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Effects of hyperthermia on goiter

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The effect of water-filtered infrared-A (wIRA) irradiation on skin temperature and skin blood flow as evaluated by infrared thermography and scanning laser Doppler imaging

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SUMMARY

Free tissue transfer, including skin flaps, is an important procedure in reconstructive surgery. During transfer of skin flaps, there is an inevitable period of ischemia. Prolonged ischemia causes significant damage and is associated with flap failure. The tolerance of tissue for ischemia increases by a process called thermal preconditioning, which can be obtained by increasing skin temperatures to supra-physiological levels. This study investigated the effect of water-filtered infrared-A irradiation (wIRA) on skin temperature and skin blood flow of the abdominal wall. Skin temperature and skin blood flow were measured with respectively, infrared thermography and scanning laser Doppler imaging. WIRA irradiation of the abdominal wall resulted in supra-physiological skin temperatures and up to a 10-fold increase in skin blood flow. No thermal discomfort during or after wIRA irradiation was registered. It is concluded that water-filtered infrared-A irradiation is a potential method for thermal preconditioning of skin flaps.

Key words: Skin temperature, skin blood flow, water-filtered infrared irradiation, infrared thermography, scanning laser Doppler imaging.

DER EINFLUSS EINER WASSER GEFILTERTEN INFRAROT-A (WIRA) BESTRAHLUNG AUF HAUTTEMPERATUR UND HAUTDURCHBLUTUNG UND DESSEN ERFASSUNG IN DER INFRAROTTHERMOGRAPHIE BZW. DEM LASER DOPPLER IMAGING

Die Gewebetransplantation, einschließlich der von Hautlappen, ist eine wichtige Maßnahme in der wiederherstellenden Chirurgie. Bei der Übertragung von freien Hautlappen kommt es unweigerlich zu einer passageren Gewebeischämie. Eine langdauernde Ischämie kann zu beträchtlichen Schäden führen und eine erfolglose Gewebetransplantation bedingen. Die Toleranz für eine Mangeldurchblutung kann durch eine sogenannte “thermische Präkonditionierung” erhöht werden, bei der die Hauttemperatur über das physiologische Temperaturniveau erhöht wird. In dieser Studie wurde der Einfluss einer Wasser gefilterten Infrarot-A (wIRA) Bestrahlung auf die Hauttemperatur und Hautdurchblutung der Bauchwand untersucht. Die Hauttemperatur wurde mittels Infrarotthermographie und die Hautdurchblutung mittels Laser Doppler Imaging erfasst. Die wIRA-Bestrahlung der Bauchwand führte zu Hauttemperaturen jenseits der physiologischen Normwerte und zu einer 10-Fachen Erhöhung der Hautdurchblutung. Es wurde keine Störung des thermischen Komforts während der Infrarotbestrahlung beobachtet. Die Wasser gefilterte Infrarot-A-Bestrahlung scheint eine geeignete Methode für die thermische Präkonditionierung von Hautlappen zu sein.

Schlüsselwörter: Hauttemperatur, Hautdurchblutung, Wasser gefilterte Infrarotbestrahlung, Infrarot Thermographie, Laser Doppler Imaging.

Thermology international 2005; 15: 89-94

Introduction

In addition to its well-known function as carrier of oxygen and nutrients to tissues, blood is the primary physiological heat exchange fluid in vertebrates. Dissipation of body heat occurs primarily by radiative loss from the skin to the environment, with blood acting as the heat transfer fluid. The main regulatory process of perfusion and heat transfer is by control of vascular tone, that is, the lumen of blood vessels. The autonomic nerve system regulates the vascular tone and thereby the cutaneous and subcutaneous perfusion (1). Although metabolism is normally the only source providing heat to the body, in some situations an external source may also add heat to the body. In fact, heat therapy is one of the oldest forms of treatment for different diseases that has been recognised since ancient times. Today heat therapy is used in modern medicine in a large variety of applications, especially in the area of rehabilitation (2).

Recently, researchers have gained new interest in heat therapy. For example, there are some reports showing that the tolerance of tissue for ischemia increases by a process called thermal preconditioning (3,4,5). Exposure of tissue to supra-physiological temperatures protects it transiently from a second normally lethal stress. The beneficial effect of thermal preconditioning is thought to be mediated by heat shock proteins, however, their exact role is not fully understood. The clinical application of thermal preconditioning could have important implications for example in cardio-thoracic surgery or free tissue transfer surgery. Both types of surgery are characterised by a period of ischemia. Increasing the tolerance of tissue for ischemia could significantly improve the postoperative results (6,7,8).

Heat therapy is applied in many ways, both directly via heat conduction and indirectly via convection and/or radiation. The advantage of radiation therapy is that there is no need for direct skin contact. If it is desirable to heat the skin as well as the subcutaneous tissue then the depth of penetration becomes important. Water-filtered infrared-A irradiation (wIRA) is a relatively new form of heat treatment. Several studies have shown that wIRA has sufficient depth of penetration to provide therapeutical heat treatment of deeper tissue (9).

This study investigated the effects of wIRA irradiation of the abdominal wall on both skin surface temperature as well as on skin blood flow measured with respectively infrared thermography and scanning laser Doppler imaging.

Methods

Ten healthy female volunteers (age 37.1 ± 7.9 years; BMI 22.8±1.8) participated in the study (see Table 1 for personal details). The lightly clothed subjects lay in a supine position on a conventional treatment bench at a room temperature of ca 23°C. The heat treatment consisted of a 20-minute period of wIRA irradiation of the abdominal skin using a Hydrosun® water filtered irradiator. The Hydrosun® water-filtered infrared-A irradiator allows a loco-regional heating of human tissue with a higher penetration depth than that of conventional infrared therapy (9). The unique principle of operation involves the use of a hermetically sealed water filter in the radiation path to absorb those infrared wavelengths emitted by conventional infrared lamps which would otherwise harm the skin due to activation of the OH-group at 0.94, 1.18. 1.38 and 1.87 micrometers. The Hydrosun®-irradiator used has a total effective radiation of 250 mW/cm2 (visible light + wIRA), where ca. 188 mW/cm2 is wIRA. Each session of radiation therapy lasted 20 minutes. For all treatments the Hydrosun®-radiator was placed at a distance of 25 cm from and at an angle of 90º to the skin surface on the right side of the abdomen with the left side being covered with a cloth (Fig.1).

Abdominal skin temperature was measured using infrared thermography (Nikon Laird 270 infrared camera, Nikon ltd., Japan). The images were stored electronically. For processing of the IR digital images we used image analysis software PicWin-IRIS (EBS system technik GmbH, München, Germany), which could produce measurements of skin surface temperatures to an accuracy of 0.1º. Prior to each experiment a small piece of silver tape was placed over the naval. This acted as visual reference points during the processing of the thermal images.

Skin blood flow of the entire abdominal area was monitored using a MoorLDI-2 laser Doppler imager (Moor Instruments ltd; England). The scanner was positioned on a shelf placed over the patient at a height that resulted in a total laser beam length (from skin surface to sensor opening) of approximately 90 cm (see Fig.1) The entire scanned area was adjusted to cover an area of approximately 360 cm although this varied slightly from patient to patient. Each scan took approximately 90 seconds to complete. Blood flow values are relative and are presented in perfusion units (PU).

Skin temperature and blood flow measurements were made before, and at 0, 5, 10, and 15 minutes after the period of wIRA treatment. During measurements (before and after irradiation), the coverage on the non-irradiated left side was removed to allow bi-lateral measurements of SBF and temperature. The skin temperature and skin blood flow profiles presented in Figures 2 & 3 were calculated from the stored images. An imaginary, 16 cm long horizontal lines was positioned approximately 5 cm above the naval with each half of the line spanning an equidistant portion of the left and right abdominal wall. The position of these lines and their respective temperature and blood flow profiles during different time points during a single experiment are shown in Fig.2. In addition, mean, minimum and maximum skin temperatures as well as skin blood flow were calculated from a circular area of 5 cm positioned over the centre of the heated and non-heated abdominal skin areas. The respective skin blood flow and skin temperature values were calculated for two time periods – prior to and at the end of the 20 min wIRA heating period.

Results

The results from a single experiment are shown in Fig.2. Here, the changes in skin temperature and skin blood flow at the different stages during the experiment are visualised in the IR-thermograms and the laser Doppler scanning images. The corresponding thermal and skin blood flow profiles have slightly different shapes. The differences in the skin blood flow values between the heated and unheated sides of the abdomen are more abrupt compared to the skin temperature profiles.

The pooled results for the temperature and skin blood flow profiles are presented in Figs 3. For clarity, data from 5 and 10 minutes after the end of the wIRA irradiation have been omitted. Before irradiation, SBF was on average 70 PU

Table 1
Subjects- vital statistics

ID	gender	Age (years)	Height (cm)	Weight (kg)	BMI	smoker
1	female	27	160	66	25.8	no
2	female	38	167	64	22.9	yes
3	female	47	169	63	22.1	no
4	female	30	158	53	21.2	no
5	female	34	170	64	22.1	no
6	female	33	160	56	21.9	no
7	female	52	167	59	21.2	no
8	female	32	154	62	26.1	no
9	female	35	165	59	21.7	no
10	female	43	172	67	2.6	no
Mean		37.1	164.2	61.3	22.8	
SD		7.9	5 .9	4.5	1.8	

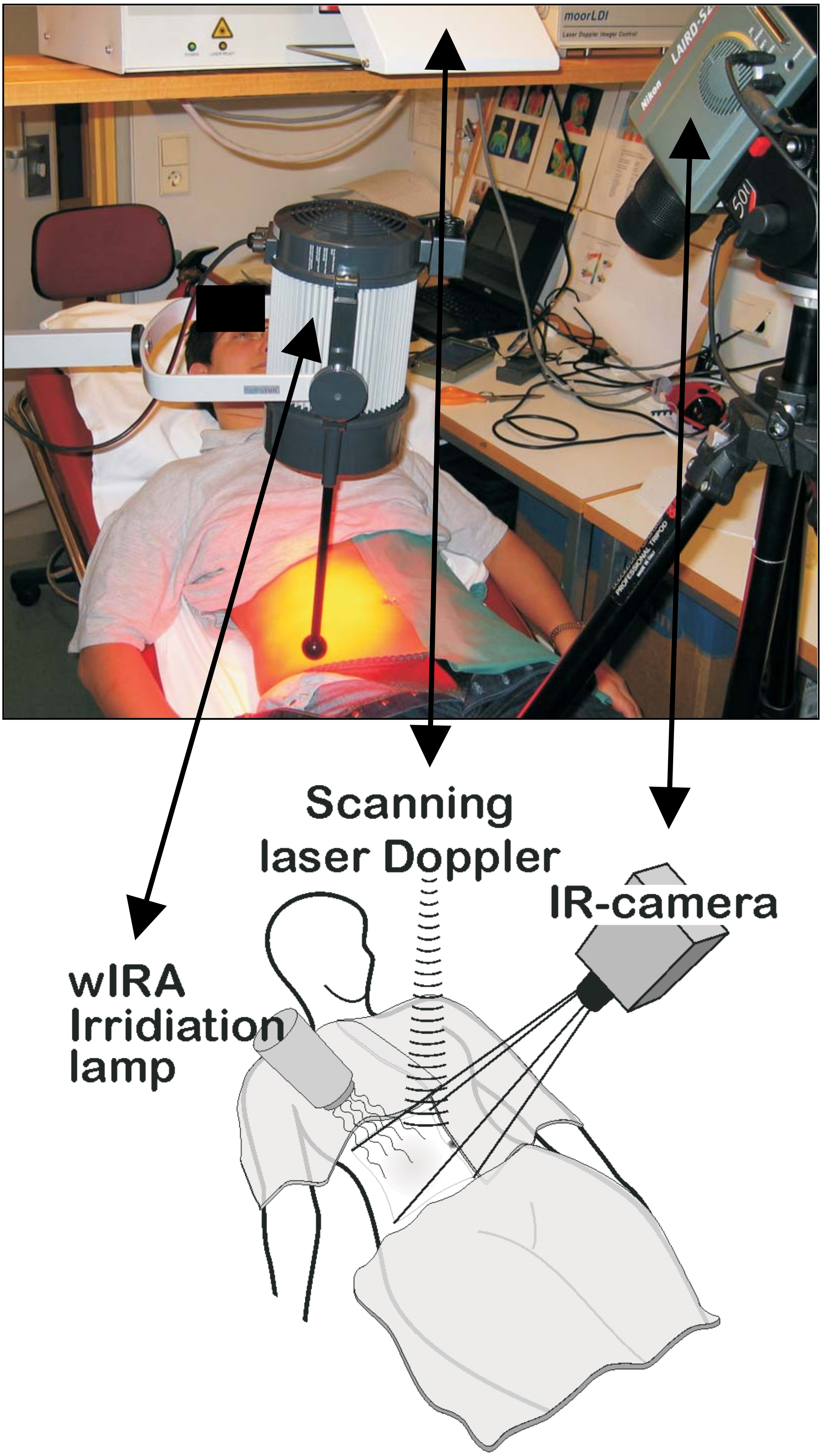


Figure1
Upper panel: Digital picture of setup during an experiment. Note that the left side of the abdomen is covered with a cloth.
Lower panel: Schematic diagram of experimental set up.

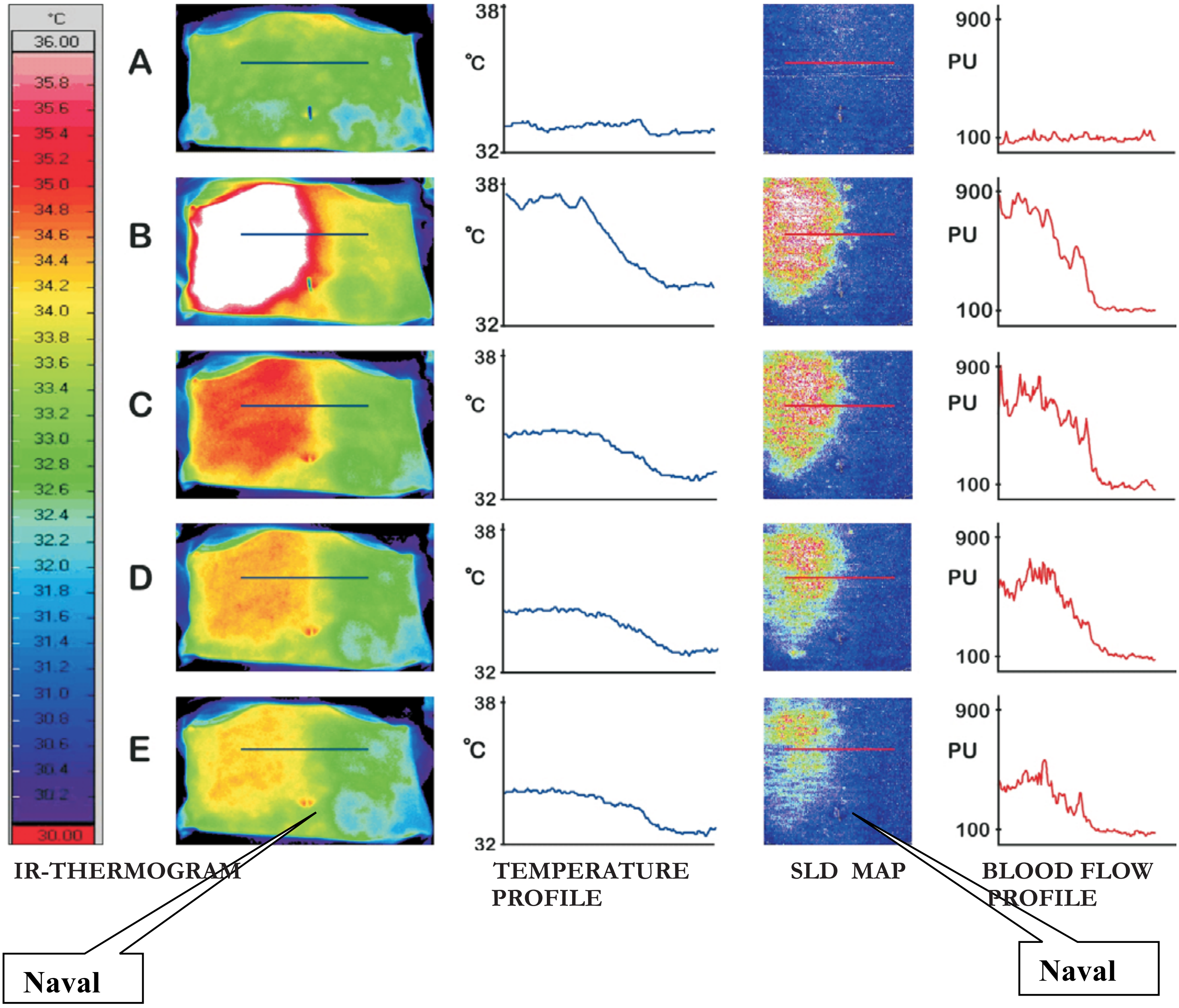


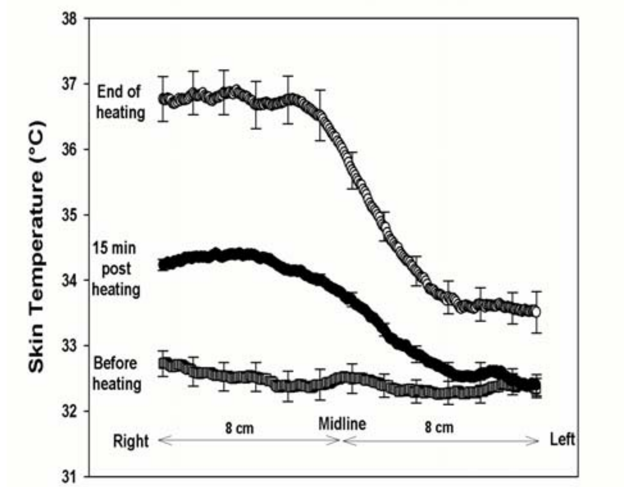
Figure 2
IR-thermograms, Temperature profiles, scanning laser Doppler scans (SLD), and blood flow profiles (perfusion units PU) of the abdominal area of a 44 year old healthy female subject before (A), immediately after (B) and 5 minutes (C), 10 minutes (D) and 15 minutes (E) after a 20 minute heating of the right side of the abdomen with a water filtered infrared-A irradiation lamp. The blue horizontal lines in the IR-thermograms indicate the position of the temperature profiles. The red horizontal lines in the SLD scans indicate the position of the blood flow profiles. In IR-thermogram B the white colour indicates skin temperatures greater than 36°C.

Table 2
Left side of abdomen (unheated side)

	Mean	Minimum	Maximum
Temperature data: Prior to heating	32.3 ± 0.2	31.6 ± 0.2	33.2 ± 0.1
Temperature data: End of heating period	33.9 ± 0.2	33.1± 0.2	35.0 ±0.1
Skin blood flow (PU): Prior to heating	79.4± 6.1	0± 0	546.4± 73.9
Skin blood flow (PU)End of heating period	88.4 ± 8.8	137.7± 32.3	1654.6 ± 678.7

Right side of abdomen (heated side)

	Mean	Minimum	Maximum
Temperature data: Prior to heating	32.5 ± 0.1	31.7± 0.1	33.3 ± 0.1
Temperature data: End of heating period	38.2 ± 0.1	36.8 ± 0.2	39.1 ± 0.1
Skin blood flow (PU): Prior to heating	79.3 ± 5.5	0 ± 0	600.4 ± 84.4
Skin blood flow (PU): End of heating period	659.9 ± 36.9	137.7 ± 32.3	1863.3 ±189.0



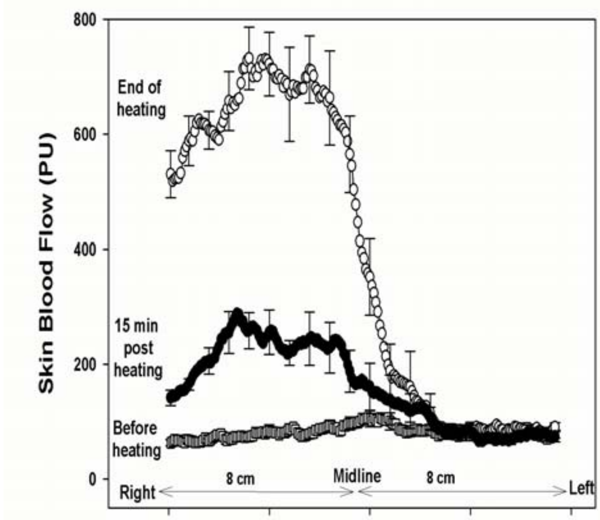


Figure 3
Abdominal skin temperatures profiles (upper panel) and skin blood flow profiles (lower panel) before, at the end of and 15 minutes after a 20 minute period during which the right side of the abdomen was heated by water-filtered infrared-A irradiation. Temperature (C°); Skin Blood Flow (PU – perfusion units.) Values means ''' SEM.

(perfusion units) and average skin temperature was 32.5°C. At the end of the irradiation period, SBF on the irradiated side of the abdomen had increased over 10 fold to ca. 710 PU while average skin temperature increased by 4.5°C to 37°C. The temperature and skin blood flow profiles represent only a small part of the heated area. Therefore, mean, minimum and maximum skin temperatures and skin blood flow over a larger area were also calculated and these results are presented in Table 2. Here, we can see that the respective mean skin temperature and skin blood flow values were very similar between the left and right side of the abdomen prior to heating. At the end of the heating period, the average skin temperature on the heated side of the abdomen had increased to 38.2°C with an average maximum of 39.1°C while mean skin blood flow increased 8.3 fold from 79.3 PU to 659.9 PU. On the non-heated side, there was only a very small increase (79.4 PU to 88.4 PU). This side of the abdomen had been covered by the cloth. Mean skin temperature on the non-heated side of the abdomen increased slightly from 32.3°C prior to heating to 33.9°C at the end of heating. Both SBF and skin temperature on the irradiated side decreased during the post-irradiation period although both were still greater at 15 min post-irradiation than their respective pre-irradiation values (ca. 200 PU and 34.2°C respectively).

Discussion

Prolonged ischemia in free flap surgery and cardio-thoracic surgery causes irreversible tissue damage and complicates surgical procedures. Tissue survival, which can range from complete to partial, depends on the length of ischemia. Pre-conditioning tissue by exposure of cells or tissues to sub-lethal stimuli in order to protect them transiently from a subsequent, normally lethal stress, are well known (3,4 ,5,6). Many forms of pre-conditioning have been investigated, in particular, ischemic, pharmacological and thermal. Pre-conditioning optimally requires a safe and non-injurious method of pre-treatment. The fact that local heating appears to be as effective as systemic heating makes the application of this technique in human subjects particularly appealing. Due to its superficial location, the skin is very easily accessible to heat exposure and local heat application appears to be the simplest and least invasive method for pre-conditioning skin that may be at risk during surgically imitated ischemia.

The results of this study clearly show that water-filtered infrared-A irradiation provides an easy and efficient way of heating the skin to supra-physiological levels without undue discomfort to the subjects. The temperature changes were paralleled by changes in skin perfusion as measured with scanning laser Doppler imaging. Interestingly, although there was a 10-fold increase in skin blood flow on the irradiated side, no changes in skin blood flow were registered on the non-irradiated side. A likely explanation is that this is caused by a large capacity of the vascular bed on the heated side to transport heat. This heat transport appears to be so efficient that there is no need for increase of skin blood flow at the neighbouring non-irradiated side. It is worth noting that the intensity of the infrared-A radiation emitted from the wIRA irradiator is approximately 3 times that emitted from the sun. Without adequate blood circulation to dissipate this high heat load, skin temperatures would rapidly increase to painful and damaging levels. If the non-irradiated side had shown an increase in skin blood flow, this might suggest that the maximum capacity of heat transfer by blood vessels at the irradiated side had been reached. This was not the case, and heating to higher skin temperature levels might therefore be possible without causing tissue damage.

The skin temperature profiles shown in Figs 2 & 3 also showed a clear difference between the irradiated and non-irradiated side of the abdomen. The transition between the irradiated and non-irradiated area was not as well defined for skin temperature as for the skin blood flow profiles. A possible explanation for this slight increase in skin temperature without a concomitant change in skin blood flow might be due to the insulative effect of the cloth which covered the skin on the non-irradiated side of the abdomen during the period of wIRA irradiation.

Minh et al. (10) described two optimal periods of protective effects after hyperthermic pre-conditioning of skin flaps in mice, one at 6hrs and one at 48 hrs.. They conclude that the first period involves the haemodynamics of the skin, because it is well-known that under increased temperature, the cutaneous vessels dilate to promote heat loss. This is supported by the observation of an average increase in skin temperature of 4.5°C that was accompanied by a 8.5–fold average increase in skin blood flow (Table 2). At 24 hours post-heat, such an effect fades away. During the second protective period, Minh et al. (10) suggest that this effect is mediated by the production of heat shock proteins. Recently, McCormick et al. (11) demonstrated that repeated low-level elevations of temperature have a similar preconditioning effect than a sub-lethal thermal stimulus. This effect was associated with an increased expression of heat shock proteins. Moreover, Wang et al. (12) showed that local heating was as effective as systemic heating in inducing the production of heat shock proteins. Although the exact role that heat shock proteins play in this process is unclear, there seems little doubt that they are, in some way, associated with an increase in flap survival.

The results of this study show that wIRA irradiation of the abdominal wall causes a significant increase in skin temperature. In our experiments, which were carried out in healthy subjects, the wIRA irradiator was positioned at the recommended distance of 25 cm from the skin surface. By reducing this distance it is possible to achieve even higher skin temperatures without thermal discomfort (9). These findings combined with the results of other studies showning that local heat treatment of the skin can induce a protective effect on tissue for ischemia suggest that wIRA treatment can be a potential method for thermal pre-conditioning of skin flaps.

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The effect of hyperthermia on the Na+/K+- ATPase activity of follicles from the health thyroid tissues, toxic and non-toxic nodules

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SUMMARY

The aim of this study was to determine the influence of hyperthermia on the activity of thyroid ATPase Na^+/K^+ in thyrocytes isolated from the healthy tissues, toxic- and non-toxic nodular goitre.

10 patients with toxic nodular goitre , non-toxic nodular goiter and 20 patients with pathologically unchanged glands were studied . Na^+/K^+-ATPase activity was defined by measurement of inorganic phosphate released from ATP hydrolysis using the method described by Bartosz et al. The highest Na^+/K^+-ATPase activity was observed for toxic goitre (about 151% of the health tissues activity). For non-toxic goitre it was 83%. This study shows that Hyperthermia significantly reduces enzyme activity in thyroid follicles. The highest decrease of activity was observed in patients with toxic goitre (55%).

Keywords: hyperthermia, thyroid gland, nodular goiter, Na^+/K^+- ATPase activity

EINFLUSS VON HYPERTHERMIE AUF DIE AKTIVITÄT DER NA^+/K^+ ATPASE IN SCHILDDRÜSEN- FOLLIKEL, DIE AUS GESUNDEM GEWEBE, TOXISCHEN ODER NICHT TOXISCHEN KNOTEN STAMMEN

Der Zweck dieser Arbeit ist die Beurteilung des Einflusses der Hyperthermie auf die Na^+/K^+ ATPase in den Schilddrüsezellen, die aus gesunden Gewebe,toxischen und nicht toxischer nodulärer Struma isoliert worden waren.

Das Untersuchungsmaterial wurde von Patienten mit toxischer nodulärer Struma (10 Patienten), mit nicht toxischer nodulärer Struma (10 Patienten) und aus der nicht pathologisch veränderten Schilddrüse (20 Patienten) entnommen. Die Na^+/K^+ ATPase Aktivität wurde aus der ATP Hydrolyse anorganischen Phosphates nach der Methode von Bartosz et al. bestimmt. Die größte Na^+/K^+ ATP Aktivität wurde in toxischen Strumaknoten beobachtet (ca. 151% der Aktivität des gesunden Gewebes). Die Na^+/K^+ ATP Aktivität für den nicht toxischen Knoten betrug 83%. Die Hyperthermie verringerte die enzymatische Aktivität in den Schilddrüsenfollikel bedeutend . Die größte Reduktion der enzymatischen Aktivität wurde in toxischen Strumaknoten beobachtet.

Schlüsselwörter: Hyperthermie, Schilddrüse, Knotenkropf, Na^+/K^+ ATPase Aktivität

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Introduction

Thyroid diseases continue to be a serious therapeutic problem in Poland. Multicentre studies, performed from 1992 to 1993, indicate that iodide deficiency in the majority of Poland, results in a moderate and minor goitre endemia (1).This is related to an increased incidence of patients referred for surgical treatment with the goitre diagnosis, based on a routine diagnostic algorithm (clinical examination, ultrasound - US, fT4, TSH, FNAB). FNAB - fine needle aspiration biopsy, commonly applied in thyroid tumours). Regarding the considerable risk of surgical procedures, and the low probability of misdiagnosis of malignancy after repeated FNABs, it is necessary to continue the search for novel non-surgical methods of the treatment of patients with thyroid nodules. Currently, the most common non-invasive treatment of benign, neutral thyroid tumors is hormonal therapy with thyroxine, whereas in case of hyperfunctioning adenomas thyreostatic and radioactive iodide are applied. Tetracycline injections are used for thyroid cyst obliteration (2). Recently, benign thyroid tumours have been more frequently treated with injections of absolute ethanol (3).

Since the early 1970s, there has been an increased interest in influence of hyperthermia on cells and the clinical application of this technique. Currently hyperthermia is one of novel, non-invasive methods used for the treatment of benign prostate adenomas and chronic prostatitis. The pioneers of this therapy are the Israeli urologists Yerushalmi and Servadio (4). Hyperthermia has also been applied in cancer therapy (5).

Until now, the influence of hyperthermia on neutral or hyperactive thyroid tumours, and also benign and malignant thyroid neoplasms has not been studied in vivo. Amsolik et al. (6) examined the influence of Hyperthermia on the thyroid gland in the rat. No microscopic changes were observed in non-heated tissues. But in heated thyroid lobes changes such as reduction in volume of the thyroid follicle, disturbances of the follicle structure, hemosiderin deposits and diffuse necrosis were noted

It is known that iodide uptake is increased in patients with toxic goitre. The accumulation of iodide (I-) by the thyroid epithelium is a secondary active transport. The recently cloned sodium-iodide symporter (NIS) represents a key molecule for thyroid function. NIS is an integral basolateral cell membrane protein that catalyzes the active transport of I into the follicular cells. The I- ions are co-transported with Na+ ions gradient and is strictly coupled with the activity of thyroid ATPase Na+/K+ (7,8,9,10).

Functional blocking of Na^+/K^+- ATPase leads to reduction of I- uptake and thyroid hormones synthesis. In thyroid gland the sodium-iodide symporter has not only a physiological importance but plays also a key role in diagnostic and treatment of thyroid diseases.

The aim of the present study was firstly to determine Na^+/K^+- ATPase activity of follicles from healthy thyroid tissues, toxic and non-toxic nodules and secondly to study the influence of hyperthermia on the enzyme activity.

Method

Subjects

The tissue material was taken from 20 patients who had had surgery for toxic and non-toxic nodular goitre. There were 10 cases of toxic goitre and 10 cases of non-toxic goitre in the studied group (all patients were female). The thyroid tissue was obtained during surgery from sites with nodular changes and also from healthy tissue.

Isolation of follicles

Thyroid follicles were isolated by a modification of the collagenase digestion / Percoll separation technique of Iwai et al. (11). The fragment of thyroid gland (about 2 g) was cut into small pieces and next crumbled with the preparation needles. The homogenate was suspended in 1 ml of 0.5 mmol/l TRIS-HCL-0.001 mmol/l EDTA buffer, pH 7.4 with 25 mg of collagenase (C-0130- 1g, SIGMA Clostridiopeptidase A: EC 3,4,24,3 from Clostridium histoliticum). The whole homogenate was shaken for 60 minutes at 37° C with the amplitude of 160 rpm. The suspension was centrifuged (700-100 rpm) for 10 minutes. After supernatant separation, the precipitate was washed twice in the same cold TRIS-HCL buffer and fractionated using a Percoll (Co.Nb.G-1.129 g/ml 17-0891-01) density gradient technique in order to remove connective tissue, including blood vessels. Percoll solutions with densities of 1.060 and 1.072, at pH 7.0 and 300 mosmmol / kg were used. The solution carried on Percoll was centrifuged about 30 minutes with 2500 rpm. After centrifugation the follicle layer obtained from the gradient of Percoll was skimmed and incubated at 47° C for 40 minutes.

Measurement of Na^+/K^+- ATPase activity

Na^+/K^+- ATPase activity, a sodium-potassium pump necessary for iodide transport was defined by measurement of inorganic phosphate released from ATP hydrolysis using the method of Veldhoven & Mannaerts (12). Isolated thyroid follicles were homogenized in ice-cooled TRIS-HCL buffer containing (5 mmol TRIS and 0.001 EDTA, pH 7.4). All samples were stored at -20 °C until assay. On the day of assay, the samples were centrifuged at 18000 rpm for 30 minutes at 4 °C enzyme activity was measured.

Na^+/K^+- ATPase activity was defined as the ATP hydrolysis observed in the absences of ouabain minus that observed in the presence of ouabain.

Protein concentration was measured by the method described by Lowry et al. (13).

Statistical analysis

Data are expressed as means ± SD. Differences between groups in mean values were assessed by the Student's t-test for paired multiple comparison procedures. A $p < 0.05$ was considered statistically significant.

Results

Table 1. presents the values of Na^+/K^+-ATPase activity for the healthy thyroid tissues, toxic and non-toxic nodules. There are significant differences observed in enzyme activity between health tissues, toxic and non-toxic goitre (figure.1). The highest Na^+/K^+-ATPase activity was observed for toxic goitre (about 151% of the health tissues activity). For non-toxic goitre it was 83%.

The hyper-functioning goitres are known to synthesize and secrete much more thyroid hormones than normal thyroid

Table 1.
Thyroid Na^+/K^+ ATP-ase activity in toxic, non-toxic goiter and healthy thyroid tissue

	ATP-ase *	% Control
Control	1251.66 ± 180.68	-
SNN	1034.88 ± 121.08	83
SNT	1888.75 ± 297.09	151

(*) ATP-ase activity is expressed as nmol P released/mg protein during 60 minutes. Results are presented as mean ± SD.
Thyroid follicles were incubated at 47 degrees Centigrade for 40 minutes
SNN – non toxic goiter. SNT – toxic goiter, Control – healthy thyroid tissue

Table 2. Effect of hyperthermia on Na^+/K^+ ATP-ase activity in toxic, non-toxic goiter and healthy thyroid tissue

	ATP-ase *	% Change
Control	757.61 ± 169.54	-40
SNN	591.28 ± 129.07	-43
SNT	853.6 ± 133.12	-55

(*) ATP-ase activity is expressed as nmol P released/mg protein during 60 minutes. Results are presented as mean ± SD.
Thyroid follicles were incubated at 47 degrees Centigrade for 40 minutes
SNN – non toxic goiter, SNT – toxic goiter, Control – healthy thyroid tissue

Fig. 1.
Thyroid Na+/K+ ATP-ase activity in toxic, non-toxic goitre and healthy thyroid tissueThyroid follicles were incubated at 47 degrees Centigrade for 40 minutes.
SNN – non toxic goitre, SNT – toxic goitre, Control – healthy thyroid tissue

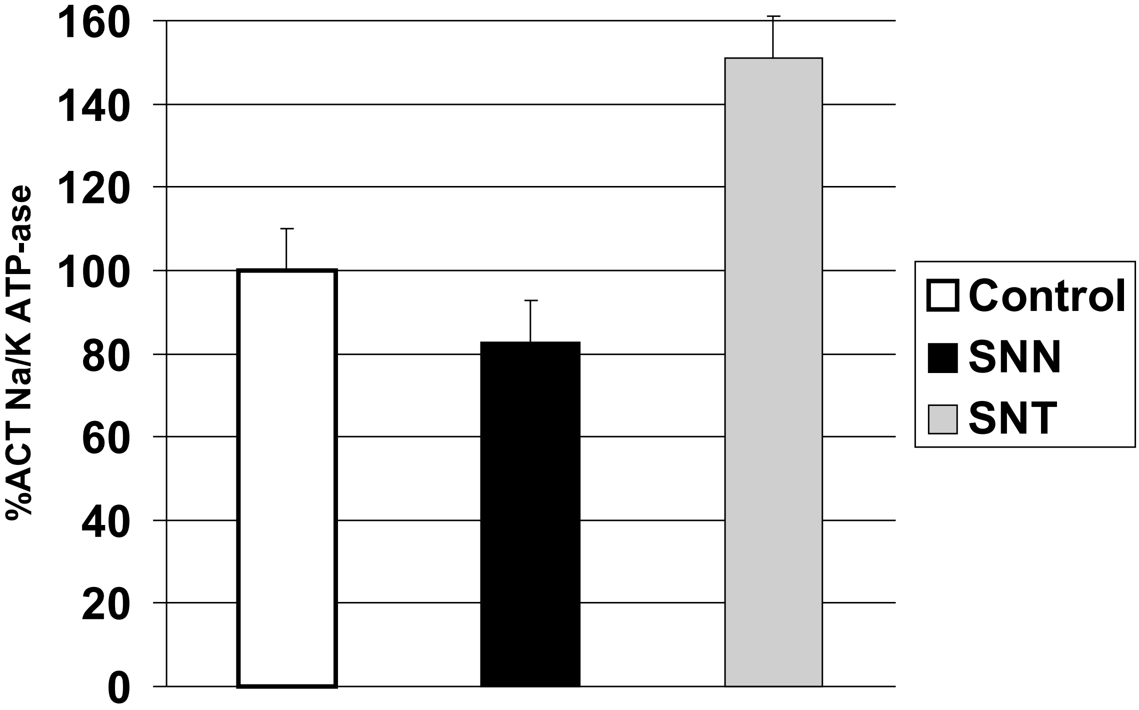
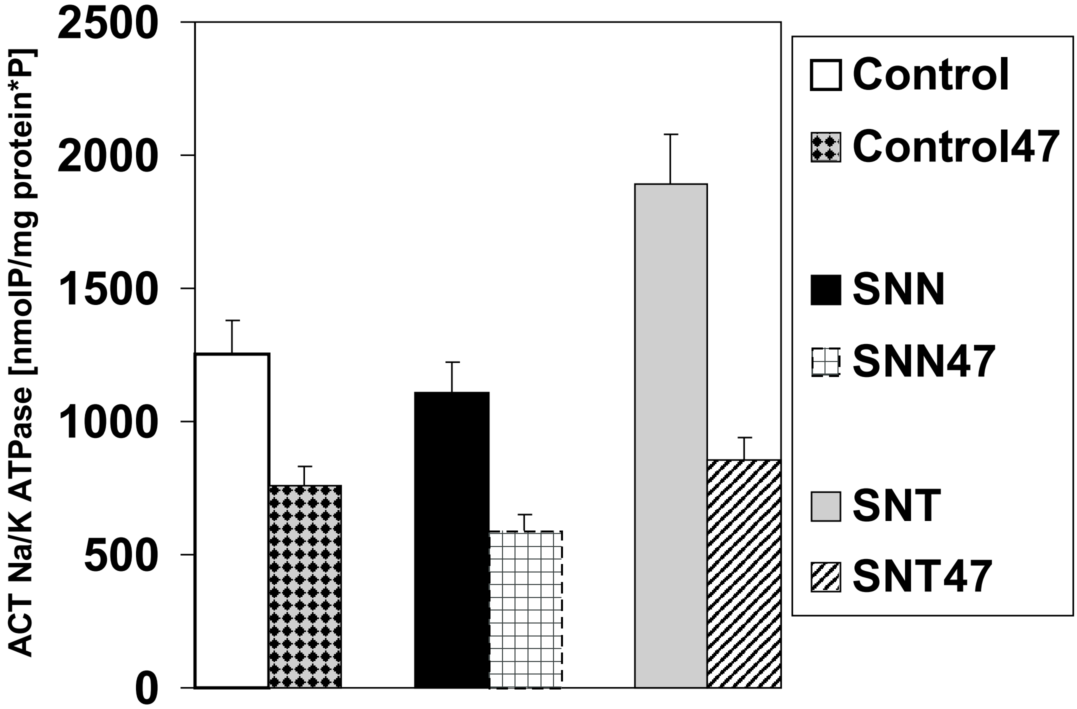


Figure.2
The influence of hyperthermia on Na+/K+ ATP-ase activity in toxic, non-toxic goitre and healthy thyroid tissue
Thyroid follicles were incubated at 47 degrees Centigrade for 40 minutes
SNN – non toxic goitre, SNT – toxic goitre, Control – healthy thyroid tissue, SNN 47 – non toxic goitre incubated at 47 degrees Centigrade for 40 minutes, SNT 47 – toxic goitre incubated at 47 degrees Centigrade for 40 minutes, Control 47 – healthy thyroid tissue incubated at 47 degrees Centigrade for 40 minutes



follicles. For that reason I- uptake in toxic nodules is increased and Na^+/K^+-ATPase activity is higher than in normal tissues and in non-toxic goitre.

Table 2. shows Na^+/K^+-ATPase activity in heat-treated follicles. The thyroid follicles were incubated at 47° C for 40 minutes. Hyperthermia reduced significantly enzyme activity in the health tissues, toxic and non-toxic goiter respectively by 40%, 43%, 55% of non-heated follicles (figure 2). The highest decrease of activity was observed for toxic goitre

Discussion

In a parallel study (in press). we observed, that critical temperature for the follicles viability is 44.7 ° C. In this study we used the temperature of 47 °C, which caused decrease of follicles viability to 10%. We also observed, that heating of follicles at 44.7°C for 40 minutes causes an increase of plasma membrane fluidity of thyrocytes from toxic and non-toxic nodules. The plasma membrane becomes more flexible and the ions transport is disordered (14,15). Bates & Mackillop (16) observed disturbances in the Na^+/K^+-ATPase functioning. Burdon et al. (17) registered a reduction in the cellular level of Na^+/K^+-ATPase of HeLa cells exposed to brief heat shock at 45° C. Mouser LM lung fibroblasts exposed to 44° C were found to release K+ ions and loss of lungs vesicles containing the Na^+/K^+-ATPase (18). However, the activity of this enzyme itself was found to be remarkably heat resistant in HA-1 cells exposed to 45° C for 30 minutes inhibited the binding of epidermal growth factor to the membranes of Rat-1 fibroblasts apparently due to a decrease of affinity of receptors for ligand (19,20). The experiments performed in a animal model (6) provided the positive results. In heated thyroid lobes (45° C, 40 minutes) the following changes were observed: a reduction in volume of the thyroid follicles or atrophy in their lumen and disturbances of the follicle structure. No microscopic changes were observed in tissues surrounding the thyroid and in non-heated lobes. Our studies, show that hyperthermia effects much more toxic and non-toxic nodules (causing increase of plasma membrane fluidity and a significant reduction of Na^+/K^+-ATPase activity by 55% in case of toxic goiter) than health thyroid tissues.

The possibility of limiting the effects of hyperthermia to tissues involved, encourages the use of hyperthermia as a new non-operative treatment modality of thyroid nodules.

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Influence of the field of view on temperature readings from thermal images

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SUMMARY

The aim of this study was to investigate the influence of the shape of measurement areas and of moderate variations in the field of view on the repeatability of temperature readings from thermal images. At the practical session in the 6th Course on Medical Thermal Imaging at the University of Glamorgan, 9 participants measured the temperature from thermal images of the face and the upper back of the same subject, but in slightly different field of view. In the standard view "Face" the region of interest named" left" and "right half of the face" were twice defined based on the protocol of the University of Glamorgan . In the standard view "Upper Back" the circular regions over the shoulder joint and an alternative, polygonal shaped measurement area were defined. Then the mean temperature and standard deviation in these regions of interest were recorded. Reliability coefficients and interclass correlations were calculated and temperature readings were further analysed by non parametric tests.

The results indicated that temperature readings from the circular region of interest over the left shoulder joint in the standard view "upper back"appeared to be better reproducible than an alternatively polygonal shaped measurement area over the deltoid muscle. It was also obtained that moderate variations in the field of view can lead to significant differences of temperature readings from these images.

Key words. thermal image, repeatability, field of view, temperature readings

EINFLUSS DES BILDAUSSCHNITTES AUF DIE TEMPERATURAUSWERTUNG AUS WÄRMEBILDERN

Ziel dieser Studie war es , den Einfluss der Form von Messarealen und von moderaten Varianten des Bildausschnittes auf die Wiederholbarkeit der Temperaturauswertung aus Wärmebildern zu untersuchen. Während der praktischen Übungen beim 6. Kurs über Medizinische Thermographie an der Universität Glamorgan, führten 9 Teilnehmer Temperaturmessungen an Wärmebildern der Standardaufnahme "Gesicht" und "Oberer Rücken, durch, die sich im Bildausschnitt mäßig unterschieden. In der Standardaufnahme "Gesicht" wurden die Messareale mit Namen "linke" und "rechte Gesichthälfte" entsprechend der Standarddefinition der Universität Glamorgan definiert. In der Standardaufnahme "Oberer Rücke" wurden kreisförmige Messareale über dem Schultergelenk und alternativ ein polygonal geformtes Messareal in dieser Region beschrieben. Dann wurden die mittlere Temperatur und die Standardabweichung dieser Messareale aufgezeichnet. Reliabilitätskoeffizienten und Korrelationskoeffizienten in Klassen wurden berechnet und die Temperaturwerte mit Hilfe nicht parametrischer Tests analysiert.

Die Ergebnisse zeigten, das die Temperaturauswertung aus einem kreisförmigen Messareal über dem linken Schultergelenk in der Standardaufnahme "Oberer Rücken" besser reproduziert werden kann als aus einem alternativen, polygonal geformten Messfeld über dem Delta-Muskel. Außerdem wurde gefunden, dass bereits moderate Variationen des Bildausschnittes zu signifikanten Unterschieden in der Temperaturauswertung aus Wärmebildern führen können.

Schlüsselwörter: Wärmebild, Wiederholbarkeit, Bildausschnitt, Temperaturauswertung

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Introduction

It has been shown in previous work, that the repeatability of standard views vary according to the body regions investigated (1,2). For the standard view "face" a high repeatability of the field of view was found, and the standard view "upper back" showed only slightly more variation (2). However, the influence of this variation in the field of view on temperature readings has not yet been determined.

Previous investigation of the reproducibility of temperature readings from defined shapes of regions of interest found high repeatability in shapes which were aligned to the outline of the body region of interest. Temperature readings from the dorsal foot and the soles and also the hour-shaped region over the knee showed reliability coefficients between 0.83 and 0.94, whilst the circular region on the elbow had a reliability coefficient of 0.73 only (2). Therefore, alternatives to circular shaped regions of interest might show a better reproducibility of temperature readings.

To answer the above questions, experiments were performed at the practical session in the 6th Course on Medical Thermal Imaging at the University of Glamorgan, where the 9 participants measured the temperature from thermal images of the face and ´the upper back of the same subject, but in a slightly different field of view.

Methods

The face and the upper back of a volunteer were imaged. After allocation into two groups of 4 and 5 subjects respec-

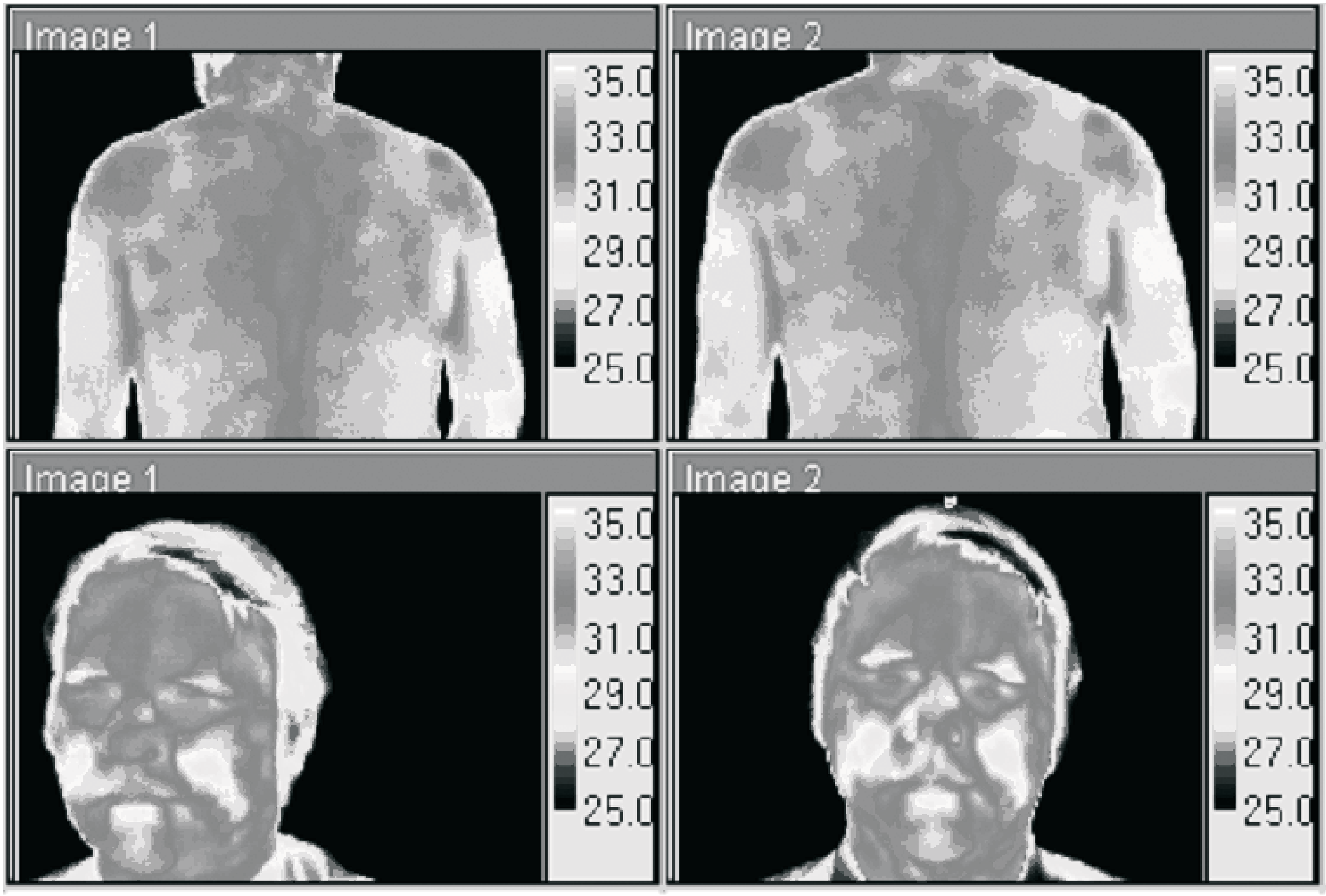


Figure 1
Image 1 and image2 selected from the series of images recorded of the upper back (top line)
Image 1 and image 2 selected from the series of images recorded of the face (bottom line)

tively, each group member was instructed to follow the instructions for standard views used in the protocol of the University of Glamorgan. In the group with 5 members, each then recorded two separate thermal images of the face of the volunteer which were then stored on the hard disk of a an image processing computer. The other group imaged the upper back of the volunteer in a similar way.

From both series of images, the two consecutive images that showed the highest variation in body position were selected for further measurements (Figure 1a-d). In all images the background temperature was not increased to bring the resulting isotherm close to the outline of the object. Therefore any defined measurement area, that crossed the outline of the object, added background temperatures to the results of the measurement.

In the standard view "Face" the region of interest named "left" and "right half of the face" were twice defined based on the protocol of the University of Glamorgan and the mean temperature and standard deviation in these regions of interest were recorded. The repeated determination of temperature was performed in a blind manner as the readers were unable to use any information from the previous measurement.

In the standard view "Upper Back" the circular regions over the shoulder joint and an alternative shaped measurement area were defined (Figure 2). This alternative region of interest was generated by drawing a horizontal line from the lateral outline of the shoulder through the highest point of the posterior axillar fold. Then, a vertical line from the upper outline of the shoulder was created to the highest point of the posterior axillar fold and finally, the vertical and the horizontal line were linked by following the outline of the shoulder. Mean temperature and standard deviation of the regions of interest were recorded and the measurement was repeated in a blinded manner as described above.

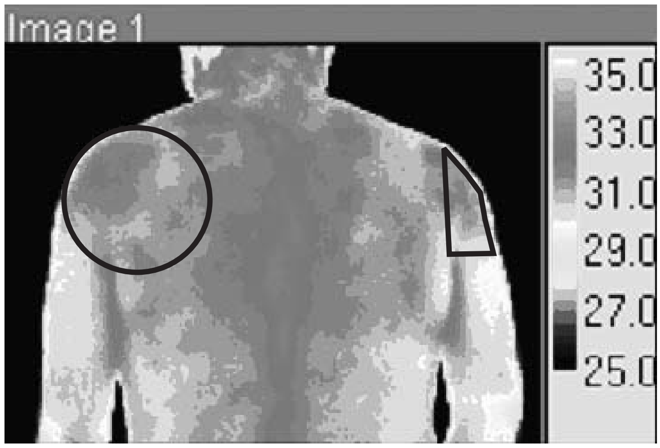


Figure 2
Circular region over the left shoulder and polygonal measdurement area at the right shoulder

Data were analysed with the spreadsheet Software Excel 2000 by calculation of mean, and standard deviation values of all measurements. The same analysis was performed for the measurements of each individual participant. The mean value of all measurements was regarded as being closest to the true value. The individual measurement error was defined by the difference between the mean value of all measurements and the mean value of individual measurements.

Further statistical analysis was performed with the software package SPSS 10, where the reliability coefficient alpha and the average interclass correlation were determined. The recorded values from the regions of interest were compared by non-parametrical tests between image 1 and image 2, and also between first and second measurement. The level for significant differences was set for 2-tailed p=0.05.

Results

Repeatabilty. Upper back

The mean individual error of measurements was small with maximal deviation of 0.13. However, individual errors up to 0.49 °C were found. Figures 3a & 3b show the mean individual errors for the regions of interest at the right shoulder.

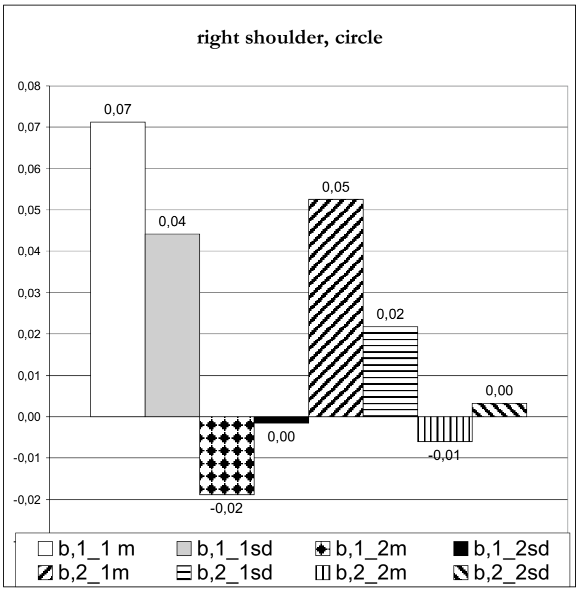
Circle

The reliability coefficient between first and second measurement was high with alpha values of 0.9399 for the left shoulder, but with 0.1385 (poor) for the right shoulder Interclass correlations showed a wide confidence interval (Table 1).

Calculation of reliability coefficients between image 1 and image 2 revealed lower coefficients than the comparison between first and second measurement (Table 2).

Alternative shape-Polygon

The alpha coefficients for polygonal regions of interest was higher than the coefficient of the circular measure-



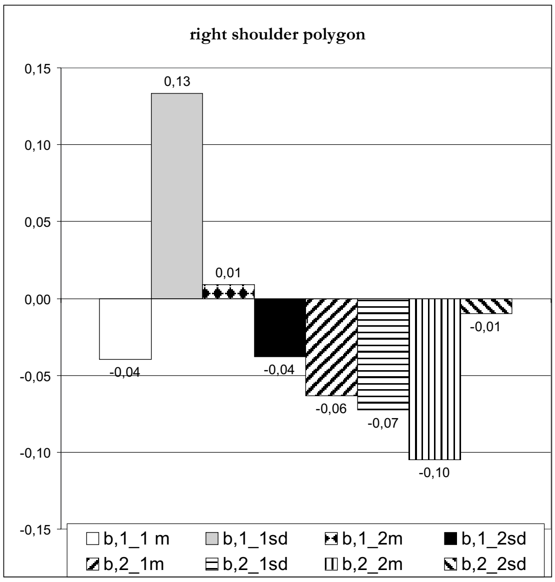


Figure 3
Mean individual errors in the circular and the polygon region of interest over the right shoulder
b,1-1, m: Image 1, 1st measurement, mean value
b,1-2, m: Image 1, 2nd measurement, mean value
b,2-1, m: Image 2, 1st measurement, mean value
b,2-2, m: Image 2, 2nd measurement, mean value
b,1-1, sd: Image 1, 1st measurement, standard deviation
b,1-2, m: Image 1, 2nd measurement, standard deviation
b,2-1, sd: Image 2, 1st measurement, standard deviation
b,2-2, m: Image 2, 2nd measurement, standard deviation

Table 1
Reliability coefficient alpha and average interclass correlation comparing measurement 1 and measurement 2

	Reliability coefficient	interclass correlation	
	Standardised item alpha	average	95% confidence interval
Right shoulder circle, mean value	0.1385	0.1407	-1.3702 to 0.6823
Right shoulder circle, standard deviation	0.6622	0.6569	0.1082 to 0.8703
Left shoulder circle, mean value	0.9399	0.9420	0.8447 to 0.978
Left shoulder circle, standard deviation	0.3901	0.4012	-0.7006 to 0.7808
Right shoulder polygon, mean value	0.7014	0.7004	0.1503 to 0.8925
Right shoulder polygon, standard deviation	0.5819	0.5670	-0.1236 to 0.8395
Left shoulder polygon, mean value	0.5829	0.5648	-0.3161 to 0.8507
Left shoulder polygon, standard deviation	0.4652	0.4778	-0.5966 to 0.,8216
Right half of the face, mean value	0.9745	0.9440	0.9217 to 0.9895
Right half of the face, standard deviation	0.0175	0.0185	-2.0300 to 0.6576
Left half of the face, mean value	0.8813	0.8725	0.6541to 0.9535
Left half of the face, standard deviation	0.0175	0.0502	-1.4580 to 0.6475

Table2
Reliability coefficient alpha and average interclass correlation comparing image 1 and image 2

	Reliability coefficient	interclass correlation	
	Standardised item alpha	average	95% confidence interval
Right shoulder circle, mean value	-1.0172	-0.6320	-4.3942 to 0.7972
Right shoulder circle, standard deviation	0.6486	0.6501	0.0313 to 0.8708
Left shoulder circle, mean value	0.4863	0.4827	-0.4445 to 0.8095
Left shoulder circle, standard deviation	0.4045	0.3836	-0.4936 to 0.7602
Right shoulder polygon, mean value	0.6041	0.5901	-0.1033 to 0.8536
Right shoulder polygon, standard deviation	0.5055	0.5172	-0.4654 to 0.8346
Left shoulder polygon, mean value	0.1303	0.1029	-0.9412 to 0.6468
Left shoulder polygon, standard deviation	0.3385	0.3434	-0.9009 to 0.7715
Right half of the face, mean value	0.8055	0.7913	0.4243 to 0.9261
Right half of the face, standard deviation	-0.0116	-0.0039	-1,3148 to 0.6170
Left half of the face, mean value	0.6823	0.6746	0.0926 to 0.8853
Left half of the face, standard deviation	0.0094	0.0026	-1.8241 to 0,6502

ment area at the right shoulder, but lower at the left shoulder. Repeatability of polygons was similar affected as circular regions of interest in the comparison between image 1 and image 2.

Repeatability-Face

Determination of mean temperature from regions of interest of the face showed the highest reliability coefficients. However, the reliability of of the standard deviation of this highly reproducible mean values was poor (alpha between 0.0175 and 0.0175).

Non-parametric tests

Comparison first and second measurement

The only significant differences between first and second measurements were detected for the standard deviation of the left half of the face in image 2 (2-tailed p=0.041) and the standard deviation of the polygon at the right shoulder in image 1.

Comparison image 1 and image 2

The Mann-Whitney Test revealed twice significant differences in measurements from image 1 and image 2. The first measurement of the mean value of the polygon at the left shoulder (2-tailed p=0.04) and the standard deviation of the mean temperature of right half of the face appeared to be different at the second measurement. The mean temperature of the circular region of interest at the right shoulder slightly failed significance (2-tailed p= 0.057) at the second measurement.

Discussion

Different to previous investigations, the region of interest following the outline of the anatomical region were less reproducible than a circular measurement area at the left shoulder. The poor reproducibility of the polygonal measurement area may be caused by the difficulty to follow the outline of the object correctly. If this is the case, the polygonal measurement area should appear with higher standard deviation of the measurement area than the circular region of interest (Figure 3), because the circle will acquire less background temperature pixels than the curved side of the poly gon.

However, this does not explain the large difference in repeatability of temperature readings from circular regions of interest at the right and the left shoulder. Differences in the field of view between image 1 and image 2 may have contributed to poor reproducibility. Although significant differences in temperature readings from the first and the second measurement were only detected for the standard deviation of the mean value, the mean value of the polygonal region at the shoulder was significantly, and the circular region of the right shoulder nearly significantly different between moderate variations of the field of view. The contribution of the field of view to the reproducibility of temperature readings from thermal images is further supported by lower reliability coefficients between images than within the same image.

The high repeatability of the standard view face (3) is confirmed by high reliability coefficients and a narrow 95% confidence interval of temperature readings from half of the face. The poor repeatability of the standard deviation of the mean temperatures of the face is probably caused by variation and structures in the background such as hair on the edge of the region of interest.

Conclusion

Moderate variations in the field of view can lead to significant differences of temperature readings from these images.

Temperature readings from the circular region of interest over the left shoulder joint in the standard view “upper back”appeared to be better reproducible than an alternatively polygonal shaped measurement area over the deltoid muscle.

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Effect of hyperthermia on the viability and plasma membrane fluidity of follicles from the healthy thyroid tissues and non-toxic nodules

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SUMMARY

The aim of the study was to determine the influence of hyperthermia on the viability and plasma membrane fluidity of thyrocytes isolated from healthy tissues and toxic and non-toxic nodular goitre.

The material studied was taken from patients operated for toxic and non-toxic nodular goitre. The thyroid tissue was obtained during surgery from nodular changes and from sites of the gland pathologically unchanged. Thyroid follicles were isolated by a modification of the Iwai et al. method. (14) After isolation, one half of the follicles were incubated in elevated temperatures of 37ºC, 42ºC, 43ºC, 45ºC, 47ºC and 50ºC for 40 min. Follicular viability as a percentage was measured by the 0.1% Trypane blue method. No significant difference was observed in the survival of follicles between the toxic and non-toxic nodular goitre at the range of temperatures studied. The critical temperature of follicular viability obtained from the Arrhenius plot was 44.7ºC.

Fluidity of the plasma membrane of thyrocytes was measured by electron paramagnetic resonance spectroscopy using two spin labels: 5 DSA and 16 DSA. We observed that the plasma membrane of thyrocytes from toxic nodular goitre is more flexible than in the case of healthy tissues; the plasma membrane of thyrocytes from non-toxic nodular goitre has more stiffness.

Hyperthermia (47ºC, 40 min) induced a significant increase in fluidity of thyrocytes plasma membrane from the healthy tissues, toxic and non-toxic nodular goitre for the probes labelled 5 DSA. An increase of plasma membrane fluidity in the hydrophobic core was also observed in cases of toxic and non-toxic nodules.

Keywords: hyperthermia, thyroid gland, nodular goitre, plasma membrane fluidity, viability

EINFLUSS VON HYPERTHERMIE AUF DIE LEBENSDAUER UND FLUIDITÄT VON PLASMAMEMBRANEN IN SCHILDDRÜSENFOLLIKEL AUS GESUNDEM GEWEBE ODER NICHT TOXISCHEN KNOTEN

Der Zweck dieser Arbeit ist die Beurteilung des Einflusses der Hyperthermie auf die Lebensfähigkeit und die Fluidität der Plasmamembran von Schilddrüsezellen, die aus gesunden Gewebe bzw. aus toxischer und nicht-toxischer Struma isoliert worden waren.

Das Forschungsmaterial stammte von Patienten, die wegen toxischer ooder nicht-toxischer Struma operiert worden waren Das Gewebe wurde intraoperativ aus nodulären Veränderungen und aus nicht pathologisch veränderter Schilddrüse entnommen. Die Schilddrüsefollikel wurde nach einer modifizierten Methode von Iwai et al. (14) isoliert. Nach der Isolation wurde die Hälfte von den isolierten Follikel in ansteigenden Temperaturen von 37° C, 42° C, 42° C, 45° C, 47° C und 50° C 40 Minuten lang inkubiert. Die perzentuelle Lebensfähigkeit der Schilddrüsefollikel wurde mit 0,1% Trypanblau beurteiltet. Es wurde keine bedeutende Unterschiede im Überleben der Follikel aus toxischen und nicht toxischer Struma bei den untersuchten Temperaturen beobachtet. In der Arrheniuskurve fand sich eine kritische Temperatur von 44,7°C für die Lebensfähigkeit der Follikel.

Die Fluidität der Plasmamembran wurde mittels Paramagnetic Resonance Spectroscopy bei Markierung mit 5 DSA bzw. 16 DSA beurteiltet. Es wurde beobachtet, dass die Plasmamembran der Schilddrüsezellen aus toxischen Struma elastischer ist.als aus dem gesunden Gewebe Die Plasmamembran der Zellen aus der toxischen Struma ist unbiegsamer. Die Hyperthermie (47° C, 40 Minuten) verursacht eine Zunahme der Fluidität der Plasmamembran aus gesunden Gewebe, aus toxischer und nicht toxischer Struma in dem mit 5 DSA markierten Proben. Die Zunahme der Fluidität der Plasmamembran der hydrophoben Schicht wurde bei Zellen aus toxischer und nicht toxischer Struma gefunden.

Schlüsselwörter: Hyperthermie, Schilddrüse, Knotenkropf, Fluidität der Plasmamembran, Lebensfähigkeit

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Introduction

Two different aspects of the cellular response to elevated temperatures, both of which have evolved over the last three decades, have motivated studies on the effect of hyperthermia. On the one hand, renewed interest in hyperthermia as a possible modality for cancer therapy has focused research on the phenomenon of cell destruction by hyperthermia. Thus, a significant effort has been extended towards documenting and analysing the pleiotropic effect of hyperthermia to uncover the mechanisms of heat-induced cell death. Currently hyperthermia is one of the novel, non-invasive methods used for the treatment of benign prostate adenomas and chronic prostatitis (30). Hyperthermia has also been applied as anti-cancer therapy (9). It has been shown that the simultaneous use of pharmacotherapy and hyperthermia increases the activity of anti-cancer drugs (6, 12). Until now, the influence of hyperthermia on neutral or hyperactive thyroid tumors has not been studied in vivo. The existing reports refer to the influence of elevated temperatures on thyroid cancer cells in vitro. These experiments revealed that thyroid cancer cells heated at 44ºC for 20 minutes and subsequently incubated at 37ºC for 18 hours displayed a significant increased tendency to spontaneous lysis in response to allogenic T lymphocytes (10). Another study describes the influence of temperature 42,5ºC within a 90-minute period in vitro on isolated thyroid cancer and normal thyroid cells. Hyperthermia inhibited proliferation and increased protein expression (hsp72, thyroglobulin) (29). The influence of hyperthermia on the thyroid gland in the rat was studied by Amsolik et al. (3). In this study both lobes were heated at 45ºC for 45 minutes. A reduction in the volume of the thyroid follicles or atrophy of their lumen was observed. No microscopic changes were observed in the tissues surrounding the thyroid. The mechanism of cell damage induced by hyperthermia has not been precisely known. Heating causes disturbances in cell functions leading to their death. Many authors think that the plasma membrane and the nucleus are the main targets of cell damage (13, 16, 17). The range of damage induced by heating depends on both the incubation time and temperature (11). Temperatures in the range of 41-46ºC cause changes in the function and structure of plasma membranes. Hyperthermia has been shown to induce an increase in plasma membrane permeability, which leads to disturbed transport of ions and other compounds (5, 7). Several studies, using different probes, have demonstrated that changes in the lipid components are probably not directly involved in heat-induced cell destruction (21, 18, 23).

The aim of this study was to determine the influence of evaluated temperatures on the viability and plasma membrane fluidity of thyrocytes isolated from health thyroid tissue and toxic and non-toxic goitre, respectively.

Methods

Subject

30 patients who had undergone surgery for toxic and non-toxic nodular goitre were studied. There were 15 cases of toxic and 15 cases of non-toxic nodules in this group. The age of the patients was from 23 to 77 years. The thyroid samples were obtained during surgery from nodular changes and from sites of the gland that were pathological unchanged.

Isolation of follicles

Thyroid follicles were isolated by a modification of the method of Iwai et al. (14). The thyroid glandular tissue was minced with scissors and then crumbled with preparation needles. The homogenate was suspended in 1 ml of 0,5mmol/ l TRIS-HCl-0.001mmol/l EDTA buffer, pH 7.4 containing 25 mg/ml collagenase (C-0130-1g, SIGMA Clostridio- peptidase A : EC 3.4.24.3 from *Clostridium histoliticum*). The minced thyroid samples were shaken for 60 min. at 37ºC with frequent agitation at 160/min. The suspension was centrifuged (700-1000 rpm) for 10 min., washed twice in the cold TRIS-HCl buffer. At this stage, a mixture of small pieces of connective tissue, floating cells and follicles were obtained in suspension. Discontinuous gradients of Percoll (Co.Nb.g – 1.129 g/ml 17-0891-01) with respective densities of 1.060 and 1.072 were prepared and the suspension was added above the top layer. The tube was centrifuged at 1000 rpm for 30 min. The cells at the interface between the solutions at densities of 1.060 and 1.072 were collected and centrifuged (700-1000 rpm for 10 min.). After centrifugation the thyroid follicles were incubated in elevated temperatures of 37ºC, 41ºC, 42ºC, 43ºC, 45ºC, 47ºC, 50ºC for 40 min.

Measurement of the viability of the thyroid follicles

For the assessment of survival of thyroid follicles the colouring method with 0.1 % Trypane blue was used. The final proportion of dead or damaged follicles and alive follicles was evaluated with the microscope and a Bürker chamber always using the same volumes of dye and the follicle homogenate.

Fluidity of plasma membrane of thyrocytes spin label study

Fluidity of plasma membrane of thyrocytes was measured by electron paramagnetic resonance spectroscopy (EPR) using two spin labels, which reside at different depths within the lipid bilayer: 5-doxylstearate (5-DSA) and 16-doxylstearate (16-DSA) (Sigma Chemical St. Louis Missouri USA). The cells were labelled with these two spin labels to final concentration 2 mmol/l and then incubated for 30 min. at room temperature. EPR measurements were performed at a temperature of 20-21ºC in a Brucker 300 Spectrometer.

From the spectrum of 5-DSA an order parameter S was derived by measuring the outer and inner hyperline splitting $2T||$ and $2T|$. The order parameter S is a measure of the distribution of molecular orientations with respect to a reference axis, which in this study was chosen to be normal to the surface of the membrane. An increase in order parameter reflects a decrease in the segmental flexibility of the spin probe. The order parameter S is given by the following equation:

$$S = \frac{T_{II} - T_{\perp}}{T_{ZZ} - \frac{1}{2}(T_{XX} + T_{yy})} * \frac{a}{a'}$$

Where: S is the order parameter; and

$$a = \frac{1}{3}(T_{XX} + T_{YY} + T_{ZZ})$$

is the isotropic hyperfine constant for nitroxide in a crystal; and

$$a' = \frac{1}{3}(T_{II} + 2T_{\perp})$$

is the isotropic hyperfine coupling constant for nitroxide in a membrane.

TXX,TYY TZZ, are the hyperfine splitting parameters determined after the incorporation of nitroxide derivatives into a host crystal, with
TXX = 6.1G, TYY = 6.1G, TZZ = 32.4G,

TII is the hyperfine splitting constant for the magnetic field parallel to the normal to the bilayer.

T⊥is the hyperfine splitting constant for the magnetic field perpendicular to the bilayer normal.

For the other spin label – 16 DSA, rotational correlation times τB and τC were measured.

τB describes motion in the direction perpendicular to the long axis, is given by equation (28, 19):

$$\tau_B = 6.5x10^{-10} * \Delta W \left[\left(\frac{h_0}{h_{+1}}\right)^{\frac{1}{2}} - \left(\frac{h_0}{h_{-1}}\right)^{\frac{1}{2}} \right]$$

and τC describes motion in the direction parallel to the long axis is given by equation (28, 19):

$$\tau_C = 6.5x10^{-10} * \Delta W \left[\left(\frac{h_0}{h_{+1}}\right)^{\frac{1}{2}} + \left(\frac{h_0}{h_{-1}}\right)^{\frac{1}{2}} - 2 \right]$$

Where: h+1 is the low-field height;h0 is the mid-field line height, h-1 is the high-field height; ΔW is the mid-field line width..

The correlation times are proportional to the flexibility of the probe. An increase in their values indicates reduced fluidity of the bilayer in the vicinity of a spin label.

Statistical analysis

Data are expressed as means ± SD. Differences between groups were assessed by the Student's t-test for pairwise multiple comparison procedures. A p value < 0,05 was considered statistically significant.

Results

Measurement of the viability of the thyroid follicles

The obtained results are presented as a percentage of survival of the follicles in particular temperatures (37ºC, 42ºC, 43ºC, 45ºC, 47ºC, 50ºC) with constant heating time of 40 min.

Table 1. shows the percentage of follicles viability isolated from health thyroid tissues and toxic and non-toxic nodules. The results of 15 patients with the histopathological diagnosis of toxic or non-toxic nodules were compared with the healthy tissues of the same of patient (Fig.1).

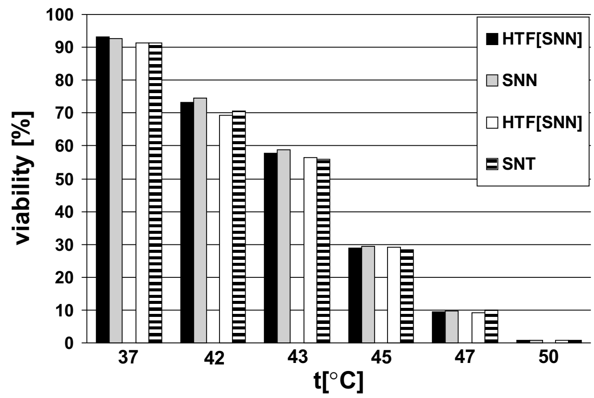
No significant difference was observed in survival of follicles between the toxic and non-toxic nodular goitre at the studied temperatures. The critical temperature of follicles

Table 1
The percentage of thyroid follicles viability.

t [°C]	HTF [SNN] %	SNN %	HTF[SNT] %	SNT %
37	91.5 ± 1.8	91.3 ± 1.7	93.1 ± 1.6	92.6 ± 1.6
42	69.1 ± 2.3	70.8 ± 2.6	73.2 ± 1.7	74.5 ± 1.6
43	56.5 ± 2.0	55.8 ± 3.6	57.8 ± 2.7	58.9 ± 3.3
45	29.1 ± 2.1	28.3 ± 3.9	28.9 ± 1.5	29.5 ± 2.1
47	9.1 ± 0.9	9.95 ± 1.3	9.5 ± 1.4	9.72 ± 1.2
50	0.77± 0.4	0.8 ± 0.4	0.68 ± 0.5	0.77 ± 0.4

The % of follicles viability was determined for the healthy tissues (HTF [SNN] – from patients operated for non-toxic nodular goiter and HTF [SNT] - from patients operated for toxic nodular goiter) and toxic (SNT) and non-toxic nodular goiter (SNN). The results are given as mean +/- SD of 15 determinations per group..

Figure. 1
The percentage of thyroid follicles viability.



The thyroid follicles were incubated in elevated temperatures of 37ºC, 42ºC, 43ºC, 45ºC, 47ºC and 50 °C for 40 min.
SNN - non-toxic nodular goiter, SNT - toxic nodular goiter, HTF [SNN] – healthy tissue from patients operated for non-toxic nodular goiter, HTF [SNT] - healthy tissue from patients operated for toxic nodular goiter

viability obtained from the Arrhenius graphs (Fig. 2a, b, c, d) was 44,7 ºC. This value was the same for the follicles from healthy thyroid tissues and toxic and non-toxic nodules.

Fluidity of plasma membrane of thyrocytes spin label study

Fluidity of plasma membrane of thyrocytes was determined in the heated (47 ºC, 40 min) and non-heated follicles.

An order parameter S and the rotational correlation times τB and τC were determined in the study of fluidity of plasma membrane of spin label thyrocytes.

No significant difference was observed in fluidity at the 5th depth of carbon atom in the fatty acid chains of phospholipids for thyrocytes from the healthy tissues and toxic and non-toxic nodular goitre (Fig.3). The spin label 16 DSA monitors the fluidity changes in the hydrophobic core of membrane. The results are presented on the graph (Fig. 4a, b). Using this spin label, we observed that the plasma membrane of thyrocytes from toxic nodules is more flexible than from healthy tissues, the plasma membrane of thyrocytes from non-toxic nodules is stiffer

Hyperthermia (the temperature of 47ºC, 40 min) induced a significant increase in fluidity of thyrocytes plasma membrane from the healthy tissues, toxic and non-toxic nodules for the probes labelled 5 DSA (parameter S) (Fig. 3). An increase of plasma membrane fluidity in hydrophobic core was also observed in case of toxic and non-toxic nodules (Fig. 4a, b)

Discussion

In the consideration of heat-induced cell killing, two aspects must be illustrated. Firstly, the total cell killing is a complex function of time/temperature combinations, which have been modelled with a single parameter (22, 25).The effects of lower temperatures (<42.5° C) are different than those of higher temperatures (>42.5°C). Secondly, there is a great difference between different species in their inherent thermosensitivities (25).

Schreck et al., (27) registered that temperature of 43°C is critical for the viability of human normal and leukaemic lymphocytes. Similar results for pig lymphocytes were obtained by Koter (20). In this study the critical temperature of viability (for healthy thyroid tissues, toxic and non-toxic nodular goiter) was 44.7°C. This may arise from the more complicated structure of follicles, which are surrounded by monolayer of thyrocytes. Such a complex of cell may be more resistant to elevated temperatures than that of other nucleid cell Andrianakis et al. (4) examined the effect of 40.4°C on thyroid structure in the late gestation foetal lamb causing increase of intra-follicular colloid area and decrease of epithelial cell height. Fujieda et al., (10) registered that the susceptibility of heat-treated thyroid cancer cell (44°C for 20 minutes, and incubation at 37°C for 18 hours) to lysis by autologous and allogenic lymphokine-actvated killer cell was significantly greater than that of untreated tumor cells. Airoldi et al., (1) observed a decrease of serum thyroid hormone in head and neck cancer patients treated by hyperthermia (42-45°C).

In membranes of mammalian cells there is sharp transition temperature in physiological temperature ranges (30-50°C), but a smooth change in fluidity between extreme temperature limits, with relatively rigid membranes at low temperatures and relatively fluid membranes at high temperatures (21). During heating, the lipid bilayer becomes more flexible. We observed no differences in fluidity at the 5th depth of carbon atom in the fatty acid chain of phospholipids of plasma membrane of thyrocytes from the healthy tissues, toxic and non-toxic nodules. However, in the hydrophobic core of plasma membrane it was observed that in the case of toxic nodules, the plasma membrane is more flexible than healthy tissues and the plasma membrane of thyrocytes from non-toxic nodules is less flexible.

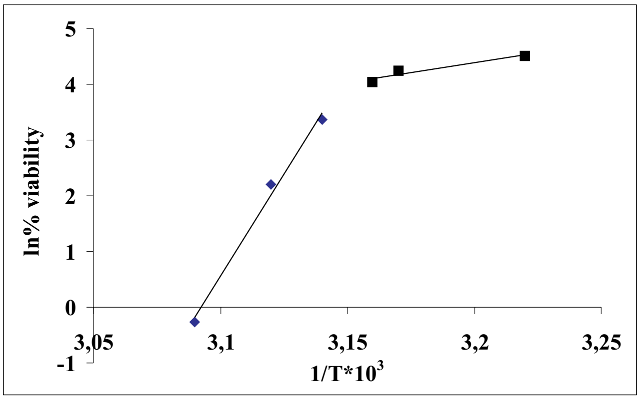


Figure.2a
The Arrhenius graph for the thyroid follicles isolated from the healthy tissues of patients operated for non-toxic (HTF [SNN]). The critical temperature of the follicles viability was determined from this graph. It was 44.7 °C.

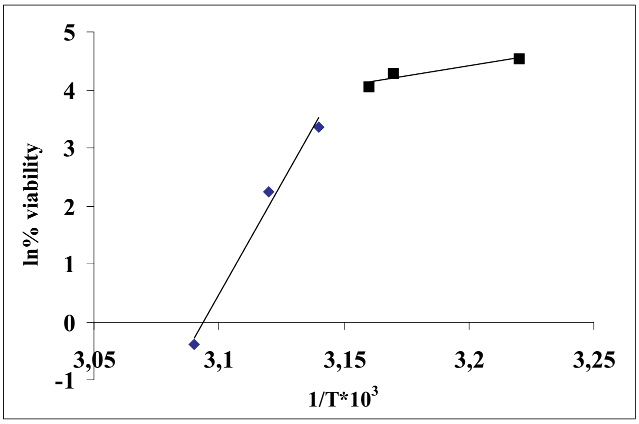


Figure.2c
The Arrhenius graph for the thyroid follicles isolated from the healthy tissues of patients operated for toxic nodular goitre (HTF [SNT]). The critical temperature of the follicles viability was determined from this graph. It was 44.7 °C

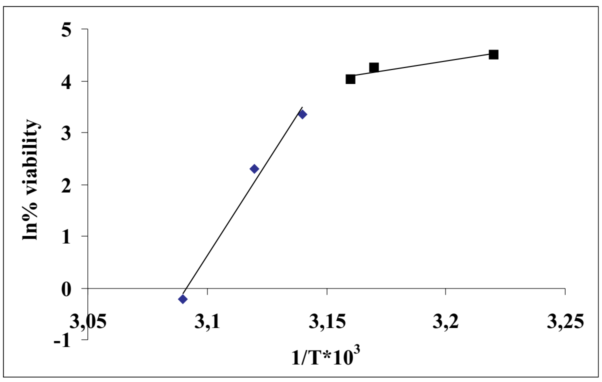


Figure 2b
The Arrhenius graph for the thyroid follicles isolated from patients operated for non-toxic nodular goitre (SNN). The critical temperature of the follicles viability was determined from this graph. It was 44.7

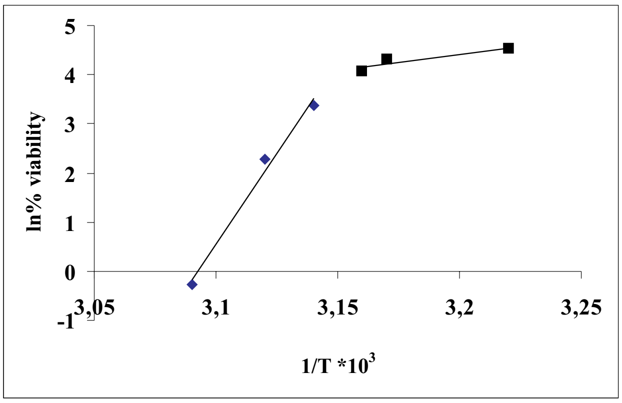


Figure 2d
The Arrhenius graph for the thyroid follicles isolated from patients operated toxic nodular goitre (SNT). The critical temperature of the follicles viability was determined from this graph. It was 44.7 °C.

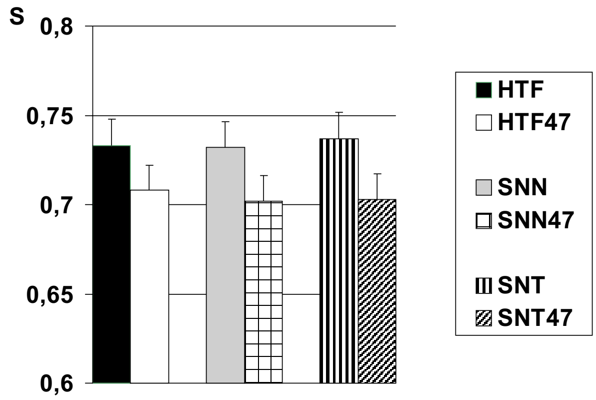


Figure 3
The effect of hyperthermia on the fluidity at the 5th death of carbon atom in the fatty acid chains of phospholipids for thyrocytes from the healthy tissues (HTF) and toxic (SNT) and non-toxic nodular goitre (SNN). The follicles were incubated at 47 °C for 40 min. (HTF47, SNN47, SNT47).
From the spectrum of 5DSA an order parameter S was determined. The results are given as mean ± SD of 15 determinations per group and compared with non-heated follicles of the same patients.

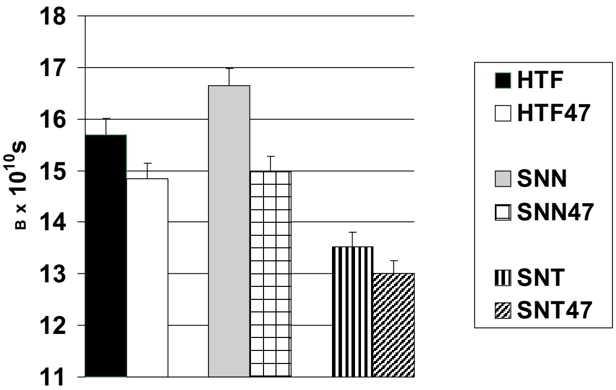


Figure4a
The effect of hyperthermia on the fluidity in hydrophobic core of plasma membrane of thyrocytes isolated from the healthy tissues (HTF) and toxic (SNT) and non-toxic nodular goitre (SNN). Incubation of follicles was carried at 47°C for 40 min.. (HTF47, SNN47, SNT47). For 16 DSA the rotational correlation times τB was measured. The results are given as mean ± SD of 15 determinations per group and compared with non-heated follicles of the same patients.

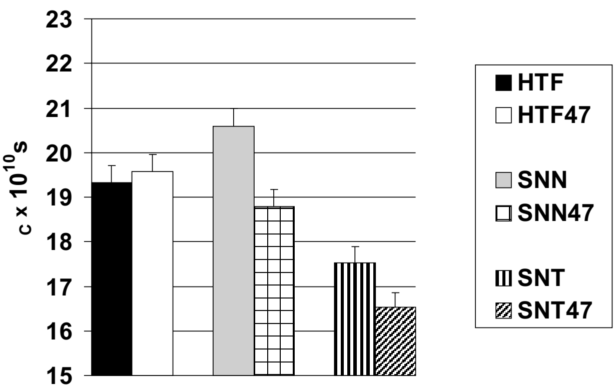


Figure.4b
The effect of hyperthermia on the fluidity in hydrophobic core of plasma membrane of thyrocytes isolated from the healthy tissues (HTF) and toxic (SNT) and non-toxic nodular goitre (SNN). Incubation of follicles was carried at 47°C for 40 min.. (HTF47, SNN47, SNT47). For 16 DSA the rotational correlation times τC was measured. The results are given as mean ± SD of 15 determinations per group and compared with non-heated follicles of the same patients.

Heating at 47°C for 40 minutes caused a significant increase of plasma membrane fluidity for erythrocytes heating at 44°C (15, 20). Hyperthermia causes an increase of plasma membrane permeability, which leads to disturbances of ion and other compounds transport (6, 7). A decrease of intracellular K+ concentration was observed (5). Mouse LM fibroblasts exposed to 44 °C were found to release K+ ions (26). The influence of elevated temperatures modifies Na+/K+ – ATPase in erythrocytes (24). The increase of plasma membrane fluidity may lead to disturbances in Na+/K+ – ATPase functioning. A decrease of enzyme activity in our study (in press) was observed.

Conclusion.

1 – There is a correlation between follicles viability and heating temperature observed on the basic of results. The critical temperature of follicles viability was 44,7 °C. No significant difference was observed in survival of follicles between the toxic and non-toxic nodular goitre at the studied temperatures.

2 – The obtained results prove that plasma membrane of thyrocytes from toxic nodules is more flexible in the hydrophobic core than from healthy tissues. Hyperthermia (the temperature of 47°C, 40 min) induced a significant increase in fluidity of plasma membrane of thyrocytes from toxic and non-toxic nodules in comparison with the healthy tissues.

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Protein Metabolism During Cryotherapy in an Experimental Rat Model

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SUMMARY

The aim of our study was to investigate the influence of cryotherapy on protein metabolism in experimental rat model. One group of rats was exposed to low temperature of -90°C for 5 days (one minute daily), the second group was exposed to the same low temperature for 10 days. The control group was not exposed to low temperature. During the study we revealed changes in the activity of some enzymes involved in protein metabolism (glutamate dehydrogenase –GDH, aspartate aminotransferase - AST and alanine aminotransferase – ALT) and we observed also changes in the plasma protein fraction. The results obtained indicate an influence of low temperatures on protein metabolism.

Key-words: low temperature, cryotherapy, protein metabolism, rat.

EIWEISSSTOFFWECHSEL UNTER KRYOTHERAPIE IN EINEM EXPERIMENTELLEN RATTENMODELL

Ziel der Studie war es, den Einfluss niedriger Temperaturen auf den Eiweißstoffwechsel zu untersuchen. Eine Gruppe von Ratten wurde 5 Tage lang (täglich 1 Minute lang) einer Temperatur von -90°C ausgesetzt, die zweite Gruppe wurde 10 Tage lang der gleichen Temperatur exponiert. Die Kontrollgruppe wurde nicht Kälte exponiert. Wir fanden bei dieser Studie Aktivitätsänderungen einiger am Eiweißstoffwechsel beteiligter Enzyme (Glutamate- Dehydrogenase –GDH, Aspartat-Aminotransferase - AST und Alanin- Aminotransferase – ALT) und wir entdeckten Veränderungen in den Eiweißfraktionen. Die Ergebnisse weisen darauf hin, das niedrige Temperaturen den Eiweißstoffwechsel beeinflussen.

Schlüsselwörter: niedrige Temperatur, Kryotherapie, Eiweißstoffwechsel, Ratte.

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Introduction

There is growing interest in the use of extreme low temperatures in medical treatment. Cryotherapy uses low temperatures applied for a short time to stimulate physiological reaction of an organism in this condition [1].

Whole-body cryotherapy results in a number of effects: analgesia, reduction of-swelling, immune reaction, hormone and circulatory system response [2,3]. Although the development of cryogenic engineering has been a widespread phenomenon for several years due substantial improvements in low temperature generation techniques, the metabolic effect and their biochemical changes in cryotherapy are not yet clear [4].

One of the fundamental functions of homoiothermic organisms is the maintenance of deep body temperature within a narrow range. Maintaining of thermal homeostasis is a necessary condition for every metabolic process [5]. Under the condition of cryotherapy, thermoregulation is affected by an increase in heat production in the organism caused by in increase in intensity of metabolism [6]. The aim of this study was to investigate the influence of cryotherapy on protein metabolism in the experimental rat model.

Material and Methods

Studies were carried out on 18 adult (3 month-old) male Wistar FL rats (body weight = 317,8 ± 24,07 g). The protocol for animal studies was reviewed and approved by Bioethical Committee of the Medical University of Silesia in Katowice. Animals for the experiment were supplied by the Experimental Animal Farm. The rats underwent two weeks environmental adaptation cycle. After this period the animals were randomly allocated into three groups (6 rats each). All groups were fed standard rat chow ad libidum

Group A - rats exposured for (-90°C) for 5 days - (-90/I)

Group B - rats exposured for (-90°C) for 10 days - (-90/II)

Group C - control rats - without exposure to cryotherapy

The rats were put individually into cold chamber in wooden cages for 1 minute. Body weight of animals was controlled before, after five and ten days of experiments.

During ether anaesthesia a sample of animals' blood was drawn from the right ventricle of the heart. The animals were then killed by spinal cord disruption. The blood was obtained from the right ventricle and collected in EDTA-containing tubes.

10% homogenates of liver from the rats were prepared in 0,9% sodium chloride solution using a Potter-Elvehjem homogenizor. Then homogenates were stored at low temperature -20°C for 36 hours. After thawing, the homogenates were subjected to ultrasound (Ultrasonic desintegrator type UD-11).

The following were measured in plasma, total protein, albumin, alpha1-globulin, alpha2- globulin, beta-globulin and gamma- globulin concentration. In plasma and liver homogenates the activity of glutamate dehydrogenase, aspartate aminotransferase and alanine aminotransferase were estimated.

Plasma proteins

Whole protein concentration was measured by the Lowry' method [7]. Albumin, alpha1-globulin, alpha2- globulin, beta-globulin and gamma-globulin concentration were measured by electrophoresis.

Enzyme activity

Activity of glutamate dehydrogenase (GDH) was measured by a spectrophotometric method described by Schmidt [8].

Activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with standard kits prepared by Alpha Diagnostics (Warsaw, Poland).

Whole-body cryotherapy: method

The cold chamber consisted of two compartments: the antechamber and the chamber. The dimensions of the cold chamber are as follows: 4.5m in length, 1.9m in width and 2.5m in height. The temperature in the antechamber reaches approximately (-60°C) during the treatment, whereas the temperature in the chamber is (-90°C) or less. The air supplied to the chamber is purified and cooled down in cryopurifiers and it consists only of oxygen and nitrogen. Oxygen concentration is maintained at a constant level of 21% to 22% and is monitored by the sensor located in the chamber. The walls are covered with multi-layer thermal insulation suitable for use in low temperatures. Cooling of the chamber to the temperature of approximately (-100°C) is possible due to heat exchangers to which nitrogen is supplied from a 5000m3 volume tank. The chamber is fitted with three doors. The two exterior doors separate the antechamber and the chamber from the surroundings, whereas the interior door separates the antechamber from the chamber. In each door there is a window through which the treatment can be monitored. The operation of the cold chamber is supervised by a controlling device linked to a computer [9].

Statistics

The data are presented as mean ± SD. Statistical analysis was performed using STATISTICA 6,0 PL. The differences between groups were analysed using U-Mann-Whitney test. We used p<0.05 for statistical significance-.

Results

A comparative assessment of plasma proteins profile of studied rats subjected to different conditions of cryotherapy is shown in Table 1.

There was a statistically significant increase in GDH activity in plasma (figure 1) and in the liver (figure 2) of the animals studied compared to the control group. The plasma activity of ALT and AST was constant and there where no changes compared to the control group. However statistically significant increase in ALT [figure 3] and AST [figure 4] activity in the liver of the animals were observed.

There were no statistically significant changes between groups exposed for 5 days and 10 days cryotherapy at a temperature of -90°C.

Table 1.
Plasma protein concentration in rats exposed to -90°C for 5 and 10 days compared to the control group

	Group (-90/I)	Group (-90/II)	Control group
whole protein [g/l]	65,8±8,57 NS	65,7±10,3 NS	66,4±9,58
Albumin [g/l]	33,2±4,71 NS	32,1±4,53 NS	33,5±3,25
alpha1-globulin [g/l]	20,7±3,74 NS	24,8±4,22 NS	23,6±2,32
alpha2-globulin [g/l]	5,98±0,69 **p<0,05**	5,27±0,97 **p<0,05**	6,82±0,55
beta-globulin [g/l]	20,0±2,44 **p<0,05**	20,3±3,39 **p<0,05**	16,9±1,31
gamma-globulin [g/l]	9,16±1,53 **p<0,05**	9,45±1,03 **p<0,05**	7,96±0,86

P<0,05 – statistically significant comparing to the control group.
NS – not significant

Figure 1
Plasma GDH activity in groups exposed to –90°C compared to the control group.

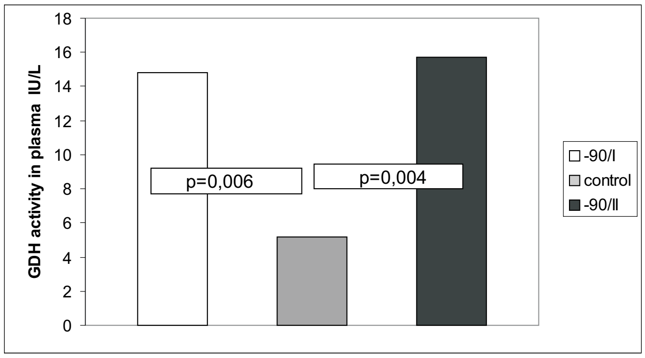
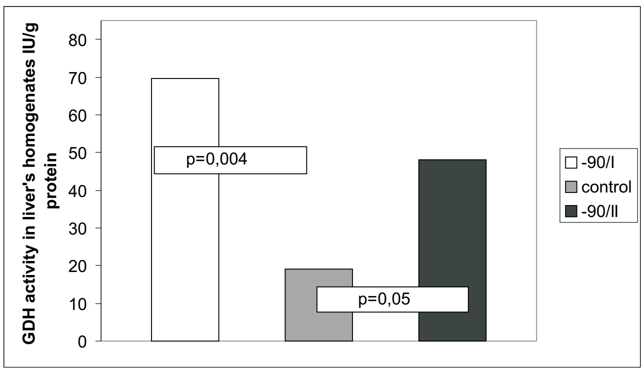


Figure 2.
Liver GDH activity in groups exposed to –90°C compared to the control group.



Discussion

A constant level of body temperature can only be preserved if the heat production by the organism is balanced by its heat loss, which is achieved by physiological mechanisms of temperature control. One of the factors determining the intensity of metabolism and consequently the rate of heat production, is the temperature of surroundings. The liver plays a crucial role in metabolic thermoregulation. The temperature of the blood in the hepatic vein is higher than that in the hepatic artery, which points to active heat production in the liver. This process is intensified when the body is cooled during cryotherapy [10,11].

The influence of cryotherapy on individual metabolic pathways is a very interesting question. In this study we have focused on protein metabolism.

An important indicator enzyme involved in protein metabolism is glutamate dehydrogenase [12,13]. In the current experiment we have observed increased activity of GDH in rats subjected to cryotherapy of -90ºC. This increase was observed in plasma and also in hepatocytes of these animals studied.

Glutamate dehydrogenase is situated in the mitochondrium. This is a regulatory enzyme which is under the influence of an activator (ADP) and inhibitors (ATP, GTP, NADH). Intensification of heat production during cryotherapy is followed by more ATP degradation in thermogenesis, resulting in higher concentrations of ADP which is an activator of GDH [14,15]. The conversion of alpha-amino groups of amino acids is catalysed by glutamate dehydrogenase in a process called transdeamination., that is a cross reversible reaction that is equal in catabolism and in glutamate anabolism. Transdeamination leads to the production of ammonia. This is a very toxic product even in minimal concentration. The main mechanism of ammonia detoxication in the liver is by the production of urea in the urea cycle [16].

Figure 3.
Liver ALT activity in groups exposed to –90°C compared to the control group.

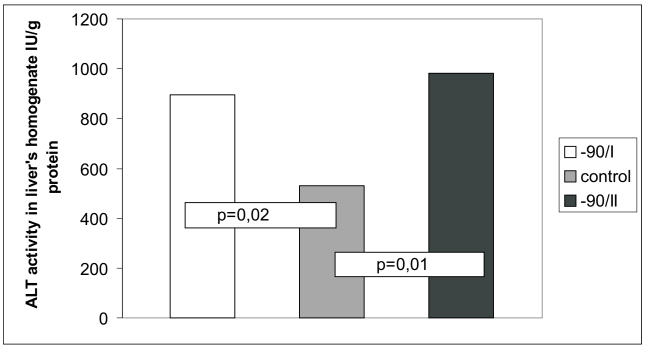
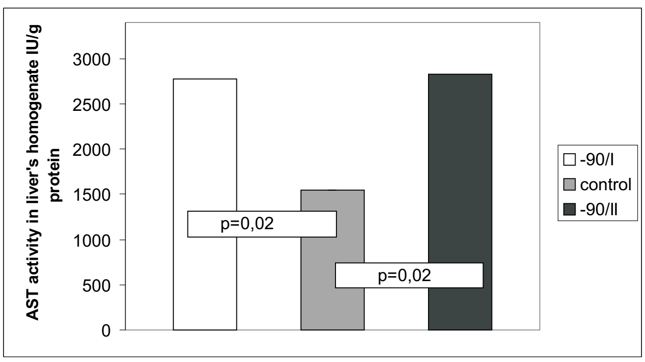


Figure 4
Liver AST activity in groups exposed to –90°C compared to the control group.



Increased GDH activity is very often accompanied by increased aminotransferase activity. Usually the removal of nitrogen during transdeamination is the first reaction in amino acid catabolism. During cryotherapy there were no changes in aspartate and alanine aminotransferase activity found in the rat plasma compared to the control group.

On the other hand, we have observed increased activity of aspartate and alanine aminotransferase in the liver of these rats during cryotherapy of -90ºC. These findings indicate an increased protein catabolism at low temperatures of -90ºC [17,18]. This observed increase in protein catabolism in low temperature conditions is related to the participation of the liver in the provision of energy [19].

We have also observed that the concentration of total protein, albumin and alpha 1-globulin in the animal groups was constant in this study, and was similar to the concentration in the control group. Our results were consistent with those obtained by Sieroñ et al in humans [20]. They observed no changes in the concentration of total protein, albumin and alpha 1-globulin during cryotherapy of patients suffering from ankylosing spondylitis. However we have observed increased beta-globulin and gamma-globulin concentration. Inversely, the concentration of alpha 2-globulin was lower than in the control group. These results are consistent with those reported by Bialy et al in humans [21]. They have observed increased concentrations of beta- globulin and gamma-globulin, and decreased concentration of alpha 2-globulin in plasma of sportsmen during 11 days cryotherapy at a temperature of -120ºC. It is therefore important that results of such research in animals correspond to findings in human research.

Data from other research confirm a constant level of total protein concentration during cryotherapy [22,23]. However, results are ambiguous concerning particular protein fractions [21,24]. The constant concentration of total protein and albumin is an argument for the maintenance of proteins' homeostasis during cryotherapy. The observed increase in protein catabolism must be balanced by an increased anabolism. We postulate that the compensatory intensification of heat production at low temperature exposure may be related to the acceleration of protein turnover [25].

Low temperature also exerts a great influence on metabolic pathways through changes in hormonal activity. Accumulation of amino acid in the blood is the main factor for adjusting the conversion of nitrogen in the liver [10]. Anabolic hormones lower the concentration of amino acid in the blood through stimulation of the capture mechanism for amino acids by skeletal muscles. This results in a reduced uptake of amino acids in the liver. On the other hand, catabolic hormones increase the supply of amino acid in the liver, the utilisation in the process of protein re-synthesis and they also stimulate the process of ureapoiesis [9]. We consider that intensification of protein catabolism during cryotherapy may be related to a modification of the hormonal regulation of this process. However, more experimental results are needed for the support of such an interaction.

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32nd Congress of the American Academy of Thermology: Abstracts

Pierre L. LeRoy, program chairman

THE INFLUENCE OF CAFFEINE ON HUMAN SKIN THERMOREGULATORY RESPONSES

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Thermologist often request that their infrared thermography subjects/patients refrain from using caffeine products for a period of at least one hour prior to imaging. This investigation examined the influence of a single dose of caffeine (120 mg) on thermal measures during standing, work (70% VO2 max, 30 minute duration), and passive recovery periods in a warm environment (30°C, 40% rh). The participant's habituation to caffeine was not determined, but regular daily use of caffeine products was recorded. Subjects refrained from using a caffeine product on the experimental days. Work rates for the five physically active college males were determined from a test for maximal oxygen consumption. During the four randomized double blind investigative trials, all participants either drank 20 ounces of Gatorade™ (G) or Gatorade™ plus caffeine (GC) prior to 60 minutes of passive standing performed at the same time of day and separated by at least 24 hours. During two trials the subjects were followed for an additional 30 minutes of standing, while the remaining two trials consisted of a 30 minute work bout followed by a 30 minute recovery test periods. Physiological measures consisted of thermographically determined skin temperatures, rectal core temperature, heart rate, Physiological Strain Index (PSI), Rating of Perceived Exertion (RPE), weight (sweat) loss, and thermal sensation. A caffeine effect was not observed until 30 minute post ingestion. Core temperature was higher during the caffeine passive trials (0.10°C) and exercise trials (0.20ºC), and recovery (0.10ºC). Post caffeine ingestion the subject's heart rates were slightly elevated, but no differences were observed in the subject's perception of thermal stress, PSI, RPE. Body weight changes indicate greater fluid retention in both the active and passive caffeine trials. Thermal imaging demonstrated slightly elevated temperatures in the chest and slightly cooler in the head with caffeine supplementation while standing in the heated climate. No difference in skin temperatures were observed for the arms and legs. During the caffeine exercise and recovery trials, the arms and legs were substantially cooler while no difference was observed for the chest region. In contrast, the head was cooler during exercise and then continued to rise in temperature during the recovery period. In conclusion, the ingestion of caffeine (120 mg) in a heated environment increased core temperature, increased heart rate, altered skin temperature, but did not disturb the thermal patterns on the skin surface.

Partial Support for the project provided by Gatorade

THERMOGRAPHIC EVALUATION OF CERVICAL DERMATOME IN THE BULL

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Neck injuries are not uncommon in some rodeo bulls. A bull was presented to the Large Animal Teaching Hospital with head carriage and slight head tilt after bucking. There were no changes in head tilt and carriage after 2 to 3 weeks post injury. The bull was in good physical condition other than the problem associated with the head carriage. Physical examination and history indicated some cervical neuropathy.

Thermographic evaluation was done using a Computerized Thermal Imaging Processor system. Facial views, along with right and left views of the neck and shoulder were obtained. Lower shoulder thermographic images left and right were similar in temperature and pattern. The left side cervical region (C1-C4) demonstrated warmer skin temperatures and a different thermal pattern when compared to the right cervical region.

Abnormal thermal patterns similar to cervical (neck) injury were observed. The bull had his head tilted more to the left and it seems that he was trying to relieve the pressure and pain presentation associated with the neck injury. In conclusion, both clinical findings and thermographic evaluation indicated neck and cervical area injury. Radiographic examination was postponed because bull weighed about 750 kg, obnoxious behavior, and it would require bull to be under general anesthesia. The bull was put on an anti-inflammatory drug and the owner was advised if condition persisted to return to the clinic in 90 days.

CRITICAL RESEARCH REVIEW OF THERMOGRAPHY IN VETERINARY MEDICINE

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After 30 years of experience and use of thermography in veterinary medicine, we have chosen this opportunity to reflect upon the critical research questions that need further investigative studies. These studies must adhere to the highest standards of scientific inquiry, while meeting the scrutiny and requirements for publication in appropriate referred journals.

1.In addition to the already recognized, normal thermographic patterns in horses, we need to establish thermal patterns and dermatome patterns for the various animal species. This will be a challenge due to the potential use of pharmacological agents that can alter thermal patterns. The thermal variation among animal species, as well as within different breeds of animals, would provide significant contributions to the field of thermology.

2.Even though we have developed considerable listings of thermal imaging standards for equine practice, we have not developed suitable standards for other animals. Indoor thermal imaging standards for environmental control are well known, but we lack meaningful guidelines and standards for outdoor imaging.

3.Skin thickness and hair coat in some animal species has been an extensively debated issue. There are some parts of the body where meaningful diagnostic thermography can be obtained, where there is a lack of hair coat, such as scrotal and perineal areas. In other cases hair clipping may be desirable to obtain diagnostic thermograms. In horses with very thin hair coats, diagnostic thermograms can be obtained without hair clippings. In other thicker or heavy coat species clipping is required prerequisite. What criterion needs to be established for hair coat and skin thickness amongst the various species

4.Some animals are not calm and quite or easily controlled during clinical examination. This requires the use of sedatives and tranquilizers to make it easy to handle them. As we know, that same tranquilizer and sedative may alter thermal patterns and temperature gradients. Thus, we need to research appropriate sedatives and dosages which can be used to calm the animal but does not have adverse effects on diagnostic value of the thermograms.

5.We know that exercise, heating, cooling, and the use of tranquilizers before and after thermal examination has been efficacious for diagnosis of various neurovascular and inflammatory conditions. What are the additional challenges of testing that we can use to enhance thermographic examination?

In conclusion, as advanced portable equipment is now becoming available for use in veterinary medicine, do we have a need for standardization of this equipment, and if so, what can we do to make it easier for the practicing veterinarian to use them?

3D VISUALIZATION OF BODY SURFACE THERMAL DATA

William Randy Adams

IRID,Inc. Virginia

IRID Inc. combines the use of Digital Signal Corporation's (DSC) Coherent Laser Radar (CLR), and a high-performance CeDip Infrared (IR) Camera.

CLR is used to measure distance to an accuracy of better than 0.1 mm., the IR camera is a 320x240 pixel array with an NETD of 20 mK.

The CLR can be used in scan mode to measure a 3D surface, or in single-point mode to measure skin movement (respiration and heart signature). The CLR can be used from 3 to 9 meters from the target.

The CeDip IR allows for visualization of small temperature differences (output is radiometric, i.e., true temperature). Time-sequence IR data can also be taken (at up to 200 frames per second) to determine responses to various stimuli. CeDip IR can be used from < ½ meter to 9 meters from the target.

Output from the CLR and IR devices can be precisely overlaid, giving a 3D model showing temperature data. This process can be used to precisely position 3D surface images taken at different times and different conditions.

Third party and proprietary software allows for measurement of volumetric, shape, and temperature data, and for comparison against previous baseline data. IRID has developed specialized software for enhancement of the thermal image data.

This technology is designed as an adjunct to existing technology, and is completely non-contact.

Uses can include

- Initial triage, reducing time for medical staff
- Respiration and heart signature data
- Thermal responses to drug, O2, CO2, etc. stimuli.
- Thermal changes over time indicating possible incipient conditions which are difficult to spot visually.
- Contact-free screening for medical conditions, e.g., SARS
- More, T.B.D.

BLOOD SUPPLY OF THE SKIN/ THE ANGIOSOMES

Srini Govindan

Medical Park, Wheeling, West Virginia.

Skin is the largest organ of the body. Temperature regulation to maintain homeostasis is one of its major roles. This important function is provided by a rich network of cutaneous arteries and veins, especially in the dermal and subdermal plexi, which supply the sweat glands and allow for heat exchange by convection, conduction, and radiation. Although the cutaneous circulation is rich and vast, the metabolic demands of the skin elements are low so that only a small fraction of the potential cutaneous circulation is necessary for skin viability. The works of Manchot, Salmon and Taylor helps us to understand the blood supply to the skin and the underlying deep tissues and segregate the body anatomically into three-dimensional vascular territories that are named "angiosomes". These three-dimensional anatomic territories are supplied by a source (segmental or distributing) artery and its accompanying vein(s) that span between the skin and the bone. Each angiosome can be subdivided into matching arterisomes (arterial territories) and venosomes (venous territories). Forty angiosomes have been described which can be subdivided further into smaller composite units.

These composite blocks of skin, bone, muscle, and other soft tissue fit together like the pieces of an intricate jigsaw puzzle. In some angiosomes there is a large overlying cutaneous "crust" and a relatively small deep tissue region, in others the reverse pattern exists. Each angiosome is linked to its neighbor, in each tissue, by a fringe of either true (simple) anastomotic arteries without change in caliber or by reduced-caliber choke (retiform) anastomotic vessels. The choke anastomotic vessels are plentiful in the integument (skin and subcutaneous tissues) and may participate in regulating the blood flow to the intact skin. On the venous side avalvular (bidirectional or oscillating) veins often define the boundaries of the angiosome.

The cutaneous arteries arise directly from the underlying source (segmental or distributing) arteries, or indirectly from branches of those source arteries to the deep tissues, especially the muscles. During their subcutaneous course the cutaneous arteries (and veins) often travel with the cutaneous nerves, either as long channels or as chain-linked system of vessels. John Hunter's hypothesis of a fixed number of cutaneous arteries and how growth and differentiation of the tissues could modify the definitive size and relationship of the arteries X and Y in different regions of the body can explain why long vessels converge on the nipple from the periphery as the breast develops in the female.

The density, size and direction of the cutaneous perforators varies from region to region of the body, being modified by growth, differentiation, and the functional demands of the part. The vessels of the head, neck, torso, and proximal limbs are larger and more widely spaced than their counterparts in the forearms, legs, hands and feet. The cutaneous perforators vary in the size and length and they all interconnect for form a three-dimensional "body carpet". The body carpet has a particularly well-developed horizontal strata of vessels in the dermis, in the subdermis, on the undersurface of the subcutaneous fat, and on the outer surface of the deep fascia.

The cutaneous veins also form a three-dimensional plexus of interconnecting channels with a dominant strata in the subdermis. Many of these veins have valves that direct the blood in a particular direction, they are often connected by avalvular veins. These avalvular (oscillating) vessels allow bidirectional flow between adjacent venous territories whose valves may be oriented in opposite direction, thus providing for the equilibration of flow and pressure. In some regions valved channels direct flow radially away from a plexus of avalvular veins as, for example, in the venous drainage from the nipple-areolar summit of the breast. In other areas valved channels direct flow toward a central focus, seen in the stellate limbs of the cutaneous perforating veins.

The skin is fed and drained by a continuous network of arteries and veins formed by vessels whose size, shape, density, and direction vary from region to region in the body.

A knowledge of the basic anatomy of the cutaneous vessels, coupled with an appreciation of the factors that influence its structure in different regions of the body has clinical application for Thermologists. In the words of Michel Salmon, between anatomy and physiology there is room for functional anatomy, for a physiological anatomy. The angiosomes concept and the neurochemical pathology specific to clinical states can be used in correlating infrared imaging clinically.

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FACIAL TEMPERATURE IN SUDDEN INFANT DEATH SYNDROME

Srini Govindan

Medical Park, Wheeling, West Virginia.

Objective: To present potential application of infrared technology in sudden infant death syndrome (SIDS).

Incidence:Most SIDS cases occur between 2-4 months of age, which coincides with important maturational changes in the central nervous system as the newborn adapts to changes in extra-uterine life.

Risk Factor:Under moderate heat stress at the level of hypothalamic preoptic region marked hypoventilation may occur in infants, an observation that has been experimentally verified in animal studies. Supine sleeping position promotes appropriate thermoregulation via the face and head, which is the major source of infant heat loss. Prone sleeping position has been shown to increase temperature around the head of infants up to 3.5°C above those measured in the supine position.

Pathophysiology:Hyperthermia in cot death was first described in 1979. The 1984 study demonstrated that 94% of SIDS victims were excessively clothed, in an unusually warm environment, hot and sweaty when found dead, or had an infective illness, which in itself, would not be expected to cause death. The infants' ability to dissipate excess heat can be compromised by combinations of overwrapping, co-sleeping, acute febrile illness, and an unusually warm heated environment. This may result in hyperthermia, which can lead to a failure of the cardio-respiratory system during sleep. du Boulay and colleagues have proposed that carotid artery mediated brain cooling is essential to maintain normal temperature homeostasis in the brain. Any failure of this process may increase brain temperature. Loss of heat is primarily via vasdilatation which predominantly occurs from the face and head in neonates. In this context the head, as the site of about 40% of the head production and up to 85% of heat loss for the infant in bed, may be particularly sensitive to thermal stress. Any disturbance of brain hypothalamic temperature homeostasis may have a prolonged effect on face and head temperature in the absence of any change in core body temperature. Hyperthermia enhances laryngeal reflex probably through temperature-dependent changes in synaptic transmission, which have a direct effect on the latency of the laryngeal adductor reflex, causing upper airway induced central apnea of sufficient severity to result in death.

Conclusion:In the first 6 months of life Thermography can help to monitor facial/head temperature over and above core temperature and indicate thermoregulatory stress at the level of hypothalamic preoptic region. This can permit administration of appropriate interventional measures to prevent events that may lead to respiratory apnea and SIDS.

Failure of neural control of respiration has been implicated in SIDS. There is complex interaction between respiratory, cardiac and thermoregulatory control centers and hemeostatic mechanisms. There is causal relationship between thermal stress and failure of respiration, but the mechanism remains unclear.

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ANATOMY, PHYSIOLOGY AND PATHOLOGY OF AUTONOMIC AND SOMATIC NERVOUS SYSTEMS AND THEIR CORRELATION TO PERIPHERAL THERMAL DYSREGULATION

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Somatosensory and autonomic neural systems interact to regulate vascular tone and thermal effects. These complex systems function non-linearly to maintain homeostatic conditions. However, in pathologic disruptions, such nonlinear adaptive processes assume a more linear, progressive pattern. (Similar changes towards linear, dynamical systems disruption can produce autonomic dysregulation and vascular sequelae in exothermic or endothermic processes of inflammation, ischemia, infarction/necrosis, neoplastic and carcinogenic angiogenesis). Characteristic thermal pattern changes frequently result from neurogenic inflammation and sympathetically mediated pain syndromes.

Such pain is clinically enigmatic as it frequently occurs without evidentiary pathology and is refractory to laboratory or imaging diagnosis. This disparity can lead to misdiagnosis and inappropriate management. Chronic pain syndromes may induce hyperactivity of C-fiber afferents evoking antidromic release of substance-P into peripheral tissues. This causes peripheral vaso- dilation both directly and indirectly. Neurogenic inflammation increases temperature within peripheral tissues, stimulating polymodal C-fibers further and augmenting the pain response. While this physiopathologic cascade has been well recognized, to date effective assessment methods applicable in the clinical (i.e. -non-experimental) setting have been unreliable, particularly during the early stages of these disorders. When more salient signs occur, they are often reflective of advanced pathology against which therapeutic intervention is more complicated, invasive, if not entirely unsuccessful. This leads to protracted disability and suffering in the patient.

It is in this light that we have renewed interest in thermographic assessment of peripheral syndromes. Current thermographic technology has significantly improved, with higher resolution, sensitivity, ease of use and cost effectiveness. While some equivocality exists regarding the acceptability of thermography, extensive peer reviewed studies support its validity in depicting thermal changes correlated to neurogenic pain, and other pathologies in which there is concomitant vascular change. By understanding specific pathologic mechanisms, we may gain additional insight to the applicability, strengths and limitations of thermographic assessment, and further study its potential utility in clinical and veterinary medicine.

3D-IR FOR ENHANCED VISUALIZATION OF THERMAL IMAGING

Prokoski F, Sebastian R L.

IRID and Digital Signal corporations, Fairfax and Alexandria, Virginia.

Simultaneous Imaging by a high performance IR camera and a coherent laser radar can produce a 3D surface thermal map that enhances visualization of thermal features and their changes over time. Custom software is being developed for the composite sensor imagery to provide true 3D measurements of the area imaged, the ability to rotate images into any desired orientation, and the ability to precisely register images taken at different times.

3D-IR visualization offers potential benefit in many applications such as: detection of incipient pressure ulcers prior to stage one; precise 3D measurements of lymphedema for design of custom compression garments and to determine the effectiveness of treatments; rapid whole-body scans to document skin condition including texture; non-contact quantitative evaluation of on-going burn treatment.

Infrared Identification Incorporated (IRID Inc.) and Digital Signal Corporation (DSC) are collaborating on the IR/CLR integration which is the subject of several issued and pending patents. The companies are currently focused on security uses of their technologies, but hope to also pursue medical applications in collaboration with other organizations.

3D-IR FOR REMOTE MONITORING OF VITAL SIGNS

Prokoski F . Sebastian R L

IRID and Digital Signal corporations, Fairfax and Alexandria, Virginia

Integration of a coherent laser radar and a high performance IR camera allows certain vital signs to be continuously monitored from a distance without contact. Respiration rate, pulse, heart signature, and skin temperature can be directly obtained at one or more locations on the body, and instantaneous blood pressure can be deduced. Anatomical features extracted from the thermal imagery can be used to continuously re-aim the CLR to remain focused on a particular spot in spite of involuntary movements associated with cardiac cycle, respiration, swallowing, blinking, spasms, etc. That reduces movement artifacts in the output signals.

The non-contact aspect of this approach has particular application to telemedicine, and to monitoring of persons under quarantine. It would reduce the risk of exposure to health care workers, and reduce the volume of medical waste created compared to contact procedures. The continuous imaging aspect of this approach provides for tracking the effects of administered drugs over time. The lock-on aspect of this approach allows patient-specific alarm indicators to be set at specific locations on that patient's body. For example: localized bleeding, swelling, temperature change, tremour, break in cardiac or respiratory rhythm could trigger an alarm.

Infrared Identification Incorporated (IRID Inc.) and Digital Signal Corporation (DSC) are collaborating on the IR/CLR integration which is the subject of several issued and pending patents. The companies are currently focused on security uses of their technologies, but hope to also pursue medical applications in collaboration with other organizations.

THE AUTONOMIC CHALLENGE AND ANALYTIC BREAST THERMOLOGY

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The basis of thermology is physiologic and, thus is unique among the diagnostic imaging techniques. Our laboratory employs an adaptive physiologic challenge procedure to differentially indicate the abnormal vasculature associated with malignant breast disease. The adaptive challenge response is used as an analytic element along with pattern recognition, temperature differential and time-based positive evolution the evaluation of breast thermology. To establish the power of the adaptive challenge procedure, I retrospectively reviewed two thousand patient studies that were five to seven years from the time of analysis. One thousand of these patients were normal studies (TH-1 & TH-2) and one thousand patients were abnormal studies (TH-4 & TH-5). Each and every of these patients had received annual multi-modality follow-up (clinical examination, thermology & mammography) from the time of their original thermology studies. The original thermology studies were reviewed in a blinded manner independently by two thermo- logists in a manner that the diagnostic contribution of the adaptive challenge response was separate from that of pattern recognition and temperature differentials. This study concluded that the response of the autonomic challenge increased the diagnostic sensitivity by seven percent and increased the specificity by twenty-eight percent.

SURGICAL THERMOLOGY- A REVIEW OF PREOPERATIVE, INTRAOPERATIVE, AND POSTOPERATIVE APPLICATIONS

Pierre L. LeRoy

Wilmington, Delaware

Increasing indications for medical infrared radiation characteristic thermal pattern recognition and expanding surgical fields of neuropathic; vascular; skeletal; and myopathically related disorders represents significant progress for the surgical patient's management in several specialties.

Since previously reported by Abernathy and Umatsu in 1986, by clinical and bioengineering advances in design miniaturization; noncooling; portability and visual analog radiometrics documenting technology. Results from declassified dual military technologies provide enhanced pixel resolution. This now allows for handheld monitoring sensor cameras to be safely employed in a variety of pre and postinterventional surgical settings and procedures.

These new capacities provide Surgeons directly with an immediate selective virtual reality of the outcome of the procedure not requiring a third party interpretation. This allows the Surgeon to determine if the interventional procedure is satisfactory in its outcome or whether additional decompression or other techniques are necessary. This is especially true in the field of neurocompression and endovascular stents.

A review of these four categories is presented by clinical case examples documented by telethermography in the author's experience.

Especially valid is the following:

1.Neurosurgical entrapment neuropathies such as axial radicular decompression; peripheral neuropathy entrapment such as seen in brachial plexus thoracic outlet syndrome and carpal tunnel syndromes.

2.Orthopedic applications provide for the monitoring of cast applications, which may be too tight to help prevent undesired neurovascular compressions leading to reflex sympathetic dystrophy (RSDS) or complex regional pain syndrome (CRPS) we have called the thermal castograms.

3.Vascular surgeons for cardiac arterial bypass; arterial anastomosis for peripheral vascular disease seen especially with diabetic patients and endovascular stents and employed in re- vascularization procedures.

4.General surgery in the evaluation of GI and bowel resection viability. Although the author has no personal experience in this latter, the principals appear to be applicable in this situation.

It is hoped that others will improve on this legacy contributed by so many that have preceded us and also that we have benefit from the critics who have helped to improve our total fund of knowledge.

Improved understanding of the basic pathophysiology as reported by J. Giordano; T. Yakash and others provided the clinical and bridging the basic science concepts elucidated by H. Ringermacher; and V. Klemas reported elsewhere in this symposium firmly establishing the credibility in assessing these orthoptic systems for the surgical interventionalist and beyond, to see that was not perceived through our limited senses.

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THERMOGRAPHY IN PHANTOM PAIN

Srini Govindan

Medical Park, Wheeling, West Virginia.

Thermography is usually done with the understanding that the primary somatosensory cortex is intact. Thermal asymmetries are correlated with nervous system lesions after excluding other causes e.g., local vascular etc.

Phantom limb pain is a frequent consequence of the amputation of a body part. Phantom limb pain is closely associated with plastic changes in the primary somatosensory cortex. Animal data indicate that behaviorly relevant training alters the cortical map. The somatosensory system is capable of functional reorganization following peripheral denervation or training. Studies on human amputes with phantom limb pain provided evidence that these reorganizational changes are modulated through nociceptive input. The pain induced hyper responsiveness can cause acute reorganization. Soros et al observed acute pain can elicit phantom sensations in healthy subjects and suspected an underlying pain- induced hyper responsiveness of the cortical hand representation to somatotopically adjacent input from the lip. Flor et al indicated that phantom limb pain is related to and may be a consequence of plastic changes in primary somatosensory cortex. Thermography studies in phantom pain can give us a method to image a peripheral correlate of cortical plasticity and reorganizational changes in the primary somatosensory cortex of neuropathic pain syndromes and can contribute to the understanding of pain pathophysiology. This can help in the development of new treatments.

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THE CORRELATION OF OCEANOGRAPHIC AND TERRESTRIAL THERMAL INFRARED ANALYSIS WITH THE BIOSCIENCES

Vic Klemas 1, Pierre L. LeRoy [2]

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Delaware Pain Clinic, University of Delaware, Newark, DE 19711

Depending on its temperature, every object or life form on Earth radiates thermal infrared energy. Infrared radiation can be used to detect natural or man-made features and to accurately measure their temperature. In nature, rattlesnakes use infrared radiation to find their prey, such as desert squirrels which also have countermeasures. Man uses infrared sensors on space satellites to locate forest fires, track hurricanes and weather fronts; to map ocean currents and temperatures. It is also used to detect enemy troops or vehicles at night. Extensive peer-reviewed data has been accumulated since the 1940 post-war era.

The intensity of the infrared signal our sensors detect depends on both the emissivity and temperature of the target. The emissivity of land surfaces varies depending on the land cover. For instance, dry desert sand has a lower emissivity and forests exhibit a higher emissivity. Therefore, the temperature of land surfaces is difficult to determine since their emissivity is highly variable and frequently unknown. On the other hand, oceanographers are quite successful in accurately mapping sea surface temperatures with thermal infrared sensors on satellites, because the emissivity of seawater is constant and rarely deviates from 98%. Similarly, since water is one of the major constituents of the human body, it is not surprising that physicians can use thermal infrared radiation to precisely outline body regions which are inflamed due to injury or illnesses. For instance, at medical pain clinics they may determine and differentiate if regional muscle, skeletal, vascular and neuropathic injury has occurred based on their characteristic diagnostic thermal pattern, a feat x-rays cannot accomplish. Thermal infrared is also used extensively by veterinarians in equine applications.

Oceanographic and medical applications of infrared radiation are based on the same physical principles and face similar issues. For instance, just as an oil slick on the ocean surface can produce errors in temperature readings obtained from infrared radiation, an oily lotion covering a body part can prevent accurate pain diagnosis and detection. Therefore, continued close collaboration between environmental scientists, oceanographers and medical specialists may prove beneficial to all concerned.

ADVANCES IN INFRARED IMAGING: A GENERAL ELECTRIC PERSPECTIVE

Harry I. Ringermacher,

Schenectady, New York

Infrared Imaging can be divided into two broad categories: Passive IR and Active IR. Passive IR simply "observes" existing radiant heat. Active IR generates its own heating from some transient heat source. The Medical profession utilizes passive IR technology for diagnoses of disease or conditions of the human body. Industry has long benefitted from the same passive technology, but has recently witnessed advances in active IR which, for the first time, permit quantitative imaging of the physical properties of materials and components other than temperature. I will overview both technologies and focus in on recent active IR applications at General Electric. Perhaps the medical community can come up with some good applications of their own!

FEED DEPRIVATION STUDY IN SWINE: VALIDATION OF THERMOGRAPHY IN ASSESSING CHANGE IN METABOLIC RATE

Sophia Wilcox

USDA-Agricultural Research Service-Livestock Behavior Research Unit and Purdue University, West Lafayette, IN

Introduction: Thermal imaging is a possible alternative to the calorimeter in assessing change in metabolic rate. The primary study was designed to quantify hunger in swine.The validation study was designed to establish the thermal camera as a method for assessing change in metabolic rate.

Method: Sixteen pigs were housed individually in a temperature-controlled building. They were fed ad libitum, twice a day (0800 and 1600). One hour after the a.m. feeding (0900), the feed was withdrawn and the pens were cleaned. Pigs were assigned to five treatment groups: 0, 6, 12, 24 and 36- hours of food deprivation. Thermal images were taken of the flank and the cortical-cranial view of the rump, including the stifle where the saphenous vein comes to the surface; using a FLIR Therma CAM™ PM695.. The summary findings comprise three repetitions of the above measurements.

Images were taken in darkness, 2.3 meters from the animal's surface, accounting for the ambient temperature at each exposure and holding the thermal camera level with, and perpendicular to, the cranial-cortical line for the flank view and the dorsal-ventral line for the rump view. While the pigs were still on regular feedings, a set of control images at four 6-hour intervals were captured to allow for diurnal temperature fluctuations.

After the appropriate feed deprivation period, another set of images was taken under the same conditions prior to sacrificing the animal. Hence each animal served as its own control.

Analysis:Using 2000 ™ software, I took two measurements from the flank view and two from the cranial-cortical rump view. From the flank view I used the maximum temperature found in the hind ham joint as well as the average temperature of the animal's surface area. From the cortical-cranial view of the rump I used the maximum temperature in the right and left stifle where the saphenous vein comes to the surface. I found the difference between the control and experimental value for each of these four measurements. I then plotted this difference against the hours of feed deprivation.

Conclusions:Of all the data collected, the average surface- area measurement from the flank view most clearly reflects the expected trend of decreasing surface- temperature with increasing hours of feed deprivation. Temperature from the control images show the fluctuation expected due to diurnal rhythms. Hence, this method of measuring differences in metabolic rate in swine appears to warrant further study.

SALMONELLA DUBLIN CHALLENGE IN DAIRY CALVES:VALIDATION OF THERMOGROPHY TO ASSESS FLUCTUATIONS IN CORE BODY TEMPERATURE

Sophia Wilcox

USDA-Agricultural Research Service-Livestock Behavior Research Unit and Purdue University, West Lafayette, IN

Introduction: Thermal images can conceivably replace rectal thermometers to obtain accurate assessment of body temperature. The primary study examined ß-glucan as effective prophylaxis for Salmonella. The validation study correlates thermal images to rectal temperatures.

Method: Nine calves were assigned to three treatment groups (n=3 per treatment) and inoculations of Salmonella dublin were administered to each calf, varying the dosage according to group; high, medium and low. We took thermal images of the calf eye and rectal temperatures simultaneously, hourly for eight hours prior to, and 72 hours post Salmonella challenge. I took the thermal images with a FLIR®™ PM695 2.2 meters from the animal, holding the camera level to but 45° from the median line in an anterior-posterior view of the face. I noted the ambient temperature and adjusted the camera.

Analysis: Using Reporter 2000™ I framed the tear duct and used the maximum temperature found in this frame to correlate to the rectal temperature. These I plotted in a regression scatter plot and calculated the correlation coefficient.

Conclusions:The amalgamated correlation coefficient was 0.83. ($P<.05$) The correlation between the two methods of measurement increased as the Salmonella dosage increased varying from 0.52 to 0.92. ($P<.05$)

The high correlation of rectal temperatures to thermal image temperatures in this preliminary study indicated that the thermal camera may be a reliable replacement for the rectal thermometer in dairy calf research.

POULTRY WELL-BEING RESEARCH: VALIDATION OF THERMOGRAPHY IN THE DIAGNOSIS OF SUB-CLINICAL BUMBLE FOOT

Sophia Wilcox

USDA-Agricultural Research Service-Livestock Behavior Research Unit and Purdue University, West Lafayette, IN

Introduction: Bumble foot is a large ball-like staphylococcus infection of the foot pad. When infection occurs, synovial membranes in the joints and tendons of the hock and feet become thickened and oedema is seen. Inflammation occurs and a fluid may be produced around the joints and tendon sheaths. Wounds heal on the outside to leave a hard core of pus inside. If the condition becomes chronic, fibrous tissue can form around the foot. The thermal camera may have the ability to detect sub-clinical levels of bumble foot. The primary objective of this study is to validate the use of the thermal camera to detect sub-clinical levels of Bumble-Foot.

Methods: 150 hens housed in pairs in standard battery cages were randomly selected from a peak-production population. Using a FLIR ThermaCAM®™ PM695 I took thermal images of the footpads at 0.91 meters from each selected hen. Fourteen days later I scored the same hens for signs of clinical bumble foot and, took digital photos.

Analysis: I inspected 150 thermal images and identified those that showed an abnormality that may be the onset of bumble foot. I correlated this to the clinical bumble foot score for each of the 150 hens 14 days later.

Conclusions:Based upon the correlation coefficient 0.93 ($P<0.05$) of incidence of clinical bumble foot to abnormal thermal images, it appears that thermal imaging can be relied upon to identify sub-clinical cases of bumble foot in Chickens. (This was designed as a preliminary study and further study would include culture and positive identification of the staphylococcus infection)

News in Thermology

Certification at the AAT-Meeting

As in 2004, the American Academy of Thermology organised a Course and Examination at the 2005 AAT meeting in Baton Rouge.:

The following participants passed the Course and Examination

1.Roman Chrucky M.D.

2.Gregory Bart M.D.

3. Jim Osterwise D.C.

4. Martin Bales, Medical Thermology Technician.

5. Sophia Wilcox, Veterinary Thermology Technician.

6. Pamela B. Rice, Medical Thermology Technician.

7. Terry Addison, Medical Thermology Technician.

8. Pat Rhea, Medical Thermology Technician.

AITA Meeting in Italy

The 8th International Workshop on Advanced Infrared Technology and Applications (AITA) will take place on September 7-10, 2005 in Rome.

Following the International Workshop held in Pisa (Italy) in September 2003, the 8th AITA conference will be held this year in Rome. The main objective of this meeting is to assess the state of the art of thermal infrared technology and to present its most interesting applications. Main topics of the workshop are advanced technology and materials, smart sensors, thermofluid dynamics, non-destructive testing, image processing and data analysis. The sessions will include advanced system applications on cultural heritage, biomedicine, environment, aerospace and industrial applications of thermal imaging.

The Chairman of the Workshop is Laura Ronchi Abbozzo and co-chairmen are G. M. Carlomagno, C. Corsi, I. Pippi, N. H. Rutt, O. Salvetti . The scientific committee includes distinguished experts in the field from France, Italy, U.K, Canada, Japan, Russia, Germany and Switzerland

The papers are to be written and presented in English, which is the official language of the Workshop.

The Workshop will be held in Rome, at the Faculty of Engineering, very close to The Coliseum, from September 7th to 11th, 2005. The registration fee will be €300,00 and will include: social dinner, coffee breaks, lunches, abstract booklet and Proceedings. A limited number of qualified students may apply at the reduced price of €150,00 (social dinner and Proceedings excluded).

Proceedings of the Workshop (extended abstracts) will be distributed on site. After the Workshop, selected full papers will be published in the International Journal of Infrared Physics and Technology (Elsevier).

For further information, please contact the technical secretariat: Anna Maria Meriggi, Fondazione Giorgio Ronchi, Via S. Felice a Ema 20, 50125 Firenze, Italy

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18th Symposium of the Austrian Society of Thermology

The Austrian Society of Thermology will return to the SAS Radisson Hotel for the 18th Thermological Symposium in October 2005. As the Ludwig Boltzmann Institute for Physical Diagnostics will cease all research activities by the end 2005, this will be the perfect opportunity to summarise the achievements made by this institution in the field of thermology. The Austrian Society of Thermology and the European Association of Thermology will continue with most of the activities of the Boltzmann Institute, especially with the publication of Thermology international.

The main theme of the symposium is " Advances in Thermal Imaging and Temperature Related Therapy", but contributions to any other topic are welcomed.

Deadline for Abstracts is September 15, 2005. For further information, please contact

Prof Dr Kurt Ammer PhD,

Ludwig Boltzmann For schungsstelle für Physikalische Diagnostik, Hanuschkrankenhaus, Heinrich Collinstr.30, A-1170 Wien

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Papers from United Kingdom, Poland, Slovakia, Germany, Italy and Hungary are expected. A recent co- operation with the German Society for Hyperthermia is likely increase the interest an topics in temperature dependent treatment. An update on current developments in therapeutic hyperthermia will also be presented at the symposium..

Medical Thermography and Thermometry

at the National Physical Laboratory, Teddington, nr London on the 9th November 2005 (UKTA & Institute of Physics and Engineering in Medicine, York). In the call for papers, the organizing committee has given the following introduction:

Recognition of body temperature as an indication of fever and disease is as old as medicine itself. Patient temperature is a fundamental physiological measurement used for observation and diagnosis.

A variety of temperature measuring devices are used clinically and these can be separated into two categories, either contact (e.g. oral thermometers, axillary thermometers, temporal strips) or non-contact devices, for example ear thermometers and thermal imagers, which detect emitted infrared radiation. Recent developments have realised high-speed and high-resolution two-dimensional imaging for body temperature assessment: medical infrared thermography.

Abstracts are invited describing all clinical applications of temperature measurement. Contributions are also encouraged which address issues of instrument calibration, quality assurance, and future developments in medical thermography and thermometry.

A limited number of bursaries are available to contribute to the travel expenses of researchers under the age of 35 attending from overseas. Please contact the organiser with your abstract by 1st August 2005 if you would like to be considered for this support.

New Board of the German Society of Regulation-Thermography

The longest existing thermographic society in Europe, which has celebrated its 50th anniversary last year, elected a new Board this year. Prof. Dr Reinhold Berz was confirmed in the position of the president, but all other positions have been changed compared to the previous board

The new vice-president is Dr Helmut Sauer, who has served for many years as secretary of the society. He was awarded with the Ernst-Schwamm-Medal in 2003.

The new secretary is a Swiss member, Dr. Luc Wyss. He is a psychiatrist by profession and lives in Fribourg.

The treasurer of the society is now Dr. rer. nat. Ronald Dehmlow. He is the author of several books on complementary treatments such as ozone.-oxygen therapy and bio-resonance.

It has been announced that Dr Arno Rost, former president of the DGTR has recently died at the age of 86.

Veranstaltungen (Meetings)

September 7-9, 2005

8th AITA Workshopp in Rome-Programme

Wednesday, September 7

10.00 Opening Session G.M. Carlomagno, C. Corsi, H. Rutt

Advanced Sensors, Chairman : G.M. Carlomagno

10.30 C. Corsi (CREO, Italy) (invited lecture)
IR Smart Sensors

11.00 C. Corsi, A. Dundee, N. Liberatore, A. Mercuri, S. Perri, A. Pifferi, G. Tosone, R. Viola, D. Zintu, M. Severi, G. Cardinali, I. Elmi, M. Passini (CREO; CNR- IMM, Italy)
Large area bolometer arrays

11.20 A. Piotrowski, P. Madejczyk, W. Gawron, K. Klos, J. Pawluczyk, M. Grudzien, J. Piotrowski, A. Rogalski (VIGO System; MUT-IAP, Poland) (invited lecture)
MOCVD HgCdTe heterostructures for uncooled infrared photodetectors

11.50 Coffee Break

Advanced Sensors, Chairman : A. Rogalski

12.10 H. Zogg, M. Arnold (Swiss Federal Inst. of Technology, Switzerland) (invited lecture)
Narrow spectral band monolithic lead- chalcogenide-on-Si mid-IR Foto- detectors

12.40 C.Trouilleau, A.Crastes, O. Legras, J.L. Tissot (Ulis, France) (invited lecture)
Infrared Uncooled Detector - Recent improvement

13.10 Lunch Thermo-fluid-dynamics, Chairman: C. Corsi

14.30 G.M. Carlomagno (DETEC, Univ. of Naples, Italy) (invited lecture) Heat transfer in complex fluid flows

15.00 E. Caffagni, P. Levoni, M. Piraccini, A. Muscio, M.A. Corticelli, G.S. Barozzi (DIMeC; Minardi F1 Team, Italy)
Thermographic analysis of flow distribution in compact heat exchangers for a Formula 1 car

Advanced Technologies and Materials, Chairman: H. Zogg

15.20 H.N. Rutt, S. Uppal (DECS, UK) (invited lecture)
Design of an electrically operated mid-infrared solid-state modulator

15.50 S. Brugioni, R . Meucci (INOA, Italy)
The measurement of liquid crystal refractive indices in the infrared 16.10 H. Polakowski, M. Morawski, H. Madura, E. Powiada, M. Kastek (MUT-IO, Poland)
The test-bed for measurement angular parameters of passive infrared systems

16.30 G. Paez, M. Strojnik, S. Scholl (Centro de Investigaciones en Optica, Mexico)
Cavity effects in coiled coil IR reference source

16.50 Coffee Break

Non-Destructive Evaluation, Chairman: E. Grinzato

17.10 M.P. Luong (CNRS-LMS, France) (invited lecture)
Introducing infrared thermography in soil dynamics

17.40 W. Swiderski, V. Vavilov (MIAT, Poland; Tomsk Polyt . Univ., Russia)
IR thermographic detection of defects in multi-layered composite materials used in military applications

18.00 F. Garrido, A. Salazar F. Alonso, I. Sáez-Ocáriz (Uni. País Vasco; CTA, Spain)
Characterization of buried cylinders by infrared thermography

Thursday, September 8

Infrared in Medicine, Chairman: G. dalla Volta

09.30 E.F.Ring (MIR Group, Univ. of Glamorgan, UK) (invited lecture)
The historical devolopment of temperature measurement in medicine

10.00 R. Bettaglio (FSM, Italy) (invited lecture)
Thermography and pain

10.30 A. Di Carlo (S. Gallicano Dermat. Inst. for Res. and Care, Italy) (invited lecture)
Skin circulation: a thermographic appraisal

11.00 Coffee Break

Infrared in Medicine, Chairman: G. L. Romani

11.30 G. Dalla Volta (U.O. Neurologia, Istit. Clinico Città di Brescia, Italy) (invited lecture)
The usefulness of thermography in headache diagnosis

12.00 A. Merla (ITAB Fond. Univ. 'D'annunzio', Italy)
Raynaud's phenomenon: functional infrared imaging applied to diagnosis and drug effects

12.20 A. J. Panas, M. Preiskorn, S. Zmuda, M. Dabrowski (MUT; MMI, Poland)
Validation of hard tooth tissue thermal diffusivity measurements applying an infrared camera

12.40 M. Dabrowski, R. Dulski, P. Zaborowski, St. Zmuda (MUT-IO; MIHS, Poland)
Emissivity of the popular dental materials

13.00 Lunch

Cultural Heritage, Chairman: P . G. Bison

14.30 L. Consolandi, D. Bertani (INOA; Univ. Studi di Milano, Italy) (invited lecture)
A prototype for high-resolution infrared reflectography of paintings

15.00 A. Kandemir-Yucel, A. Tavukcuoglu, E. N. Caner- Saltik (METU, Turkey)
In situ assessment of structural timber elements of a historic building by infrared thermography and ultrasonic velocity

15.20 M. Gargano, N. Ludwig, G. Poldi
(IFGA, Univ. di Milano, Italy)
Comparisons and complementarities of different IR camera for infrared reflectography

15.40 N.P. Avdelidis, C. Ibarra- Castanedo, M. Koui, X. Maldague, A. Moropoulou (GEL-GIF, Univ. Laval, Canada; MSES, Nat. Techn. Uni. Athens, Greece)
Thermography approaches on plastered mosaics

16.00 R.A. Palmer, E.K. Nakayama, A. deCruz, P. Igram, C. Culberson, M.L. Wolbarsht (Dept. of Chem., Dept. of Biol., Dept. of Biomed. Eng., Duke Uni.,USA)
The use of infrared spectroscopy to understand the mechanism of the Er:YAG laser (2.94 µm) ablation of surface films, contaminants and encrustations in the conservation of two- and three-dimensional works of art

16.20 Coffee Break

Buildings and Infrastructures, Chairman: X. Maldague

16.50 Ch. Maierhofer, R. Arndt, M. Röllig (BAM, Germany) (invited lecture)
Influence of concrete properties on the detection of voids with impulse-thermography

17.20 E. Grinzato, V. Vavilov, P.G. Bison, S. Marinetti (ITC-CNR, Italy; Tomsk, Russia)
Hidden corrosion detection in thick metallic components by transient IR thermography

17.40 U. Galietti, V. Luprano, S. Nenna, L. Spagnolo, A. Tundo (IMG – Polit. Bari; ENEA; CETMA, Italy)
Non destructive defect characterization of concrete structures reinforced by means of FRP

18.00 M. Acebes Pascual, A. Poblete (Valladolid University, Spain)
Thermography measurement of the effect of humidity in mortar porosity

18.20 C. Meola (DETEC, Univ. of Naples, Italy)
Infrared thermography of masonry structures

20.00 Social Dinner

Friday, September 9

Remote Sensing & Astronomy, Chairman: O. Salvetti

09.00 P. Salinari (INAF, Italy) (invited lecture)
Present and future of IR astronomy

09.30 M. Strojnik, G. Paez (Centro de Investigaciones en Optica, Mexico) (invited lecture)
Infrared detection of a planet next to a bright star

10.00 P. Salinari (INAF, Italy)
Correction for the effects of atmospheric turbulence

10.20 J. Simpson (Digital Image Analysis Lab., USA)
Parallel processing of remotely sensed data: application to the ATSR-2 and the modis instruments

10.40 A. Barducci, F. Buongiorno, D. Guzzi, P. Marcoionni, I. Pippi (IFAC-CNR; INGV, Italy)
Validation of SPA algorithm by high temperature events monitoring in remotely sensed images

11.00 Coffee Break

Image Evaluation & Data Processing, Chairman: I. Pippi

11.30 G. Pieri, S. Colantonio, M. Benvenuti, O. Salvetti (ISTI-CNR; TD Group, Italy) Object tracking in an infrared vision system

11.50 R. Dulski, H. Madura, T. Piatkowski, T. Sosnowski (MUT-IO, Polland)
Analysis of a thermal scene using computer simulations

12.10 A. El Maadi, X. Maldague (ECE, Université Laval, Canada)
An adaptive system for outdoor infrared video surveillance
Industrial Applications, Chairman: Ch. Maierhofer

12.30 P.G. Bison, F. Cernuschi, E. Grinzato, S. Marinetti, D. Robba (CNR, CESI, It) Ageing evaluation of thermal barrier coatings by thermal diffusivity

12.50 G. Cuccurullo, G. Sorrentino, L. Cinquanta, V. Pierro (DIMEC; Uni. Molise; Uni. Sannio, Italy)
A procedure to achieve fine control in MW processing of foods

13.10 J. Zmywaczyk, H. Madura, P. Koniorczyk, M. Dabrowski (MUT, Poland) Estimation of thermophysical properties by an inverse method with experimentally determined temperature distribution of a heater

Closing Session

September 12–16, 2005

SPIE Europe International Symposium
Optical SystemsDesign

Friedrich-Schiller-Universität Jena, Germany

Symposium Chair. Andreas Tünnermann, Fraunhofer-Institut für AngewandteOptik und Feinmechanik (Germany)

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Theme: Imaging osteo-articular pathologies

Registration deadline: 1st September 2005

Information:
Dr Tiu Calin

Tel/fax +40 244 373 108
Email: office@srt.ro)
Website: srt.r5

October 22, 2005

18th Thermological Symposiumof the Austrian Society of Thermology and the Ludwig Boltzmann Research Institute for Physical Diagnostics

Venue: SAS Radisson Hotel, Vienna

Main theme. Advances in Thermal Imaging and Temperature Related Therapy

Deadline for abstracts: September 15, 2005

Information: Prof Dr Kurt Ammer PhD

Ludwig Boltzmann Forschungsstelle für Physikalische Diagnostik, Hanuschkrankenhaus, Heinrich Collinstr.30
A-1170 Wien
Tel: +43 1 914 97 01 Fax: +43 1 914 92 64

Email. KAmmer1950@aol.comt

November 9, 2005

Medical Thermomography & Thermometry
at National Physical Laboratory, Teddington, UK

Organised by the IPEM Physiological Measurernent and Ernerging Technology Special Interest Groups

Co-sponsored by National Physical Laboratory Thermal Measurement Awareness Network and the United Kingdom Thermography Association

Deadline for abstracts. 10- August 2005

Information: Dr John Picket, Clinical Physics
Bart´s and the London NHS Trust
The Royal London Hospital
London E1 2BL

Tel: +44 20 7377 7000 ext 2002 Fax: +44 20 7377 7100
Email: j.a.picket@qmul.ac.uk

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ZAKOPANE POLAND 15th –17th Sepember 2006

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Topics

Human body temperature, thermal physiology, applications of thermal imaging in medicine, veterinary medicine and biology.
Infra red imaging systems, software systems for thermal image processing,
Technological and practical standards.

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