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Evaluation of Thermal Variation of Skin Wounds In Rats Subjected To Biomodulatory Therapies

Thermographic Study of the Nasolabial Fold Region ofWomenSubmitted To Filling With Hyaluronic Acid

XVI EAT Congress Announcement

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In general, manuscripts hould be organized as follows: Introduction, methods, results, discussion, acknowledgements, references. A short abstract in English and, if possible, German (translation will be offered) should head the manuscript. Following the abstract, up to 5 key-words should characterize the paper.

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Gautherie M, HaehnelP, Walter JM, Keith L. Long-Term assessment of Breast Cancer Risk by Liquid Crystal Thermal Imaging. In: Gautherie M, Albert E, editors. Biomedical Thermology. New York Alan R.Liss Publ; 1982. p. 279-301.

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References:

[1] International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Medical Education 1999; 33; 066-078

[2] www.consort-statement.org

[3]www.strobe-statement.org

[4]www.prisma-statement.org

[5] www.stard-statement.org

[6] www.care-statement.org

[7] www.spirit-statement.org

[8]www.equator-network.org/wp-content/uploads/2013/03/SAMPL-Guidelines-3-1

[9]Moreira DG et al. Thermographic imaging in sports and exercise medicine: a Delphi study and consensus statement on the measurement of human skin temperatur. J Thermol Biol 2017, 69: 155-162

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Contents

Evaluation of Thermal Variation of Skin Wounds In Rats Subjected To Biomodulatory Therapies

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SUMMARY

INTRODUCTION: Biomodulatory therapies able to help tissue repair such as laser photobiomodulation and ozone therapy, have aroused the interest of many scientific communities. Infrared thermography is a diagnostic tool adept for verifying dysfunctions resulting from the healing process, especially when it is undergone to the influence of different therapeutic resources.

AIMS: To evaluate the effects of laser photobiomodulation, ozone gas and ozonized oil on the thermal variation of tissue in skin wounds in rats through thermographic analysis.

METHODS: This was an in vivo experimental study in which 40 male Wistar rats were submitted to cutaneous wounds to create standardized dorsal wounds using a circular scalpel of 6 mm diameter. The specimens were allocated in 4 groups containing 10 animals each. Three groups received treatment with laser photobiomodulation, ozone gas and ozonated oil, respectively. And were compared to the Control Group through thermograms of the wound area on the 5th and 10th post-operative day. The ANOVA test was used and the significance level was $p<0.05$.

RESULTS: A reduction in the average temperature in the wound area was observed in all experimental groups with greater variation of thermal coefficient in the treated groups ($p<0.05$). On the 5th day, Gas ozone and ozonated groups presented lower main temperature in comparison with control group and laser group (33.9°C±0.7, 34.7°C±0.4, respectively), as well as with thermal variation coefficient (ΔT= -1.8 and 1, respectively). On the 10th day, the thermographic averages of the basal temperature became closer, exception of the ozone gas group which maintained the average temperature higher at 35.1°C±0.4 ($p<0.05$) and higher ΔT (+1.8).

CONCLUSION: Infrared thermography proved to be effective for monitoring the thermal variation resulting from the inflammatory process, during cutaneous tissue repair undergone to the biomodulatory therapies using. Overall, with thermal imaging, ozone gas stood out from the other groups since it showed lower mean average temperatures during the inflammatory phase of the repair and also for raising the average temperature on the 10th study day for the remodeling phase.

KEYWORDS: Healing; ozone; Laser; Low power laser therapy; Infrared thermography.

BEWERTUNG DER THERMISCHEN VARIATION VON HAUTWUNDEN BEI RATTEN, DIE BIOMODULATORISCHEN THERAPIEN UNTERZOGEN WURDEN

EINLEITUNG: Biomodulatorische Therapien, die in der Lage sind, die Gewebereparatur zu unterstützen, wie z. B. die Laser-Photobiomodulation und die Ozontherapie, haben das Interesse vieler wissenschaftlicher Gemeinschaften geweckt. Die Infrarot-Thermografie ist ein diagnostisches Instrument, das geeignet ist, Funktionsstörungen zu überprüfen, die sich aus dem Heilungsprozess ergeben, insbesondere wenn dieser unter dem Einfluss verschiedener therapeutischer Maßnahmen erfolgt

ZIELE: Es sollten die Auswirkungen von Laser-Photobiomodulation, Ozongas und ozonisiertem Öl auf die thermische Variation von Gewebe in Hautwunden bei Ratten durch thermografische Analyse untersucht werden.

METHODEN: Hierbei handelte es sich um eine experimentelle In-vivo-Studie, in der 40 männliche Wistar-Ratten mit einem kreisförmigen Skalpell von 6 mm Durchmesser Hautwunden unterzogen wurden, um standardisiert Wunden am Rücken zu erzeugen. Die Proben wurden in 4 Gruppen mit je 10 Tieren eingeteilt. Drei Gruppen erhielten eine Behandlung mit Laser-Photobiomodulation, Ozongas bzw. ozonisiertem Öl. Diese wurden mit der Kontrollgruppe durch Thermogramme des Wundbereichs am 5. und 10. postoperativen Tag verglichen. Der ANOVA-Test wurde verwendet und das Signifikanzniveau betrug p<0,05

ERGEBNISSE: In allen Versuchsgruppen wurde eine Reduktion der mittleren Temperatur im Wundbereich beobachtet, wobei der thermische Koeffizient in den behandelten Gruppen stärker variierte (p<0,05). Am 5. Tag wiesen die Ozongas- und ozonisierten Gruppen im Vergleich zur Kontrollgruppe und der Lasergruppe (33,9 °C±0,7, 34,7 °C±0,4) sowie zum thermischen Variationskoeffizienten (ΔT= -1,8,bzw.1) eine niedrigere Haupttemperatur auf. Am 10. Tag näherten sich die thermographischen Mittelwerte der Basaltemperatur an, mit Ausnahme der Ozongasgruppe, welche die Durchschnittstemperatur mit 35,1 °C±0,4 (p<0,05) und einem höheren ΔT (+1,8) höher hielt.

SCHLUSSFOLGERUNG: Die Infrarot-Thermografie erwies sich als wirksam zur Überwachung der thermischen Schwankungen, die sich aus dem Entzündungsprozess während der Hautgewebereparatur während der biomodulatorischen Therapien ergeben. Insgesamt hob sich Ozongas mit Wärmebildaufnahmen von den anderen Gruppen ab, da es niedrigere mittlere Durchschnittstemperaturen während der Entzündungsphase der Reparatur und auch eine Erhöhung der Durchschnittstemperatur am 10. Untersuchungstag für die Umbauphase zeigte.

SCHLÜSSELWÖRTER: Heilung; Ozon; Laser; Lasertherapie mit geringer Leistung; Infrarot-Thermografie.

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Introduction

Biomodulatory therapies represented by laser photobiomodulation and ozone therapy have increasingly been investigated looking for obtaining standardized clinical protocols. Such therapies are able to induce analgesic, anti-inflammatory and tissue biomodulator effects that help to improve the healing pattern of different types of wounds [1-3].

With a wavelength that lies in the visible and invisible light spectrum, laser photobiomodulation positively impact on healing process through increased adenosine triphosphate (ATP) biosynthesis, collagen biosynthesis, lymphocyte proliferation and functional activity and number of fibroblasts [4,5]. Laser photobiomodulation may be characterized as triggering or inhibiting physiological, biochemical and metabolic processes through its photophysical or photochemical effects [6]. Such effects are interdependent and may vary according to the type of treated tissue, the fluence and irradiance of laser, as well as the time and intervals of application designed for the patient [6,7].

Ozone therapy has a therapeutic action based on activating redox mechanisms, protein synthesis and increasing the number of ribosomes and mitochondria in cells [8,9]. Thus, changes at the cellular level explain the increase in functional activity and regenerative potential of tissues and organs submitted to ozone treatment [10,11].

During the wound healing process, the microcirculation in the wound bed undergoes a complex change. In the first days, there is a predominance of the acute inflammatory phase which results in important hemodynamic shift with intense exudation and subsequent vascular proliferation [12,13]. Currently, an image resource has been used to assess the thermal coefficient variation resulting from the increase of circulatory pattern in a given tissue - infrared thermography [14]. This diagnostic tool captures the image with a special camera that maps the patient's body and transforms the detection of infrared emission from each anatomical site into temperature [15]. It was introduced as a diagnostic method in Medicine since 1960 [16]. However, with the advancement of technology in data collection, transfer and temperature measurement, it has significantly evolved in terms of sensitivity, specificity and image resolution [17]. This non-invasive, irradiation-free, non-contact and painless technique does not provide information on morphological characteristics, but on functional, thermal and vascular changes in the tissue [14, 18-21].

It might be applied in the diagnosis of different diseases such as dermatitis, vascular dysfunctions, lesions in sensitive nerves, inflammatory processes and also in the monitoring of breast cancer [22-24]. However, there are still no studies which have comparatively documented the thermal coefficient variation in different phases of wound healing, especially when these are undergone to the action from the biomodulatory therapies already described.

The aim of the present study was to evaluate the effects of 670 nm laser photobiomodulation, ozone gas and ozonated oil on the pattern of thermal variation in the skin tissue of rats during repair, using infrared thermography.

Material and methods

Experimental study using an animal model

This study was forwarded to the Commission for Ethics in Animal Use (CEUA) with register number 67/2019 and obtained a favorable opinion. The sample included 40 male Wistar rats with an average weight of 250 grams (g). The rats were housed in specific plastic cages in groups of five, with good lighting conditions (light/dark cycle of 12/12 h) under a temperature of ± 22-24 °C, and were fed a commercial diet (Nuvilab, Quimtia, Colombo-PR, Brazil) and water ad libitum.

Surgical procedure

The animals were weighed, anesthetized with 10% ketamine hydrochloride (Dopalen®, Sao Paulo, Brazil) 75 mg/ml and 2% xylazine hydrochloride (Anazedan, Sao Paulo, Brazil) 5 mg/ml, with dosages of 2mg/kg and 3mg/kg respectively. After verified the loss of flexion reflex obtained from the paw, dorsal trichotomy and antisepsis with povidine iodine (Rioquímica, São Paulo, Brazil) were performed. Then, a circular incision was made with the aid of a metallic scalpel, (Biopsy circular scalpel, Stiefel, Germany) with 6 mm in diameter to obtain a uniform and standardized wound, which was performed by a single duly calibrated operator.

Experimental groups

A sample size calculation was carried out to determine what was n required for the present study. Therefore, statistical significance levels of $p<0.05$ and a coefficient of variability of 20% were used, based on studies with a similar design to the present research that found an increase in collagen (primary outcome) greater than 30%. Animals were randomly allocated into 4 experimental groups of 10 rats each, as described below and were sacrificed on the 5th and 10th days (20 animals in each period) after beginning the tests.

Group 1 - Control Group (CG): did not receive any type of treatment.

Group 2 - Laser Group (LG): rats were subjected to 4 punctual applications of 1 J/cm2, with a total dosimetry of 4 J/cm2, per day of application. It was used an aluminum and gallium arsenide laser semiconductor device (AlGaAs, 9 mW, 670 nm, 0.031 W/cm2 diode laser) with continuous emission and active tip area of 0.28 cm2 (VR-Laser), KC-610- Dentoflex, Brazil). The final dosimetry was 12 J/cm2 since the rats were irradiated for 3 consecutive days after the surgical procedure.

Group 3 - Ozone Gas Group (GG): rats received gaseous ozone therapy through subepithelial insufflation. The ozone was produced by the Philozon® generator (Philozon - Indústria e Comércio de ozone generators - LTDA, Santa Catarina, Brazil) at a concentration of 13?g/mL of ozone, from medical oxygen, with a constant flow of 1L/min. The mixture with oxygen was captured in a 5mL syringe and by using an insulin needle, the gas was insufflated at the edge of the lesion with a volume of 1mL in each application. This procedure was repeated for three consecutive days after the surgery.

Group 4 - Ozonated Oil (OG): rats was treated with 100% ozonized sunflower oil at 100% concentration (Philozon - Indústria e Comércio de Geradores de Ozone - LTDA, Santa Catarina, Brazil) dripped onto the lesion surface. For three consecutive days 50 µl was applied to the surface of the lesion in the same manner as in the previous groups.

The animals were submitted to biomodulatory therapies on days 1, 2 and 3 of the study. Each group with 10 animals had half of them sacrificed on the 5th day and the other half on the 10th day.

Infrared Thermography evaluation

The infrared camera used was the FLIR T430sc, focal plane array 320×240 pixel and image frequency of 30 Hz. The equipment has an 18 mm FOL lens, carries out studies in the temperature range of - 40 °C to 650°C, had an emissivity set to 0.98 and thermal sensitivity (NETD.) less than 30mK. It operates in the spectral range of electromagnetic waves between 7.5 and 13?m. The accuracy of the equipment is ±1°C or ±1% (limited range) ±2°C or 2%, whichever is greater, at 25°C nominal. Also, the time between switching the camera on and capturing the images was 15 minutes. The calibration of the camera is carried out using radiation sources that are traceable to International Standards at the Rede Brasileira de Calibração (Brazilia National Calibraton Satndards) FLIR Systems Brazil, Sorocaba, São Paulo, Brazil.

To obtain a pattern of images, the following protocol was adopted: the rats were placed in an orthostatic position on a fixed object 0.5 m in relation to the ground and acclimatized for a minimum period of 15 minutes before data collection. The camera distance from the animals was 0.5 m, and the temperature of the environment was controlled at around 20°C ± 1°C".

On the first day, rats were photographed at the following times: 10 minutes after shaving; immediately after the surgery and, immediately after therapy (except the control group). On the fifth and tenth days, animals of each group were anesthetized again and photographed with the thermographic camera 10 minutes after anesthesia. The normality and abnormality criteria adopted were based on studies by Uematsu [25,26], in which the pattern of clinical abnormality was established according to temperature variation. If the coefficient of thermal variation is greater than 0.3 the change is considered as significant.

Statistical analysis

To identify the association between the thermal variables, a database was created in Microsoft® Excel® 2010 (version 14.0.7132.5000), Microsoft® Office Professional Plus 2010, USA and analyzed in the R software (version 3.1.1). Data distribution for normality was tested using the Shapiro-Wilk test. The ANOVA test was used followed by the post hoc Bonferroni test. The significance level was $p<0.05$.

Results

The mean basal temperature of the rats was measured with a mercury thermometer introduced into the rectum and recorded at 33.2°C. Table 1 illustrates the temperature values of the animals at different moments during experiment. It was noticed the mean basal temperature of all rats was 33.2°C. After shaving, there was a raise in mean basal temperature to 34.3°C in the 40 animals participating of the experiment ($p<0.05$), with Delta T (ΔT) of +1.1°C. Right after the surgical procedure, there was a decrease in temperature in all rats, with a average of 33.7°C and delta T of -0.6°C ($p>0.05$).

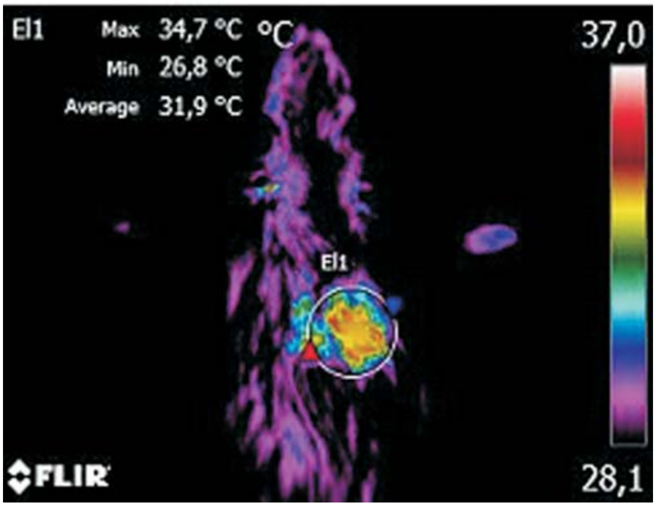

Figure 1:
Control Group (CG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

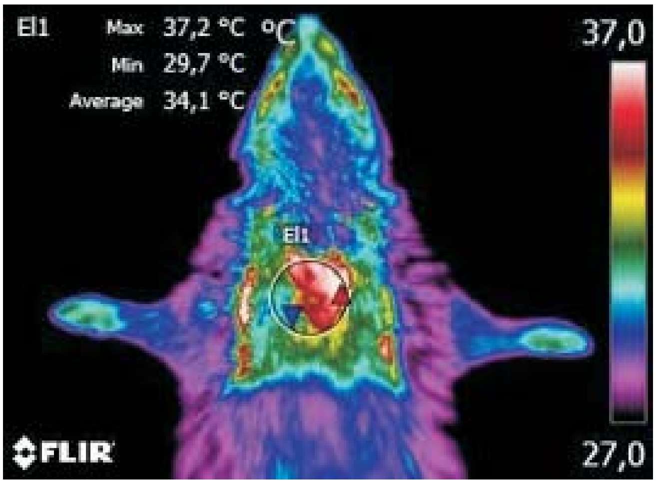

Figure 2
Gas Group (GG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

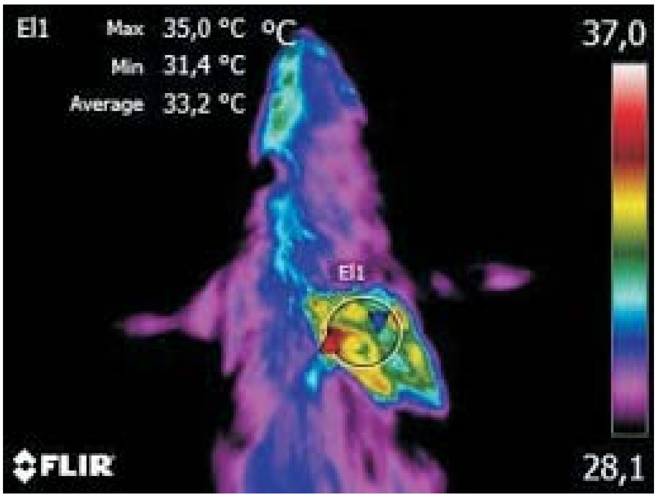

Figure 5:
Control Group (CG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days.

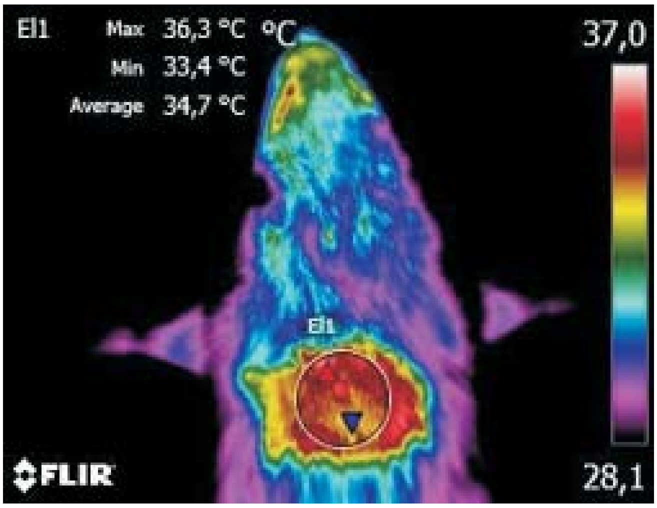

Figure 6
Gas Group (GG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days.

Table 1:
Mean temperature with standard deviation of the experimental groups in different periods of the study.

EXPERIMENTAL GROUPS	**MEAN TEMPERATURE IN °C AND STANDARD DEVIATION**			
	POST SKIN INCISION	POST 1st THERAPY SESSION	5 DAYS	10 DAYS
CONTROL GROUP (CG)	33.7^{A}±0.8	-	35.7^{B}±1.0	33.3^{C}±1.1
OZONE GAS GROUP (GG)	33.7^{A} ±0.8	33.1^{D}±0.4	33.9^{E}±0.7	35.1^{F}±0.4
OZONATED OIL (OG)	33.7^{A} ±0.8	31.1^{G}±0.7	34.7^{H}±0.4	33.3^{I}±2.2
LASER GROUP (LG)	33.7^{A} ±0.8	32.7^{J}±0.6	35^{L}±0.7	33.7^{M}±1.3

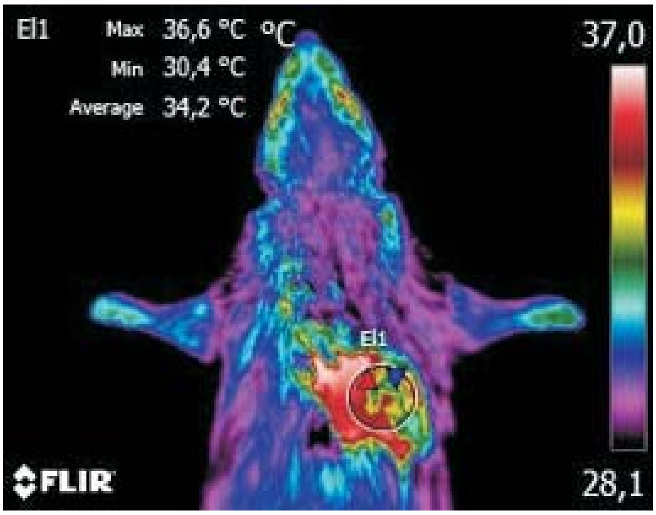

Figure 3
Ozone Group (OG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

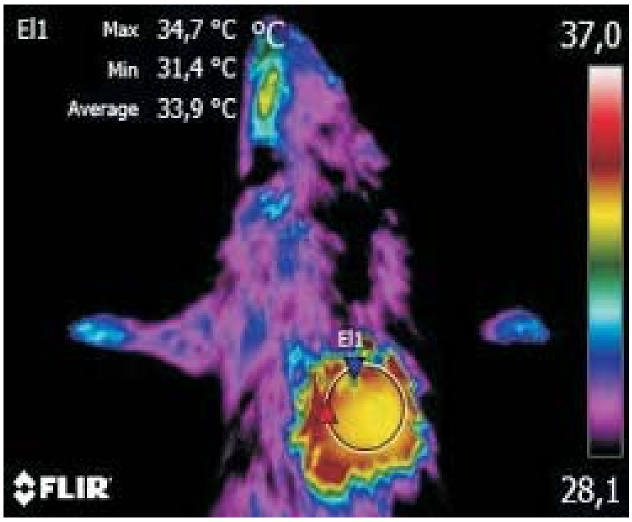

Figure 7
Ozone Group (OG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days

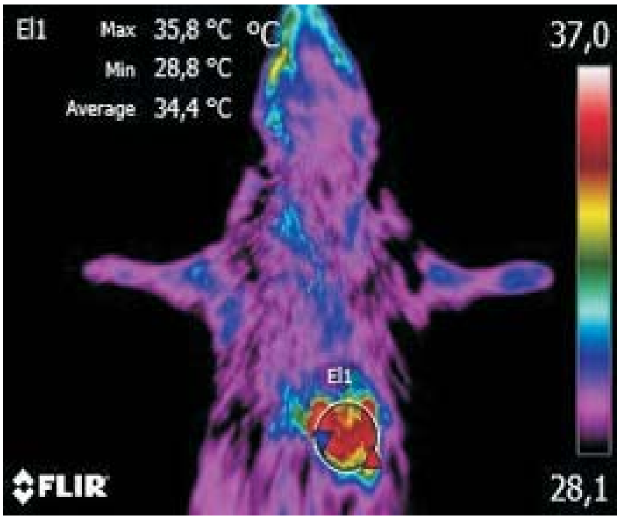

Figure 4
Laser Group (LG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

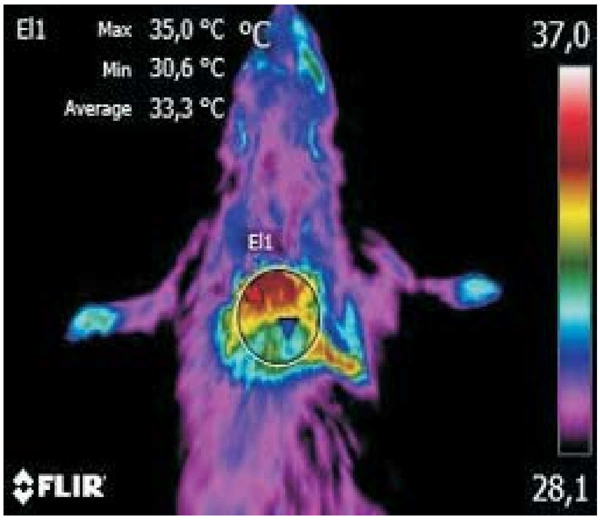

Figure 8
Laser Group (LG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days.

Table 2:
Intra and intergroup comparison of the thermal variation coefficient in the two periods of analysis.

EXPERI-MENTAL GROUPS	**MEAN TEMPERATURE in °C**											
	Post 1st Therapy Session				**5 days**				**10 days**			
	CG	**GG**	**OG**	**LG**	**CG**	**GG**	**OG**	**LG**	**CG**	**GG**	**OG**	**LG**
CONTROL GROUP (CG)	-	-0.6	-2.6*	-1.0*	-	-1.8*	-1.0*	-0.7*	-	+1.8*	0.0	+0.4
OZONE GAS GROUP (GG)	-0.6	-	-2.0*	-1.6*	-1.8*	-	+0.8	+1.1*	+1.8*	-	-1.8*	-1.4*
OZONATED OIL (OG)	-2.6*	-2.0*	-	-1.6*	-1.0*	+0.8	-	+0.3	0.0	-1.8*	-	+0.4
LASER GROUP (LG)	-1.0*	0.4	-1.6*	-	-0.7*	+1.1*	+0.3	-	+0.4	-1.4*	+0.4	-

*ANOVA, statistically significant difference ($p<0.05$) between CG/GG, CG/OG, CG/LG, GG/OG, GG/LG, OG/GG e OG/LG, just after fist therapy session, after 5 days, and after 10 days.

Once it was defined that the mean temperature to be adopted as a basis for the evaluation of biomodulatory therapies corresponded to that calculated after cutaneous surgery, the results of each experimental group are described below.

A. Control Group

At the end of the 5th day, the mean temperature was 35.7°C, with a temperature increase of around +2.0°C (ΔT=+2.0°C; $p<0.05$). On the 10th day, the average temperature was 33.3°C. The thermal coefficient variation was negative in this group with ΔT of -2.4°C ($p<0.05$); (Figures 1 and 5).

B. Ozone Gas Group

It was found that immediately after the use of ozone gas, the average temperature of the treated animals dropped to 33.1°C, with a ?T of -0.6°C ($p<0.05$). At the end of the 5th day, the mean temperature was 33.9°C, with a temperature increase of around +0.8°C ($p>0.05$). Regarding this group, around the 10th day the mean temperature was 35.1°C. The variation of the thermal coefficient was ascending in this group, for this period (ΔT =+1.2°C; $p<0.05$); (Figures 2 and 6).

C. Ozonated Oil Group

It was verified that soon after using the ozonized oil, the average local temperature of the treated animals dropped to 31.1°C, with a delta T of -2.6°C ($p<0.05$). At the end of the fifth day, the average temperature was 34.7°C with a temperature increase of around +3.6°C ($p<0.05$). Around the 10th day, in the group treated with ozonized oil the mean temperature was 33.3°C. The thermal coefficient variation was negative (ΔT =-1.4°C; $p<0.05$); (Figures 3 and 7).

D. Laser Photobiomodulation Group

In this experimental group, it was noticed that the use of laser photobiomodulation right after the surgical procedure promoted a decrease in mean temperature compared to the postoperative period of around 32.7°C, with a delta T of -1.0°C ($p<0.05$). On the 5th day after starting therapy, the mean temperature at the wound site rose to 35°C, with a delta T of +2.3°C. Ten days after the initial procedures, the average temperature was 33.7°C (ΔT =-1.3°C; $p<0.05$); (Figures 4 and 8).

Table 1 comparatively illustrates the coefficients of thermal variation (ΔT) of the experimental groups in the different periods of the study. It was observed that in the immediate period after carrying out the 3 biomodulatory therapies, all animals showed negative delta T in relation to the Control Group (GG ΔT=-0.6°C; OG ΔT=-2.6°C and LG ΔT =-1.0°C); ($p<0.05$).

Five days after therapies has started, when the mean temperatures obtained in the experimental groups were compared to those in the Control Group, it was found that thermal coefficient variation was significantly negative for all treated groups, with the highest ΔT observed between the CG and the GG (CG/GG ΔT=-1.8°C; CG/OO ΔT=-1.0°C and CG/LΓ ΔT=-0.7°C); ($p<0.05$); (Table 2).

On the 10th day, it was verified that the mean temperatures of the OG and LG groups were close to those of the Control Group, and the delta T showed a few variation (CG/OG ΔT=0.0°C; CG/LG ΔT=-0.4 °C); ($p>0.05$). The Ozone Gas group exhibited an increase in mean temperature in relation to that of the control group and ascending delta T, with a statistically significant difference (CG/GG ΔT=+1.8°C); ($p<0.05$); (Table 2).

Discussion

This study was the first to document the pattern of thermal variation under the light of infrared thermography in the area corresponding to experimentally induced cutaneous wounds in rats, submitted to biomodulatory therapies with 670 nm laser photobiomodulation, ozone gas and ozonated oil. The effects induced by such therapeutic agents in different types of tissue have been described as potentially anti-inflammatory, analgesic and tissue repair stimulators [27-29]. On the other hand, infrared thermography utilization in experimental healing models has also been explored [30]; however, its use for chronological assessment of the thermal gradient observed during tissue repair associated with biomodulatory therapies is still scarce in the literature.

Infrared thermography is an imaging test that was first introduced in medicine by Lawson in 1956, initially intended for the auxiliary diagnosis of breast cancer [19]. The literature assures that thermograms provide information about possible dysfunctions and, therefore, could help in the diagnosis of pathological states [16]. With a camera containing infrared detectors, the thermogram tracks the thermal coefficients on the body surface defined from the heat dissipation. Thermal variation may be related to circulatory factors and suffer alterations due to variations in blood flow and volume [19,22,31]. Vascular changes registered by the thermogram in an injured region may help to identify the probable origin of pain. Infrared thermography is a technique that provides real-time images of the temperature of a surface, in a non-invasive, non-radioactive and painless way. Therefore, has no restrictions on its use, despite its applicability limitations on morphological characteristics [18,23,32,33].

In the present study, a significant boost in local temperature was observed through Infrared thermography in all rats shortly after the shaving procedure, which represented a physical injury that triggered inflammation of the subcutaneous tissue. It is known that changes occur in the physical-chemical composition of the tissue in the lesion microenvironment such as low oxygen tension, pH reduction, presence of reactive nitrogen and oxygen species. These reactions trigger the biosynthesis of chemical mediators that activate the cells involved in the tissue repair process, as well as the vasodilation of the capillary net [34].

Although trichotomy has caused a significant increase in temperature in all rats included in the study, thermograms were only performed after the required time for thermal equalization, which was 10 minutes. As all animals were submitted to the same hair removal procedures and surgical wound induction, it is believed that the thermal variation patterns found in this study may really be attributed to the biomodulatory used therapies.

As well as in other models that evaluated tissue repair, in this experimental model of cutaneous healing, the first events that occur in the injured tissue are vasoconstriction, in an attempt to contain bleeding and increased vascular permeability. It contributes to the transmigration of leukocytes to the wound microenvironment [35-37]. On the first day treating with biomodulatory therapies, there was a decline in mean temperatures regarding wound areas of the treated groups, with greater significance in the OG (Ozonated Oil Group). In addition to a possible anti-inflammatory effect, it is important to highlight that this apparent difference in OG may also be explained by the fact that the ozonized oil was kept cold to ensure the maintenance of ozone stability [29]. It is likely that the temperature equalization time for using the oil was insufficient for it to reach a temperature similar to that of the environment. GG and LG also showed a temperature reduction of 0.6°C and 1.0°C, respectively. The immediate anti-inflammatory action induced by these two biomodulatory therapies in the tissue has been described in the literature and may justify this result [38,39].

On the 5th day of the experiment, a growth in temperature was observed in all groups, especially in the CG. In this stage of tissue repair there is an increase in cell migration, extravasation of serum molecules, antibodies and proteins through the capillaries, which corresponds to the transition from the exudative to the proliferative phase in the connective tissue. This scenario it was possible by the increase in blood supply and vascular permeability [35]. This fact is essential to the formation of granulation tissue, responsible for new cells nutrition in the region under repair [13]. Although these tissue changes were not analyzed in this study, it might be noticed that, in the light of infrared thermo.-graphy, Delta T was lower in the groups submitted to biomodulatory therapies. There are two possible explanations for this thermographic finding. One of them is supported by the documented anti-inflammatory action of laser photobiomodulation and ozone [40]. Marchionni et al., in 2010 [27], demonstrated that low power laser irradiation (670nm) on the 5th day of healing was able to induce a lower degree of exudative inflammation, with a lower percentage of polymorphonuclear cells. Also, authors found that there was a greater presence of myofibroblasts and collagen biosynthesis as histological results showed faster healing for the 5-day period. Although this research focused only on thermoscopic and thermographic analysis, it is possible to suggest that in this phase of skin repair corresponding to the 5th day, the treated groups had already accelerated the healing process and proliferative phenomena were established in the tissue.

Thus, it could be hypothesized that there was a balance between the tissue biostimulating action and the potential anti-inflammatory effect of biomodulatory therapies. The present study has limitations since there is a need to correlate variations in thermal coefficients with morphological aspects. And it may be done through a histopathological study of the skin corresponding to cutaneous injuries to comparatively evaluate the temperature captured by the thermogram and tissue changes, such as the degree of inflammation and fibroblastic and endothelial proliferation.

In this study, the animals in the OG (Ozone Gas Group) were the ones that presented a temperature closer to the initial baseline, on the 5th postoperative day. In turn, the OG group also showed a lower result than the control and laser, although higher than the gas group. Based on studies that demonstrate the chronology of the repair process in rats, during this period, the healing process transits between granulation and collagen production to compensate for tissue loss. The modulation of the inflammatory response reflects the ability to reduce pro-inflammatory agents such as TNF-α, IL1-β and COX-2 in the lesion area, mediators related to the increase in temperature [30]. This fact could also explain the lower temperatures in the groups submitted to biomodulatory therapies shown in the 5th. day. Some research studies have shown that ozonized oil is efficient in modulating the inflammatory process, stimulating angiogenesis and promoting enzymatic reactions that favor oxygen metabolism, in order to improve skin wound healing [29,41]. In the present study, it was observed that the ozonized oil was able to change the thermal pattern in the wound area showing signals of better healing results. These quantitative and qualitative specifications could be better observed from the morphological study of the injured and treated areas in the study.

On the 10th day of the study, it was verified that the Control Group, oil and laser, registered temperatures close to the initial average temperature of the animals, basal temperature. This information indicates that in this period, the wounds of these groups of animals were already in an advanced healing process. However, Ozonated Gas Group showed a higher temperature value when compared to the other groups. There are reports that ozone produces systemic biological effects and not just local ones, and that it is able of interfering with cell activity for a long period of time, despite having a rapid initial reaction in tissues [42, 43].

The present research strongly suggests that using thermograms might be useful to qualitatively and quantitatively monitor the pattern of microcirculation during tissue repair, in a non-invasive and painless way. Additionally, it proved the action of the biomodulatory therapies applied in the skin tissue subjected to physical injury, through the thermal coefficient variation.

It should be noted that this study has some limitations, since the specific analysis of only two repair periods could have been expanded. As matter of fact, the authors agree that it would be useful to study the early period of wound repair and do encourage the development of new studies in this area. Additionally, it is necessary to analyze the histopathological changes occurring in the animals' skin tissue to verify the occurrence of the biological processes already mentioned, such as neoangiogenesis, fibroblastic proliferation and collagen biosynthesis. Other limitation of the present study was the investigation of just one single dose of each therapy. It would be also important to study different application doses of the therapies, so that their effectiveness in establishing a safe protocol for using these technologies can be proven. Even so, it is also advisable to carry out similar studies in humans and comparative analyzes between the thermographic image and the histological characteristics observed in the tissue.

And finally, the authors also recommend the development of studies that evaluate the bactericidal potential of the biomodulatory therapies used to correlate it with thermoscopic and thermographic changes that can be documented by infrared thermography.

Conclusion

Infrared thermography proved to be an effective tool for capturing temperature variations during all stages of the research. It was possible to notice that the biomodulatory therapies applied caused vascular sensitive changes to the thermogram. Analyzing the images allowed concluding that there was a significant thermal variation in the groups tested with the therapies in comparison to the control one. Overall, with thermal imaging, ozone gas stood out from the other groups since it showed lower mean average temperatures during the inflammatory phase of the repair and also for raising the average temperature on the 10th study day for the remodeling phase.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Thermographic Study of the Nasolabial Fold Region of Women Submitted To Filling With Hyaluronic Acid

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SUMMARY

INTRODUCTION: The increase in the incidence of complications caused by the hyaluronic acid (HA) facial fillers must be evaluated. Infrared Thermography (IRT) can be a valuable tool for the diagnosis of local dysfunctions suggestive of inflammation and ischemia.

AIMS: Using IRT to describe the thermal coefficient of the nasolabial fold (NLF) region of patients who underwent HA filling.

METHODS: Thermal image were acquired with a thermal camera attached to a smartphone, before, immediately after, 1 hour, 3 hours and 28 days after NLF region HA filling.

RESULTS: Just after HA injection, there was a significant increase in temperature at the right side for the regions of interest (ROIs) 1, 2 and 3 ($p<0.05$). The same pattern was observed at the left side; however, without statistical significance. Three hours after the procedure, there was a decrease in temperature on both sides, with significant differences for ROIs 3, 4, 5 and 6 ($p<0.05$). Twenty-eight days after the procedure, ROIs 1 and 4 showed higher temperature than the other ROIs ($p<0.05$).

CONCLUSION: Cutaneous thermometry assessment as part of the clinical assessment protocol of individuals submitted to HA filling can be used as an objective method to monitor the microvascular network.

KEYWORDS: Thermography; Skin Temperature; Hyaluronic acid; Dermal fillers.

THERMOGRAFISCHE UNTERSUCHUNG DER NASOLABIALFALTENREGION VON FRAUEN, DIE EINER AUFFÜLLUNG MIT HYALURONSÄURE UNTERZOGEN WURDEN

EINLEITUNG: Die Zunahme der Inzidenz von Komplikationen, die durch die Faltenauffüllung mit Hyaluronsäure (HA) verursacht werden, muss bewertet werden. Die Infrarot-Thermografie (IRT) kann ein wertvolles Instrument für die Diagnose lokaler Funktionsstörungen sein, die auf eine Entzündung und Ischämie hindeuten.

ZIELE: Verwendung von IRT zur Beschreibung des thermischen Koeffizienten der Nasolabialfaltenregion (NLF) von Patienten, die sich einer HA-Füllung unterzogen haben.

METHODEN: Die Wärmebilder wurden vor, unmittelbar nach, 1 Stunde, 3 Stunden und 28 Tagen nach der Auffüllung der NLF-Region mit HA mit einer Wärmebildkamera aufgenommen, die an einem Smartphone befestigt war.

ERGEBNISSE: Unmittelbar nach der HA-Injektion kam es zu einem signifikanten Temperaturanstieg auf der rechten Seite für die Messareale (ROIs) 1, 2 und 3 ($p<0,05$). Das gleiche Muster wurde ohne statistische Signifikanz. auf der linken Seite beobachtet. Drei Stunden nach dem Eingriff kam es zu einer Temperaturabnahme auf beiden Seiten, mit signifikanten Unterschieden bei den ROIs 3, 4, 5 und 6 ($p<0,05$). Achtundzwanzig Tage nach dem Eingriff zeigten die ROIs 1 und 4 eine höhere Temperatur als die anderen ROIs ($p<0,05$).

SCHLUSSFOLGERUNG: Die Beurteilung der Hautthermometrie als Teil des klinischen Bewertungsprotokolls von Personen, die einer HA-Auffüllung unterzogen wurden, kann als objektive Methode zur Überwachung des mikrovaskulären Netzwerks verwendet werden

SCHLÜSSELWÖRTER: Thermografie; Hauttemperatur; Hyaluronsäure; Hautfüllstoffe

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Introduction

The search for aesthetic procedures using Hyaluronic Acid (HA) for the prevention and treatment of facial aging has increased in contemporary society. HA is one of the most used dermal fillers in the correction of expression lines and facial furrows due to its already proven safety and efficacy [1,2]. HA injections are the second most popular non-surgical cosmetic procedure after botulinum toxin treatment [1].

HA is a polymer that binds extensively to water, capable of retaining water up to 1000 times its volume [3]. It maintains the longevity of collagen fibers, which are responsible for tissue support, hydration and elasticity. HA content in tissues and its production gradually declines with age, and this process constitutes a complex and continuous biological event that is characterized by cellular and molecular changes [4]. Due to the decrease in the HA biosynthesis process, senile folds develop [5].

During the aging process, structural changes occur in the face related to a decrease in volume, with atrophy and migration of fat compartments to the lower regions of the face, prominent Nasolabial Fold (NLF) [3,6]. The NFL is considered one of the most characteristic signs of facial aging and begins at the alar groove and alar facial crease and ends at 1 to 2 cm laterally to the lip commissure [7]. However, this is an area of high risk for aesthetic procedures because of its close proximity to the Facial Artery (FA), a vessel that comprises most of the blood supply of the face. Therefore, FA may be vulnerable to vascular injury during injections for aesthetic treatments [8]. Complications resulting from wrong manipulation of injections in the NLF have been reported in the literature [8]; therefore, knowledge of the anatomy of the area to be treated and the indicative signs of vascular alterations during and after the procedure are essential to avoid adverse events that may be subjective and observer dependent [9]. Among the most common complications are the involvement of facial blood vessels by compression or embolism [10].

Infrared Thermography (IRT) is an important image method which detects the infrared radiation emitted by the body through the generation of heat. Several factors can influence the registration of the thermal pattern of a given anatomical site. The blood flow is highlighted as the main factor and it is normally regulated by the autonomic nervous system (ANS). IRT is very sensitive and detects small changes in the temperature distribution of the face, contributing to the diagnosis and monitoring of several clinical conditions in which the skin temperature may reflect an inflammatory process and/or dysfunction in the underlying tissues, leading to an increase or reduction of the blood flow [11,12].

In a recent bibliographical search, there was a lack of studies that associate IRT with the use of dermal fillers in the daily clinical practice of health professionals such as physicians and dentists [13,14]. Due to the increasing cases of immediate and late complications resulting from procedures using facial fillers such as HA, there is an urgent need to evaluate these aesthetic-functional procedures. Hence, the use of IRT as an auxiliary tool for the diagnosis of local dysfunctions becomes relevant [15,16]. This assessment method allows the health professional to verify the condition of the microcirculation of the treated anatomical site and intervene in early stages in cases of adverse effects such as development of microemboli, vascular compression, among other conditions. The present study evaluated the variation in the thermal coefficient of the NLF region before and after HA filling using IRT.

Methods

This prospective analytical descriptive study involved 25 female patients, randomly selected from a private clinic. This study was submitted to the University's Ethics Committee and is in accordance with the declaration of Helsinki (protocol number 4.333.341). To calculate the sample size, the GPower (Universitat Kiel, Germany) was used, with ?=5%, power of 80% and effect size of 0.25, plus 20% in case the case of patient losses to follow-up. Initially, the patients were submitted to a detailed anamnesis and physical examination, to confirm and guarantee that they all adhered to the inclusion criteria of the study. The research protocol described below has been previously published [17] and the checklist with 15 points for the collection of tsk using IRT in sports and exercise medicine was also used [18].

All patients should present depression or deepening of the NLF, aged between 45 and 55 years, without comorbidities and have signed the Informed Consent Form. Volunteers that were breastfeeding, pregnant, with autoimmune diseases, smokers and with thermal asymmetry (Δt) $\geq$ 0.3°C between the regions of interest (ROIs) of the hemifaces were excluded from the sample.

Patient's sociodemographic data were collected, including marital status, age, education, profession and family income. The patients' faces were cleaned with 70% alcohol and their hair was fastened with hair band for better facial exposure during thermal image acquisition. After a period of 20 minutes, necessary for body thermoregulation, an image was captured with an infrared camera. Then, NFL filling procedure was performed with AH Princess® VOLUME (23mg/ml, 0.3% lidocaine hydrochloride; Croma-Pharma GmbH, Leobendorf, Austria), chemically cross-linked with 1,4-butanediol diglycidyl ether (BDDE). Right after the procedure, a thermal image was acquired. Four thermal images were acquired 1 hour, 3 hours and 28 days after HA filling procedure.

All procedures were performed during the morning due to the influence of the circadian cycle on body temperature throughout the day. The HA application technique was done using a retroinjection with a 22G Pro Deep cannula (Alur Medical, China) with a single port, performed with a 22G needle, applied at the equidistant point of the ROIs that would be evaluated (Figure 1). The HA filling was placed in the subcutaneous plane, in the predetermined region, bilaterally, with a total dose of 1 ml.

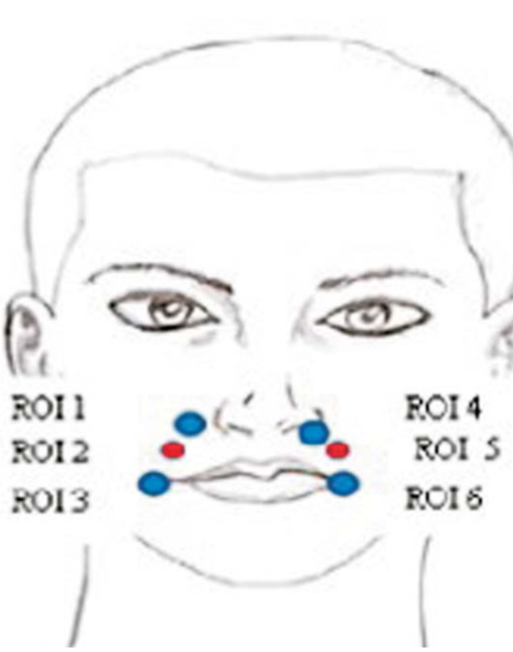

Figure 1
Mapping of the assessed regions of interest (ROI) for thermogram analysis (adapted from Haddad et al.(26))

All thermographic images were coded and saved in JPEG format (Joint Photographic Experts Group) and separated into individual folders for each volunteer. FLIR tools® software was used to assess all thermal images. To assess the local circulatory pattern, an infrared camera from FLIR ONE Pro (Victoria, Australia) with a focal plane array of 160×120 pixels and an image frequency of 8.7 Hz, which captures images in real time, was used. The equipment performs studies in the temperature range from -20°C to 400°C and has a thermal sensitivity (MRDT) of 150 mK. It operates in the spectral range of electromagnetic waves between 8 and 14?m, corresponding to the long wavelength infrared range. To obtain image standardization, the following protocol was adopted: patients positioned on a rotating bench, with their Frankfurt plane parallel to the ground. The thermal camera was attached to a tripod at 1 meter above the ground and 30 centimeters distance from the volunteer's face (Figure 2A and 2B).

Facial recordings were performed in the frontal view to assess the right and left NLF in the same image. All image acquisitions were within the thermal window of 28-37°C. The thermograms were obtained in four different times: immediately after the HA filling, one hour, three hours and 28 days after the filling in order to calculate the thermal coefficient variation (ΔT). The coefficient was calculated according to the difference between ROIs temperatures for each analysis. To calculate the temperature variation, the initial basal temperature recorded before the start of the procedure was used as the standard value. The environment had its temperature and humidity controlled by a thermo-hygrometer at 22°C ± 1°C and a maximum of 60%, respectively. To avoid thermal interferences, sources of air streams direct to the volunteer were avoided.

Microsoft Excel software was used to tabulate data for statistical analysis. With regard to qualitative variables (occupation, marital status, age, education and monthly income), data were obtained from a one-dimensional frequency table and their respective percentages were identified. For analysis of the variation of thermal coefficients obtained through thermographic records, individual worksheets were built. Initially, descriptive and exploratory analyzes of all data were performed. Categorical variables were described with absolute and relative frequencies, quantitative variables with means and standard deviations, and quartiles scores. Then, a mixed generalized linear model was applied to analyze the temperature as a function of the method. A mixed generalized linear model was also used to analyze temperature as a function of ROI and time, considering in the model that ROIs were measured in the same patients and with repeated measurements over time. The analyzes were carried out with the aid of the R program and with a significance level of 5%.

Results

Table 1 describes the sociodemographic data of the studied population. It was found that 60% (n=15) of the partic-

Table 1.
Descriptive analysis of the study participants' profile (n=25)

Variable	Category	Frequency (%)
Marital status	Married	15 (60%)
	Divorced	2 (8%)
	Single	7 (28%)
	Widow	1 (4%)
Education	Fundamental I	1 (4%)
	High school	6 (24%)
	Graduate	13 (52%)
	Postgraduate	5 (20%)
Income bracket	Up to 1	4 (16%)
(minimum wage)	2 to 5	10 (40%)
	6 to 10	7 (28%)
	Above 10	4 (16%)
Profession	Physiotherapist	5 (20%)
	Teacher	3 (12%)
	Administrator	2 (8%)
	Attorney	2 (8%)
	Room assistant	2 (8%)
	Merchant	2 (8%)
	Civil servant	2 (8%)
	Therapist	2 (8%)
	Plastic artist	1 (4%)
	Dentist	1 (4%)
	Pharmaceutical	1 (4%)
	Hospital manager	1 (4%)
	Outdoor technician	1 (4%)
Variable	Mean (standard deviation)	Median (minimum and maximum value)
Age (years)	48.9 (±3.3)	48 (45-55)

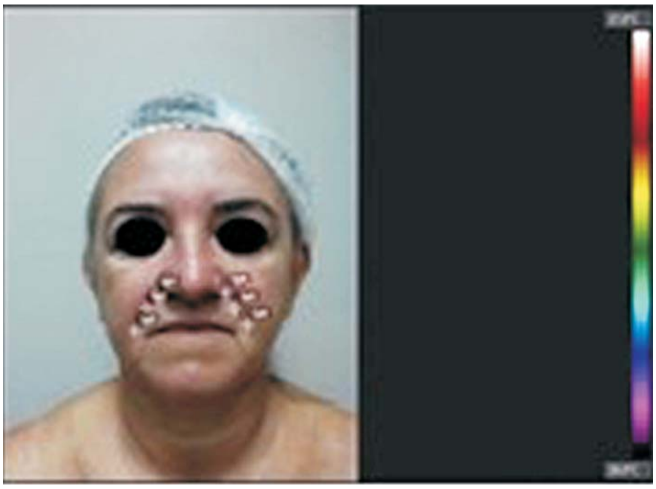

Figure 2A1
Before the procedure (photography)

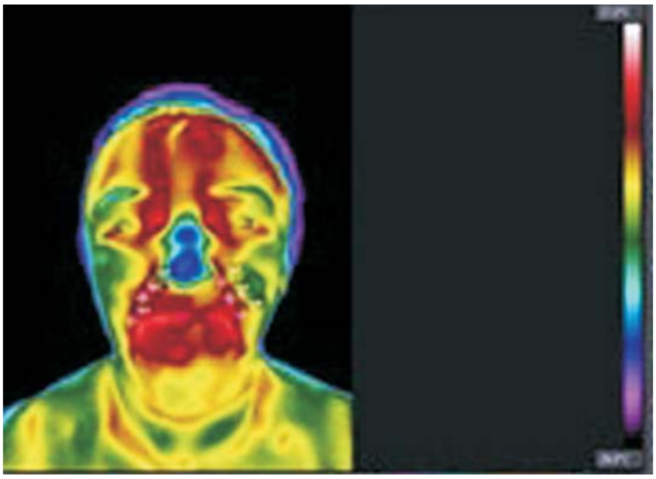

Figure 2A2
Before the procedure (thermal image)

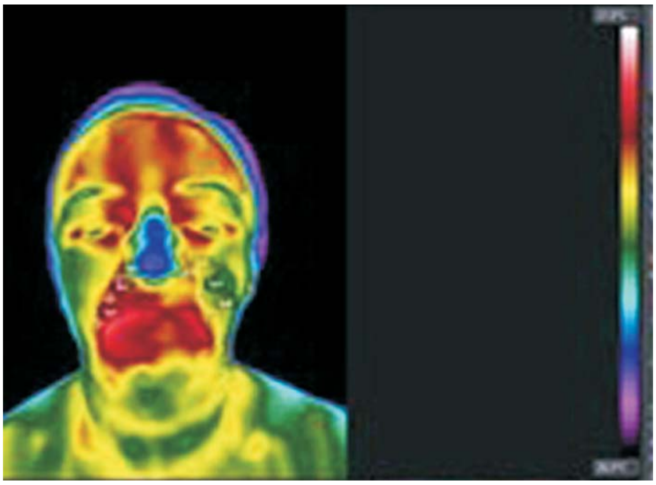

Figure 2A3
Immediatly after the procedure

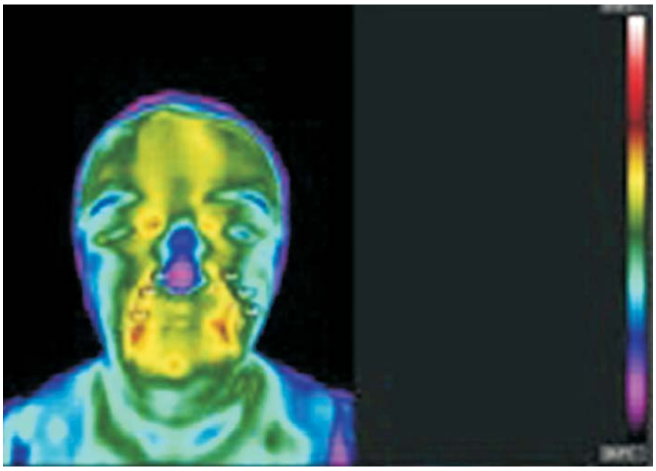

Figure 2A4
One hour after the procedure

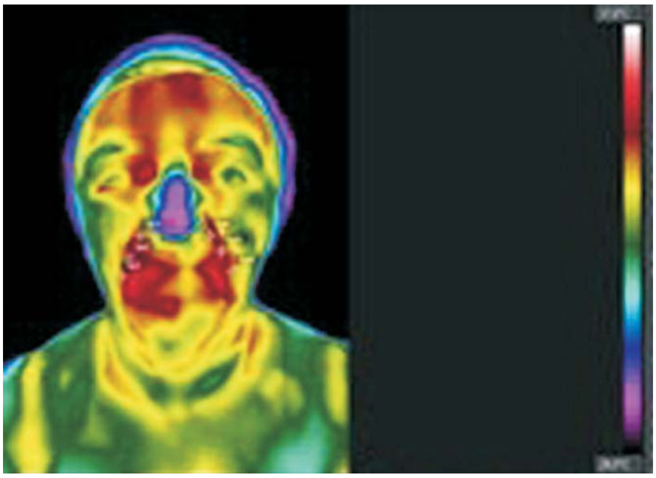

Figure 2A5
Three hour after the procedure

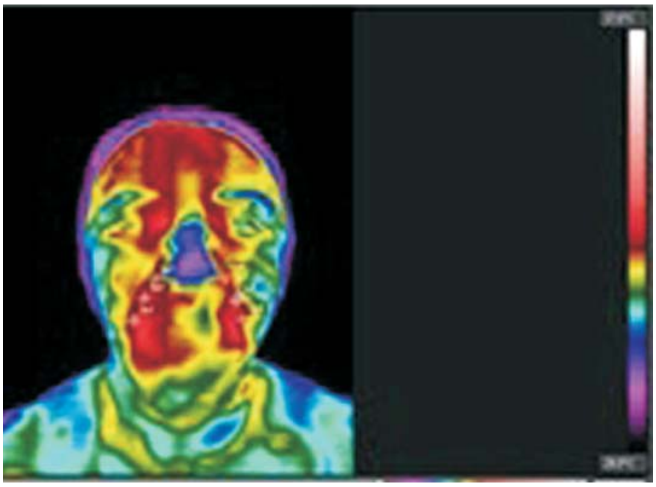

Figure 2A6
Twenty-eight days after the procedure

Figure 2B1
Before the procedure (photography)

Figure 2B2
Before the procedure (thermal image)

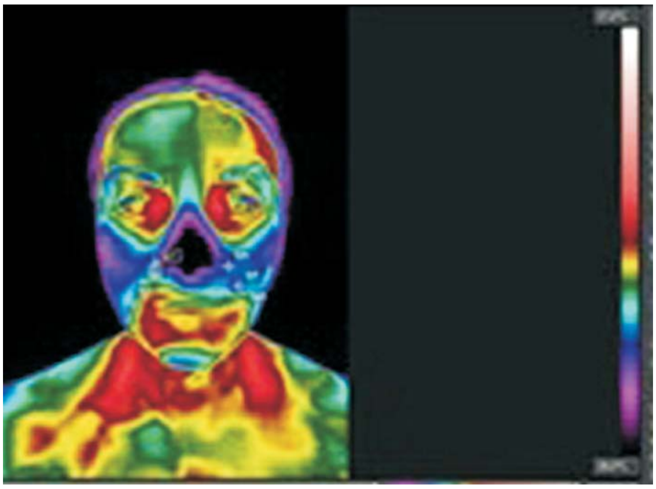

Figure 2B3
Immediatly after the procedure

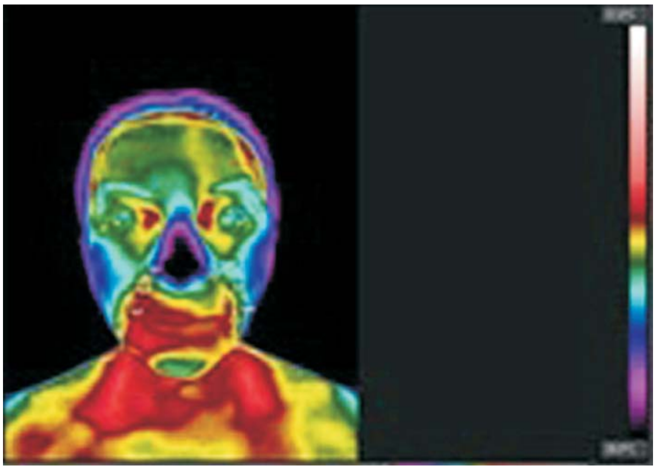

Figure 2B4
One hour after the procedure

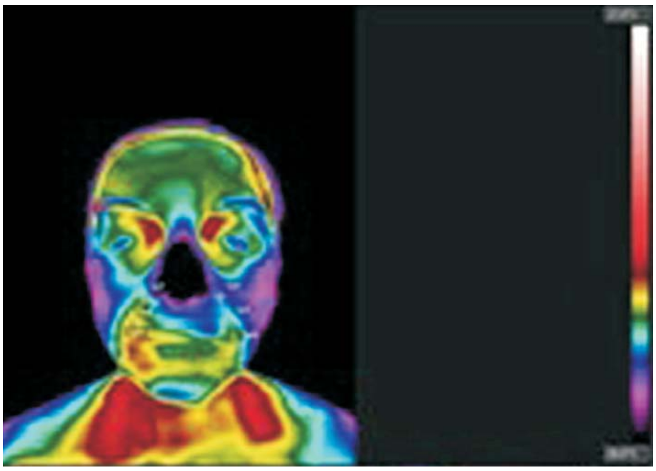

Figure 2B5
Three hour after the procedure

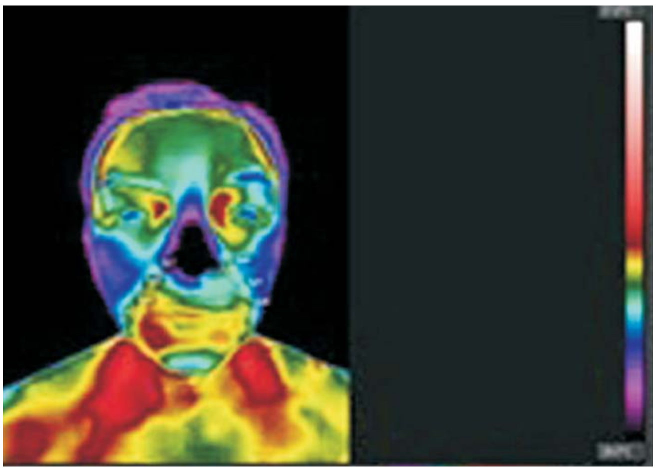

Figure 2B6
Twenty-eight days after the procedure

Table 2
Mean temperature (standard deviation) in Celsius (°C) measured in the frontal view of assessed region of interest and acquisition time.

Side	ROI	Time				
		Before the procedure	immediate post	After 1 hour	After 3 hours	After 28 days
Right	1	32.7 (1.0) BC a	33.3 (1.2) A a	32.8 (1.1) B a	32.5 (1.1) C a	32.5 (0.7) BC a
	2	32.4 (1.3) BC ab	33.1 (1.4) A a	32.6 (1.3) B ab	32.2 (1.4) C ab	32.2 (0.8) C ab
	3	31.8 (1.2) B c	32.3(1.3) A b	31.8 (1.2) BC de	31.5 (1.3) BC d	31.5 (0.9) C c
Left	4	32.6 (1.0) A a	32.8 (1.00) A b	32.4 (1.0) A bc	32.0 (1.1) B bc	32.4 (0.7) AB a
	5	32.2 (1.2) A b	32.3 (1.2) A bc	32.0 (1.2) AB cd	31.7 (1.4) B cd	31.8 (0.8) AB b
	6	31.6 (1.2) A c	31.9 (1.3) A c	31.5 (1.2) AB e	31.0 (1.4) C e	31.1 (0.9) BC c

p(ROI)<0.0001; p(time)<0.0001; p(interaction)=0.0013. Distinct letters (uppercase horizontally and lowercase vertically) indicate statistically significant differences (p=0.05)

ipants were married and 72% (n=18) reported having higher education. Regarding the average salary, it was observed that 68% (n=17) claimed to earning between 2 to 10 minimum wages per month. With regard to work activity, a variety of professions were reported; however, physiotherapy was the most prevalent profession reported by the volunteers (20%; n=5). The sample mean age was 48.9 years old, ranging from 45 to 55 years old.

Tables 2 and 3 illustrate the mean temperatures of the different ROIs in the assessed different times, mapping the thermal variation based on the initial temperature before filling with AH. Additionally, for documentation purposes, Figure 2 illustrates the thermograms of each patient in the assessed different times. It was observed that on the right side, the temperature was significantly higher in the immediate post-procedure time thermogram than in the other assessed times (p<0.05). Thermograms acquired 1 and 3 hours after the procedure showed no significant temperature difference from the pre-procedure thermogram for all ROIs (p>0.05) except for ROI 3 of the thermogram acquired 3 hours after the procedure (p<0.05). After 28 days, it was observed that only ROI 3 showed a significant negative variation in the thermal coefficient (?T) (p<0.05).

On the left side, there was a similar pattern of tissue heating in the immediate post-procedure time, although without statistical significance (p>0.05). The temperature was lower at 1 and 3 hours after the procedure compared to the pre-procedure image (baseline) and post-procedure times, with statistical significance only for the images acquired 3 hours after the procedure for all ROIs (p<0.05). 28 days after the procedure, it was found that the mean temperatures of the ROIs showed lower values than the initial control, but only ROIs 4 and 5 exhibited significantly lower ΔT (p<0.05).

When comparing the ROIs at each specific time, it can be noted that before the procedure, on both sides, ROIs 1 and 4 had a significantly higher temperature than ROIs 3 and 6 (p<0.05). Just after HA filling, on the right side, it was observed that ROIs 1 and 2 had a higher temperature than ROI 3 (p<0.05) and on the left side, ROI 4 had a higher temperature than ROIs 5 and 6 (p<0.05). One and 3 hours after the procedure as well as after 28 days, ROIs 1 and 2 had a higher temperature than ROI 3 (right side), and ROIs 4 and 5 had a higher average temperature than ROI 6 (left side); (p<0.05).

Discussion

With the increase in society's aesthetic demands, the injection of HA dermal fillers has been considered one of the most performed procedures in the practice of facial aes-

Table 3.
Variation of the thermal coefficient (ΔT in °C) in relation to the basal temperature, in the frontal view of assessed region of interest and acquisition time.

Side	ROI	Time				
		Before the procedure	immediate post	After 1 hour	After 3 hours	After 28 days
Right	1	32.67	ΔT = +0.66*	ΔT = +0.11	ΔT = - 0.22	ΔT = - 0.18
	2	32.42	ΔT = +0.66*	ΔT = +0.14	ΔT = - 0.22	ΔT = - 0.22
	3	31.80	ΔT = +0.45*	ΔT = - 0.03	ΔT = - 0.31*	ΔT = - 0.32*
Left	4	32.59	ΔT = + 0.23	ΔT = - 0.24	ΔT = - 0.57*	ΔT = - 0.19
	5	32.22	ΔT = +0.07	ΔT = - 0.21	ΔT = - 0.53*	ΔT = - 0.41*
	6	31.64	ΔT = +0.29	ΔT = - 0.12	ΔT = - 0.65*	ΔT = - 0.54*

* p<0.0001

thetics and rejuvenation, being capable of reducing rhytids and furrows; therefore, providing the individual a more youthful appearance. HA fillers constitute about 80% of all injectable dermal fillers due to their biocompatibility with human body tissues [19,20].

In the present study, all volunteers were women with an average age of 49 years. This finding corroborates with Chung and Lee [21] study, which reported that women resort to filling the NLF region with HA more frequently than men.

In general, the search for aesthetic procedures that aim to contribute to facial harmonization tends to increase from the 4th decade of life onwards, although a trend towards an increase in aesthetic facial procedures in young patients has been observed in recent years. Between 40 and 50 years of age, the synthesis of elastin begins to reduce abruptly and this reduction promotes the disintegration of the network of elastic fibers and water as the hygroscopy of the glycosaminoglycans decreases. Additionally, there is an increase in the degradation of existing collagen and a reduction in its biosynthesis. Changes in the elastic fiber network result in loss of tissue strength and architecture, manifesting both as static wrinkles and pronounced folds [6]. These events could justify the greater perception of signs of senility around the 4th or 5th decades of life.

Faced with the increasing number of facial fillers, especially with HA, the present assessed, in an unprecedented way, the mean temperatures of 6 ROIs along the NLF. The occurrence of local ischemia resulting from the accommodation of HA in the dermis has been reported in the literature [3,22-24]. More rarely, other authors have also documented exacerbation of the inflammatory process due to HA insertion [20,25].

The thermographic visual analysis of cooling and heating zones on the face performed in the present study allowed the identification of the occurrence or absence of areas of hypoperfusion or reactive hyperemia. On the right side, right after HA injection, there was a significant increase of +0.66°C in temperature compared to baseline in ROI 1, +0.66°C in ROI 2 and +0.45°C in ROI 3. These same characteristics were observed on the left side, but without statistical significance. According to the literature, variations in the thermal coefficient equal to or greater than 0.3ºC can already be considered suggestive of abnormality or indicative of dysfunction [26,27].

The increase in temperature observed in the present study can be explained by the probable inflammatory response to the manipulation of the tissue corresponding to the anatomical site where the filling material was inserted. It is known that one of the first alterations that occur in the tissue as a result of an aggression is vasodilation, which reflects an increase in local skin temperature [27,28]. In addition, such a change in temperature, just after the procedure, can also be justified by the presence of lidocaine in the HA gel, considering that it has a vasodilator action [1,24]. Lidocaine is rapidly absorbed after injection and eliminated from the body in approximately 90 minutes, with a short half-life [7] .

Three hours after the procedure, there was a decrease in the thermal coefficient on the right and left sides of the face, with a statistically significant difference in ROIs 3, 4, 5 and 6 probably due to the effect of HA filler in the dermis and its hygroscopic expansion [3]. Because it is a polymer, HA binds to water, with the capacity to retain it in up to 1000 times its volume. This hygroscopic expansion can probably reduce local microcirculation by vascular compression, with a consequent decrease in local temperature due to transient partial ischemia [29]. The degree of HA crosslinking seems to be a determining variable to justify the hygroscopic capacity of this material. As the number of crosslinks increases, the chains are held closer together and their flexibility to separate becomes more limited, reducing the gel's swelling ability. Changes in water absorption mainly occur immediately after injection and may contribute to initial volume increase [30,31].

The fact that the thermal coefficient remains negative over 28 days after the procedure in all ROIs also seems to be related to the high fixed charge density of HA, which is osmotically active. Thus, there is a progressive attraction of water molecules to the injected anatomical site, which can result in mild edema and increase the compressive strength of the filler against the normal connective tissue. This effect can lead to a slight expansion of the HA after its insertion, which will depend on the crosslink of the material. However, its duration may extend to longer periods of time such as 3 to 6 months, depending on the injection site [32].

In this study, 28 days after HA filler injection ROIs 1 and 4 showed higher temperature than the other assessed ROIs, although the coefficient of thermal variation still remained negative. It is known that at the NL thermoanatomic point that corresponds to these ROIs, the facial artery and its branches have close topographical connections with the NLF. In fact, in a study carried out by Yang et al., 2014 [8], 93.3% of the branches of the facial artery were observed close to the NLF and in the region lateral to the nose, where this artery naturally becomes superficial.

Although there are studies in the literature that have investigated the physiological effects of materials used in facial harmonization, such as Gazerani et al., 2009 [33], which assessed the effect of subcutaneous administration of botulinum toxin by measuring the skin temperature using infrared thermography, the present study is the first to use the thermographic analysis of facial skin before and after the use of HA fillers, at four different times. As mentioned by Carvalho et al., 2022 [17], there are many possible complications associated with these kind of subcutaneous biomaterials infiltrations. Thus, the information gleaned from the sequential IRT findings might have an impact on medical decision-making including - ruling out future candidates for HA and other implants, and predicting possible immediate or late effects associated to these procedures. As a matter of fact, based on our results, we also encourage the use of thermograms to evaluate potential diagnostic complications and to prescribe therapeutic recommendations post HA infiltration.

However, the study has some limitations that must be addressed. One concerns the necessary extension of the thermographic analysis period to verify the natural tendency of the skin to return to its basal temperature. The individual variability of each participant also needs to be assessed. However, the authors do recommend the use of IRT to monitor possible complications arising from aesthetic procedures, especially when they are performed on the face.

Conclusion

In this study, the NLF region temperature after filling with HA procedure assessed using IRT, demonstrated a difference in the mean temperature in the different assessed times. As the adverse effects of filling with HA represent a growing health problem, the use of preventive strategies such as cutaneous thermometry can contribute to the prevention and aggravation of this problem.

There is an urgent need for growing scientific evidence to implement IRT models for procedures that are part of the list of facial harmonization techniques, in special, HA filling.

Financial Support

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Meetings

April 26th - 28th, Wisla, Poland

XXth Conference of the Polish Society of Medical Physics with a session on" Thermography application in medicine".

Venue: Luksusowe apartamenty w Beskidach | Wisla | Vislow Resort (https://vislow.pl/)

Fee: 250.- EUR including

- accommodation (Fri till Sun)

- breakfast (Sat 27th, Sun 28th)

- lunch (Sat 27th)

- dinner (Fri 26th, Sat 27th)

Conference Webpage:

20 Slaskie Seminarium Fizyki Medycznej | PTFM o. Slaski (ptfm-slask.pl)
(https://www.ptfm-slask.pl/20ssfm/)

Contact:
prof. dr n. fiz. hab. n. med. Armand Cholewka

Email: armand.cholewka@gmail.com

May 31st, 2024, New Orleans

Live scientific session at the Roosevelt Hotel in conjunction with the 2024 new Cardiovascular Horizons (NCVH) Annual Meeting.

Webpage:
https://annualmeeting.aathermology.org/

About The Event

The 2024 Annual Scientific Session will be a live, in person meeting with opportunity for virtual presentations. It will be held in conjunction with The 2024 New Cardiovascular Horizons Proceedings, held in New Orleans, at the Roosevelt hotel. The AAT Session will be a single day event from 8am-2pm on May 31st, 2024.

The Pre-Meeting Physician's Thermography Interpretation and the Getting Started Courses will be offered subject to demand and meeting space availability. The Interpretation course is on line, and at your own pace. Typically, the Physician live question and answer session is conducted between 9:00am and 12:00pm (Eastern Time). The Getting Started course runs from 2:00pm to 5:00pm. Use the "Registration" link on the top tool bar to sign up.

Registration will be required for BOTH the NCVH meeting and the AAT Scientific Session. The registration link for the NCVH meeting is NCVH Registration (https://ncvh.org/ncvh-2024/) Use the Promo code AAT24 for the AAT discounted rate. Use the Registration link on the top tool bar of this page to register for the AAT Sessions.

If you would like to stay at the Roosevelt Hotel or have NCVH assist you with travel arrangements use this link: Hotel & Travel.
(https://web.cvent.com/event/bdc39ff6-d452-413a-bcdc-50d97ffcad4f/summary?locale=en) Make sure to list AAT in the reservation

2024 Conference Schedule

Day 1

The Pre-Meeting Physician's Thermography Interpretation and the Getting Started Courses will be offered subject to demand and meeting space availability.

9:00 am - 12:00 pm
Physician Interpretation Q & A Session
By Robert Schwartz, MD AAT COB, AIIR Imaging Alliance, Greenville, SC

2:00 pm - 5:00 pm
"Getting Started In Medical Thermology" Round Table
By Jan Crawford, RN, BSN AAT Board, Scaly Mt, NC, Robert Kane, DC AAT Member, San Francisco, CA

Day 2

PROGRAM

8:00am - 8:10am
Welcoming Comments

8:10am - 8:40am
The Emergence of Point of Care Infrared Thermography (POCIT) in Circulatory Disorders
By Ariel Soffer, MD AAT Member

8:40am - 9:10am
The Cutting-Edge Fusion: POC AI-Enhanced Dual Infrared-Visual Thermography in Vascular Imaging
By Marcos Brioschi, MD, PhD AAT Honorary Member, AIIR Imaging Alliance, ABRATERM, Sao Paulo University, Brazil.

9:10am - 9:30am
AAT POC Guidelines
By Jan Crawford, RN, BSN AAT Board, Scaly Mt, NC

9:30am - 10:00am
Panel Discussion: Soffer, Brioschi, and Crawford

10:00am - 10:15am Break

10:15am - 10:45am
Musculoskeletal Causes of Chest, Neck and Arm Pain
By Robert Schwartz, MD AAT COB, AIIR Imaging Alliance, Greenville, SC

10:45am - 11:15am
Thermal Imaging RSDS/CRPS: Case Reports
By George Schakaraschwili, MD, AAT Board, Denver, CO

11:15am - 11:45am
CCI, CCJ Procedures, and SSR Assessments
By Jaime Browning, DC, DCCJP, AAT Member, Spartanburg, SC

11:45am - 12:15pm
A Novel Approach to Dysautonomia
By Robert Schwartz, MD AAT COB, AIIR Imaging Alliance, Greenville, SC

12:15am - 1:00pm Lunch

1:00pm - 1:30pm
The AAT Collective Intelligence Initiative
By Marcos Brioschi, MD, PhD AAT Honorary Member, AIIR Imaging Alliance, ABRATERM, Sao Paulo University, Brazil.

1:30pm - 2:00pm
The Blue Highways Book Tour. Clinical Thermography in the United States 2022
By James Stewart Campbell, MD AAT Senior Member, Pfafftown, NC

2:00pm Adjourn

6th - 8th September 2024, Wroclaw, Poland

XVI Congress of the European Association of Thermology

Venue¨
Wroclaw University of Environmental and Life Sciences

The EAT and Wroclaw University of Environmental and Life Sciences are delighted to invite you to participate in the XVI EAT Congress from 6th to 8th September 2024. Following on from the most recent meetings in Porto (2012), Madrid (2015), London (2018) and online in 2021, the Congress heads to eastern Europe for 2024 to Wroclaw in Poland. The Organising Committee looks forward to welcoming you to Wroclaw University of Environmental and Life Sciences in the summer of 2024

Dr. Kevin Howell, President, European Association of Thermology

Dr. Maria Soroko-Dubrovina, Chair, XVI Congress Organising Committee

The Congress is held under the honorary patronage of His Magnificence, Rector of the Wroclaw University of Environmental and Life Sciences.

Organising Committee

This committee is responsible for all operational aspects of the Congress, including delivery of the meeting at the local venue

- **Maria Soroko-Dubrovina (POL), Chair**
- Kurt Ammer (AUT)
- Kevin Howell (GBR)
- Anna Jung (POL)
- Adam Roman (POL)
- Adérito Seixas (POR)
- Manuel Sillero-Quintana (ESP)
- Karolina Sniegucka (POL)
- Ricardo Vardasca (POR)
- Anna Zielak-Steciwko (POL)
- Paulina Zielinska (POL)

International Scientific Committee

This committee is responsible for reviewing all submitted abstracts to the Congress, and approving the final Congress programme

- **Kevin Howell (GBR), Chair**
- Kurt Ammer (AUT)
- John Allen (GBR)
- Danilo Gomes Moreira (BRA)
- George Havenith (GBR)
- Anna Jung (POL)
- James Mercer (NOR)
- Adérito Seixas (POR)
- Manuel Sillero-Quintana (ESP)
- Maria Soroko-Dubrovina (POL)
- Hisashi Usuki (JPN)
- Ricardo Vardasca (POR)

Registration fees

	Early registration (until 6 May 2024)	Late registration (after 6 May 2024)
EAT member	€360	€410
Non-member	€440	€490
One-day registration	€200	€250
Student	€200	€250
Accompanying person	€120	€120

Registration for the XVI EAT Congress is now open. Please register by completing the form here.
(https://docs.google.com/forms/d/e/1FAIpQLSeAj9BsmoTFQcFXMwuh0IYl1m0c4GKWtQClBbPDQ_lWSvUZMA/viewform)

Registration includes access to all congress sessions, congress lunch and coffee breaks, the Gala Dinner and walking tour.

Attendance at the pre-congress Short Course on Medical Thermography requires a separate registration and payment. More information, when available, will be posted here (http://www.eurothermology.org/education.html) .

Short Course in Medical Thermography

Date:6th September 2024

*Venue:*Wroclaw University of Environmental and Life Sciences

The European Association of Thermology has education as one of its key aims. To that end, we offer the EAT "Short Course in Medical Thermography," taught by an experienced faculty of thermology researchers.

The Course normally takes place every three years, along with the Congress of the EAT, at a European venue.

Main themes of the 2024 course in Wroclaw will include:

- Physical principles of heat transfer
- Principles of thermal physiology and skin blood perfusion
- Standardization of thermal imaging, recording and analysis
- Quality assurance for thermal imaging systems
- Producing a thermographic report
- Provocation tests
- Image analysis
- Educational resources

Local hotels

Below is a list of hotels near to the Congress venue in Wroclaw...

- Hotel ZOO (recommended) (https://zoo-hotel.pl/)

10% discount for congress participants: please quote "XVI Congress of the European Association of Thermology" when booking

Address: ul. Wroblewskiego 7, 51-627 Wroclaw

Phone: +48 510 091 913; 510 663 963

Reservations: biuro@zoo-hotel.pl

- Green Hostel Wroclaw

Address: ul. Mickiewicza 4, 51-619 Wroclaw

Phone: +48 71 348 06 06

Reservations: info@greenhostel.wroclaw.pl

- Radisson Blu Hotel Wroclaw

(https://www.radissonhotels.com/en-us/hotels/radisson-blu-wroclaw)

Address: ul. Purkyniego 10, 50-156 Wroclaw

Phone: +48 71 375 00 26; 71 375 00 370

Reservations: reservations.wroclaw@radissonblu.com

- Grape Hotel and Restaurant (https://www.grapehotel.pl/en/)

Address: Parkowa 8, 51-616 Wroc?aw

Phone: +48 71 73 60 400

*Reservation*s: biuro@grapehotel.pl

- Hotel Puro (https://purohotel.pl/en/wroclaw/)

Address: Pawla Wlodkowica 6, 50-072 Wroclaw

Phone: +48 71 772 51 00

Reservations: wroclaw@purohotel.pl

- Hotel Europeum (https://europeum.pl/en/)

Address: ul. Kazimierza Wielkiego 27A, 50-077 Wroc?aw

Phone: +48 71 371 44 00; 71 371 44 02

Reservations: europeum@europeum.pl

- Hotel B and B Wroclaw Centrum (https://www.hotel-bb.com/en)

Address: Piotra Skargi 24-28, 50-082 Wroclaw

Phone: +48 71 324 09 80

Reservations: wroclaw@hotelbb.com

- Hotel Mercure Wroclaw Centrum (https://all.accor.com/hotel/3374/index.en.shtml)

Address: pl. Dominikanski 1, 50-159, Wroclaw

Phone: +48 71 323 27 00

Reservations: H3374-SL2@accor.com

Secretariat Contact address

XVI Congress of the European Association of Thermology

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