irradiation of the nitrosyl complexes of cytochrome c determines the photolysis of the complexes and the release of NO.

In the absence of light, mitochondrial respiration is partially suppressed by the NO produced by the mitochondrial NO-synthase. NO inhibits respiration by binding to electron carriers such as cytochrome, cytochrome oxidase and possibly Fe-S complexes. Intense light irradiation determines the photolysis of these complexes, as well as the restoration of respiration and of the ATP synthesis (18).

High concentrations of nitric oxide can be sometimes determined in cell cultures, due to the release of NO by the mitochondria and cytochrome oxidase as a result of LLLT irradiation. It was assumed that LLLT acts through by photodissociating nitric oxide from the cytochrome oxidase, thus restoring the mitochondrial respiration inhibited by excessive concentrations of nitric oxide (24).

In addition to the photodissociation of nitric oxide by the cytochrome oxidase, as it has been described above, it can also be released from other intracellular sources, such as nitrosyl hemoglobin and nitrosyl myoglobin.(25) The light mediated vasodilation was first described in 1968 by R.F. Furchgott in his research on nitric oxide, for which he received the Nobel prize in 1998 (26). Further studies of other researchers have confirmed and extended Furchgott's work and have demonstrated that light influences the local production or the release of nitric oxide and stimulates vasodilation through the effect that nitric oxide has on GMPc.

In the calcaneal area, both in the insertion of Achilles tendon and in the insertion of the plantar fascia, a paradoxical cooling effect of the irradiated area, as well as of the contralateral area used as a control area, is obtained. It should be noted that the microcirculation of skin in this region has particular anatomic and physiological characteristics compared with other regions of the body. Due to the fact that this area, along with the hands and the ears, is exposed to temperature variations, it is rich in arteriovenous anastomoses, which are direct connections between arterioles and the venules of the deep dermal plexus. The arteriovenous anastomoses have thick, richly innervated muscular walls. They provide a low-resistance pathway for the transfer of blood from arterioles to venules for high flow rates, having a role in thermoregulation (27). Moreover, it was discovered that the diameter of arteriovenous anastomoses depends on the quantity of nitric oxide. Blocking the endogenous production of nitric oxide reduces the diameter both of arterioles and of venules, as well as that of arteriovenous anastomoses, having a significantly more pronounced effect in the latter case (28).

Therefore, through the production of vasoactive mediators, laser irradiation determines the deviation of blood flow in the calcaneal region by opening arteriovenous anastomoses from the capillary bed directly to the venous system, cooling this area as a result, as proven by performing serial thermograms.

Another area rich in arteriovenous anastomoses is the volar surface of the hands. In this case, the laser irradiation determined an initial minor rise in skin temperature, followed by a marked drop in temperature, with lower values recorded within 60 and 90 minutes of laser exposure than the initial values.

The chemically mediated vasodilation induced by exposure to laser radiation is involved in achieving the therapeutic effects of laser therapy. First of all, the active hyperemia obtained by the increased diameter and

the decreased permeability of lymphatic vessels and capillaries generate a washing out effect of inflammatory substances (histamines, bradykinin, cytokines and lymphokines) exerting an anti-inflammatory effect. Second, vasodilation increases the intake of oxygen and nutrients, which is essential for repairing damaged tissues - biostimulation effect. Third, the active hyperemia also determined by photochemical reactions promotes the drainage of algogenic substances, thus eliminating the cause of pain sensations - analgesic effect.
The observation that MLS laser therapy, which comes under the category of high-power lasers, does not produce thermal effects is relevant to confirming both the safety profile of this therapy, which does not produce skin lesions in its application, as well as its therapeutic use. Therefore, in the absence of the heating effect of irradiated tissues, laser irradiation can also be used in proximity to metallic parts (osteosynthesis materials) used in orthopaedic surgical procedures.

Conclusions

Laser therapy determines changes in skin temperature both in the irradiated area, as well as in the contralateral (control) area, therefore laser irradiation has a systemic effect.
The amplitude and the (increasing or decreasing) trend of such changes depends on the physiological characteristics of the tissues in the areas concerned: in proximal areas there is a rise in local temperature, while in the distal areas there is a drop in temperature.
The maximum amplitude of temperature variation is recorded within 30-60 minutes of exposure to laser, suggesting that the photothermal effect is not involved in this phenomenon, despite the fact that MLS therapy determines a significant energy input, using both high frequencies and power (average power 3300 mW, peak power 3x25W).
The photochemical effect of the laser irradiation of tissues generates vasoactive chemical mediators responsible for temperature variations both in the exposed area, as well as in the contralateral area, acting as a control area.
In the areas with a particular vascularization, characterized by the abundance of arteriovenous anastomosis, a paradoxical drop in skin temperature both in the exposed area and in the contralateral area used as a witness is achieved. This phenomenon can also be explained by the appearance of vasoactive mediators which shunt the blood flow to the venous system by determining the vasodilatation of arteriovenous anastomoses.
Of the three main effects of laser, photothermal, photomechanical and photochemical, the one that is most deeply involved in obtaining the biological effect in case of MLS therapy is the photochemical effect.
Even though MLS therapy has a high peak power (3x25W), it does not produce a photothermal effect, a fact which was demonstrated by the initial reduction of local temperature after irradiation in certain areas (heel).

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