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EXERCISE-ASSOCIATED SKIN TEMPERATURE DYNAMICS BY INFRARED THERMOGRAPHY. METHODS AND APPLICATIONS.

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PROLOGUE

My interest for science and research began when I was attending my Master Thesis project.
My Professor Giampietro Alberti proposed to me a project aimed to compare thoracic and diaphragmatic breathing exercises using thermography. At that time, I was particularly interested in respiratory muscles training. I did not hesitate and I accepted his proposal enthusiastically.
It was an interdisciplinary project involving various disciplines such as sport sciences, physiology, applied physics, bioengineering. In fact, the project was in cooperation with the Department of Physics of Università degli Studi di Milano. I studied the fundamental concepts of infrared physics, and I spent almost of my time in laboratory, gathering and analyzing data: a great opportunity to study physical exercise, such as breathing training, using a non-usual approach. The result was an incredible opportunity of knowledge and growth. Since that very moment, I thought that I wanted to become a researcher.
Thus, I applied for a Phd in Sport Sciences, during which my passion for research has been rising exponentially.

The main topic of my Phd project was the application of infrared thermography in the assessment of the skin temperature modifications in relation to exercise. Thermal images are recorded under nonsteady-state conditions using a thermal camera, and further analyzed to extract measures of skin temperature.

The first step of my doctoral course was the preparation of the manuscript regarding my Master Thesis project, that has been published some months later. The next steps included other studies on the same issue, such as thermography and physical exercise, of which part of them are currently in development.
Furthermore, the second topic of my Phd course was the study of the pacing strategy in cross-country skiing, which allowed me to match my cross-country skiing hobby with my interest for research.
After three years, I am now concluding my Phd course and I wish I will be able to continue doing research better and better.

ACKNOWLEDGEMENTS

I would like to thank everyone helped me in different ways during the last three years.

First, my father and my mother for constantly supporting and encouraging my studies and my choices.

I give my deepest thanks to my tutor Professor Giampietro Alberti for his constant support and guidance. Moreover, for giving me the possibility to begin a PhD course three years ago, and for still building my dreams to become a researcher.
Special thanks to Dott. Nicola Ludwig (Department of Physics, University of Milan) for his excellent guidance and constructive advice since the period of my master thesis, and Dott. Marco Gargano (Department of Physics, University of Milan) for his constant and precious support in infrared-thermography lab, and patience in explaining me infrared physics issues.
I owe many thanks to Prof. Andrea Caumo for his invaluable contribution in the development of my research projects and data analysis.
Thanks to Prof. Giovanni Michielon for his precious help in different ways, and for increasing my self-confidence constantly.
As for Professors, I would like to thank my University friends. I have had the opportunity to meet precious friends. Thanks to them for the great experience that we have spent together, even in different way: Luca, Athos, Alessio, Luca, Gabriele, Nicoletta, Jacopo, Paolino, Marco, Letizia.

During the second year of my PhD course, I had the opportunity to spend a research period in the University of Alta (Norway). There, I was involved in two research projects, tutored by Professors Andi Weydahl and Tor Oskar Thomassen, which I would like to thank a lot. Furthermore, thanks to Professor Trine Glad (Head of Department of Sport, University of Alta), Giovanna Calogiuri (University of Hedmark), and Isabella Desantis for their support and friendship during this period.

Last, but certainly not least, thanks to all my friends that have actively contributed to my growth during these years, expecially the climbing group. As someone has previously said: “we are and we will remain forever *pura razza caiana*”. Furthermore, and probably the most important citation: “one is worth one”.

PUBLICATIONS

During the three years of my Phd course, the following papers, in which I have authorship, have been accepted for publication:

I. Ludwig, N., Gargano, M., Formenti, D., Bruno, D., Ongaro, L., Alberti, G.. Breathing training characterization by thermal imaging: a case study. *Acta Bioeng. Biomech.* Vol. 14, No. 3, pp.41-47. 2012.

II. Formenti, D., Ludwig, N., Gargano, M., Gondola, M., Dellerma, N., Caumo, A., Alberti, G.. Thermal imaging of exercise-associated skin temperature changes in trained and untrained female subjects. *Ann. Biomed. Eng.* Vol. 41, No. 4, pp.863–871. 2013.

III. Ludwig, N., Formenti, D., Gargano, M., Alberti, G.. Skin temperature evaluation by Infrared Thermography: Comparison of Image Analysis Methods. *Infrared Phys. Tech.* Vol 62, No. 1, pp.1-6. 2014.

IV. Formenti, D., Trecroci, A., Cavaggioni, L., Caumo, A., Alberti, G.. Heart rate response to a marathon cross-country skiing race: a case study. *Sport Sci. Health.* DOI 10.1007/s11332-014-0187-8, 2014.

V. Formenti, D., Rossi, A., Calogiuri, G., Thomassen, T.O., Scurati, R., Weydahl, A.. Exercise intensity and pacing strategy of cross-country skiers during a 10-km skating simulated race. *Res. Sports Med.* Accepted and in press.

The present doctoral thesis focuses on two of these manuscripts: II and III. Furthermore, new data inserted in the following study, which is currently in progress, are presented. A preliminary report has been published as abstract:

VI. Formenti, D., Trecroci, A., Ludwig, N., Gargano, M., Caumo, A., Alberti, G., Thermographic skin temperature response to different movement velocity of squat exercise until exhaustion: a preliminary report. In Book of Abstracts of the 19. Annual Congress of the European College of Sport Science - ISBN 978-94-622-8477-7. 2014.

ABSTRACT

Heat dissipation during sport exercise is an important physiological mechanism that may influence athletic performance. Therefore, monitoring skin temperature during exercise provides important physiological information about thermoregulatory processes. Skin temperature measurements through infrared thermography have the advantages to be non-invasive and to record temperature data simultaneously from different points on a wide area of the body.

The aim of the present investigation were: first, to compare three methods of thermal images analysis in skin temperature evaluation, and second to study the skin temperature dynamics during two types of physical exercise. In the present thesis three studies will be presented and discussed.

The analysis of thermographic images, with the goal of obtaining a temperature value representative of a specific area, is usually performed by different methods of averaging temperature values inside a selected Region of Interest (Troi and Tot). A comparison between the methods mainly used in literature in the specific case of a muscular group of calves on a population of 33 healthy subjects is presented. Here, it is presented an alternative method (Tmax) to obtain a temperature value of a specific area based on maximal temperature detection instead of considering the average temperature on the selected area. No meaningful difference in mean temperature between Troi and Ttot was found ($p = 0.9$), while temperature values calculated using Tmax were higher than the above methods ($p < 0.001$). The high correlation among the compared methods prove that they can equally represent temperature trends in cutaneous thermographic analyses.

The second and the third study presented here are applicative study investigating the skin temperature response during physical exercise.

The aim of the second study was to test the hypothesis that differences exist in the dynamics of exercise-associated skin temperature changes between trained and untrained subjects. Thermoregulation of a local muscle area (muscle–tendon unit) involved in a localized steady-load exercise (standing heels raise) using infrared thermography was investigated. Seven trained female subjects and seven untrained female controls were studied. Each subject performed standing heels raise exercise for 2 min. Thermal images were recorded prior to exercise (1 min), during exercise (2 min), and after exercise (7 min). The analysis of thermal images provided the skin temperature time course, which was characterized by a set of descriptive parameters. Two-way ANOVA for repeated measures detected a significant interaction ($p = 0.03$) between group and time, thus indicating that athletic subjects increased their skin temperature

differently with respect to untrained subjects. This was confirmed by comparing the parameters describing the speed of rise of skin temperature. It was found that trained subjects responded to exercise more quickly than untrained controls ($p<0.05$). In conclusion, physical training improves the ability to rapidly elevate skin temperature in response to a localized exercise in female subjects.

The third study presented here is a preliminary report, since the data analysis is still in progress. It aimed to investigate the skin temperature response by using infrared thermography during slow speed low intensity exercise as compared to normal speed low intensity exercise in squat trial. We hypothesized that low intensity resistance exercise with slow movement would result in a skin temperature response slower than the one of the normal speed exercise with the same intensity.

13 active males performed 2 sessions of deep squat exercise until exhaustion, with 50% of 1 RM. The pace of movement was set in 1s eccentric / 1s concentric and 5s eccentric / 5s concentric phase in the 1st and in the 2nd session respectively. Thermal images were recorded every 20s before exercise (2min), during exercise (until exhaustion), and after exercise (10min).

Surprisingly, a different behaviour of skin temperature during and after exercise was observed among subjects: a decrease in skin temperature in 9 subjects (down group) and an increase in the other 4 (up group). Thus, statistics will be performed in each group separately.

It was shown that the response of cutaneous circulation to dynamic exercise is characterized by a initial vasoconstriction to dissipate heat from the core through the skin followed by vasodilation driving the blood flow from inactive tissue (including the skin) to active muscles involved in exercise. We speculate that the unexpected different behaviour of the skin temperature response in the 2 sub-groups was probably due to a time-dependent predominance of vasoconstriction over vasodilation or viceversa.

PART 1: INTRODUCTION

In this initial part, the background on which this thesis is based will be given.

In particular, the first part provides a brief general overview of the application of infrared thermography in biomedical field, with particularly emphasis to the application in exercise sciences and to the comparison with more traditional instruments, such as thermocouples.

The second part concerns infrared thermography technology: theoretical physics concepts on which infrared thermography is based, as well as practical and methodological features in its application are presented.

1.1. BIOMEDICAL APPLICATIONS OF INFRARED THERMOGRAPHY

1.1.1. A general overview of the use of infrared thermography in biomedical sciences

The first use of infrared thermography technology in the biomedical sciences was reported in 1960 (Ring 2007; Ring and Ammer 2012), although its application in the evaluation of skin temperature were already proposed several years before, by Hardy in 1934 (Hardy 1934).

IRT has been applied in the last 50 years in studying diseases in which skin temperature is an indicator of inflammation, or skin blood flow modifications caused to a clinical abnormality (Lahiri et al 2012; Ring and Ammer 2012).

In 1963, in a study published by the prestigious journal *Science*, Barnes showed that thermograms can provide useful information of physical anomalies and thus, it can be used for diagnosis of physical illness (Barnes 1963). Sherman and colleagues used infrared thermography for the assessment of skin temperature asymmetries between paired limbs and concluded that infrared thermography together with grid map of body is the simplest and immediate method for scanning body temperature (Sherman et al 1996). Recently, Fauci et al. reviewed the history of infrared thermography with special regard to detector systems, and its application in medical diagnosis (Fauci et al 2001) and Jiang et al. reviewed the potentialities and limits of infrared thermography in biomedical field (Jiang et al 2005). Several applications of infrared thermography in medical science has been proposed and discussed (Jung 2003; Ring and Ammer 2012).

It has been extensively used for diabetic neuropathy (Ring 2010; Bagavathiappan et al 2010), vascular disorders (Bagavathiappan et al 2009; Pauling et al 2012), breast cancer detection (Acharya et al 2012), thermoregulation study (Bouzida et al 2009), fever screening (Singler et al 2013).

Furthermore, there are also applications in dentistry and dermatology (Fikackova and Ekberg 2004), diagnosis of rheumatologic diseases (Will et al 1992; Ring 2004), detection of metastatic liver disease (Mansfield et al 1970), heart treatment (Manginas et al 2010).

Throughout the last 20 years, considerable progresses have been reached in the knowledge of the physiological mechanisms of skin temperature changes and temperature distribution over surface of specific body area (Yang and Yang 1992; Lahiri et al 2012).

Finally, it is possible to conclude that infrared thermography has a great potentiality of different applications in biomedical sciences.

1.1.2. The measure of skin temperature during physical exercise: thermocouples vs. thermography

It is well established that physical activity induces complex thermoregulatory processes where part of heat in excess is dissipated through the skin to the external environment. Physical exercise and repetitive effort is a challenge to thermal homeostasis. During exercise, the thermoregulatory control of skin blood flow has the fundamental role to maintain normal body temperature and leads to changes in hemodynamics, and, therefore, thermal signals (Kenney and Johnson 1992). Therefore, measuring skin temperature provides useful information about the complex thermal control systems (Fernandes et al 2014).

The immediate response to exercise on skin temperature, reflecting modifications of skin blood flow is usually measured using thermocouples. Thermocouples are the most common method for the measure of the skin temperature. Their system is based on the Seebeck effect, which occurs when a potential difference is created between two conductor materials at different temperature (Childs 2001; Smith et al 2010). The main advantages of thermocouples are high accuracy, sensitivity, high range of temperature measures, and the possibility to be used in the measure of skin temperature during exercise (Smith et al 2010). Disadvantages include: small measurement area of only few cm^2 (Smith et al 2010); differences in skin temperature based on the method for attaching thermistors (Tyler 2011); complications related to heat loss through convection, conduction and evaporation in the area where the thermocouples are attached, due to the contact feature of the measurement system (Smith et al 2010; Fernandes et al 2014).

Similar to thermocouples, infrared thermography has both advantages and disadvantages in the measure of skin temperature during exercise. The main advantage is the non-invasiveness of the technique that does not require direct contact with the skin, avoiding heat loss by conduction (Vargas et al 2009). Furthermore, the capability of monitoring skin temperature over a wide are of body (region of interest: ROI), the freedom of movement during exercise, high accuracy and sensitivity, and the possibility to visualize a map of temperature of the region of interest. With specific types of camera, it is also possible to record video, under non-steady state condition such as exercise (Zaproudina et al 2008; Vargas et al 2009; Fernandes et al 2012; Fernandes et al 2014). Disadvantages are the relatively high cost of the instrumentation, the need to stop exercise for recording when the camera has not video mode, and the need for specific training to control factors that may affect the measurements (Vargas et al 2009; Marins et al 2014b). Furthermore, the analysis of thermal images requires long time, training and experience for extracting temperature values of the region of interests.

A Brazilian research group has recently published an interesting study comparing thermocouples and infrared thermography in the measure of skin temperature before, during and after exercise. They found that there is low concordance between the two methods examined (Fernandes et al 2014). It is possible to conclude that both thermocouples and infrared thermography have advantages and disadvantages, which should be considered in the measure of skin temperature related to exercise. However, the present thesis is only based on the application of infrared thermography technology in the evaluation of skin temperature changes associated to exercise, and no comparison between thermography and thermocouples will be addressed.

1.1.3. The application of infrared thermography in sport and exercise sciences

Although the first paper involving the application of infrared thermography in the study of physical exercise was published in 1977 (Clark et al 1977), only in the last years infrared thermography has been widely used as technique to record variations of skin temperature associated to physical exercise.

Nowadays, thermography has been using for detecting locomotion injuries in athletes. Research suggests that one of the most beneficial application of infrared thermography is the screening of athletes for overuse injuries, and to monitor health status of osteoarticular structures (Abate et al 2010; Hildebrandt et al 2010; Hildebrandt et al 2012; Marins et al 2014a).

On the other hand, only few studies using infrared thermography have been devoted to the study of physiological responses of skin temperature to physical exercise. Using infrared camera technology, exercise-associated cutaneous temperature modifications can be visualized. Therefore, the analysis of infrared images recorded before, during and after sport-specific exercise allows a straightforward investigation of the skin temperature response to exercise. Thermal measurements of skin using infrared thermography is an indirect valid assessment of the skin blood flow (Swain and Grant 1989).

The first studies focused not only on the skin temperature changes related to exercise, but also on the skin temperature distribution over the whole body, or specifics body regions.

Authors found that during exercise the distribution of skin temperature differed dramatically from that observed before exercise (Clark et al 1977; Goss et al 1988; Hunold et al 1992). This fact may be attributed to a redistribution of the blood flow under the cutaneous tissue, caused by intervention of thermoregulatory and non-thermoregulatory mechanisms.

Another issue is the study of the skin temperature variations associated to physical activity or exercise. It is possible to distinguish two types of thermographic investigation in exercise science:

1. Recording thermal images before and after a specific exercise, aimed to assess difference in skin temperature value from pre to post exercise.
2. Recording thermal images before, during, and after exercise, to evaluate the skin temperature dynamics occurred throughout a specific exercise.

Both types of investigation are present in literature. However, it is worth noticing that the second one (i.e. evaluating the skin temperature dynamics occurred throughout a specific exercise) present the greater difficulties in the preparation of experimental protocol and setting.

This is due to the body movement of the subject under inspection. It is not possible to move the thermal camera to follow the region of interest of subject during exercise. Thus, when region of interest coincides with parts of the body in movement, it is usually required that subject interrupts exercise for 1 s to 3 s or re-achieves the starting position (Formenti et al 2013). Otherwise, it is possible to record thermal images in region of interests which do not coincide with parts of the body in movement (Zontak et al 1998).

1.1.4. A brief literature overview of the application of infrared thermography in sport and exercise

Thermographic investigations have been performed in various disciplines and sports. Most of the studies focused on cycling, probably the discipline more studied.

Two important papers investigating the skin temperature response to cycling effort have been published in 90 years. Hunold and colleagues studied skin temperature patterns (i.e. temperature distribution over the surface) of thighs in response to 10 min at 100 w. They found that i) the relatively homogenous temperature distribution at rest was replaced by a temperature pattern with well-defined warmer and cooler areas; ii) the skin temperature tended to decrease during exercise period (Hunold et al 1992). Such decrease in skin temperature during cycling exercise was also observed by Torii et al, which entitled their manuscript, published by the "*British Journal of Sports Medicine*", "*Fall in skin temperature of exercising man*". This decrease in skin temperature, occurred especially at the onset of the exercise, was attributed to vasoconstriction and not to thermoregulatory factors (Torii et al 1992).

Other authors applied infrared thermography to the study of skin temperature response to cycling exercise. Abate and colleagues examined the skin temperature response to cycling warm up in trained and untrained subjects (Abate et al 2013). Furthermore, a research group from the *University of Reims* investigated the relationship between gross efficiency, heart rate, and skin temperature of the lower limb in cycling exercise (Bertucci et al 2013; Arfaoui et al 2014).

Infrared thermography has been applied in other disciplines, such as running (Cena and Clark 1976; Clark et al 1977; Merla et al 2005; Merla et al 2010; Fernandes et al 2014) and swimming (Zaïdi et al 2007; Seixas et al 2014). It is worth emphasizing the contribution of a research group of the *Università di Chieti e Pescara*: they studied the time profiles of skin temperature of thighs before, during and after running exercise in trained and untrained subjects (Merla et al 2005), as well as the skin temperature modifications associated to running exercise until exhaustion in different parts of the body (Merla et al 2010).

Of particular interest are also those papers that have addressed the skin temperature response to resistance exercises, such as knee flexion (Ferreira et al 2008), isometric contraction of the anterior deltoid (Bertmaring et al 2008), and breathing exercise (Ludwig et al 2012). As compared to part of the others (Torii et al 1992; Zontak et al 1998; Abate et al 2013), these studies regarding resistance exercise have the peculiarity that thermal images were recorded on the muscles involved in exercise directly. This allows a straightforward evaluation of the metabolic heat dissipated by radiation through the skin.

Finally, Gold and colleagues (Gold et al 2004) measured cutaneous temperature of the dorsal hand of office workers during writing at keyboard, to assess differences between subjects with carpal tunnel syndrome and healthy subjects.

1.2. THEORETICAL AND METHODOLOGICAL ASPECTS OF INFRARED THERMOGRAPHY THECHNOLOGY

1.2.1. Theoretical aspects of infrared physics

Based on the measurement of the infrared radiation emitted from an object, infrared thermography is an accessible technique to obtain images that represent the temperature distribution of a surface.

The principle of this technique is that all objects with temperature above absolute zero (0 °K) emit electromagnetic radiation. For room temperature ranging between 0 °C and 100 °C these are mainly in infrared (i.e. below red) radiation or thermal radiation (Jones 1998; Maldague 2001). The intensity of this radiation is a function of temperature.

Conventionally, the band of this thermal infrared radiation lies within a range of 3 –14 μm (Figure 1).

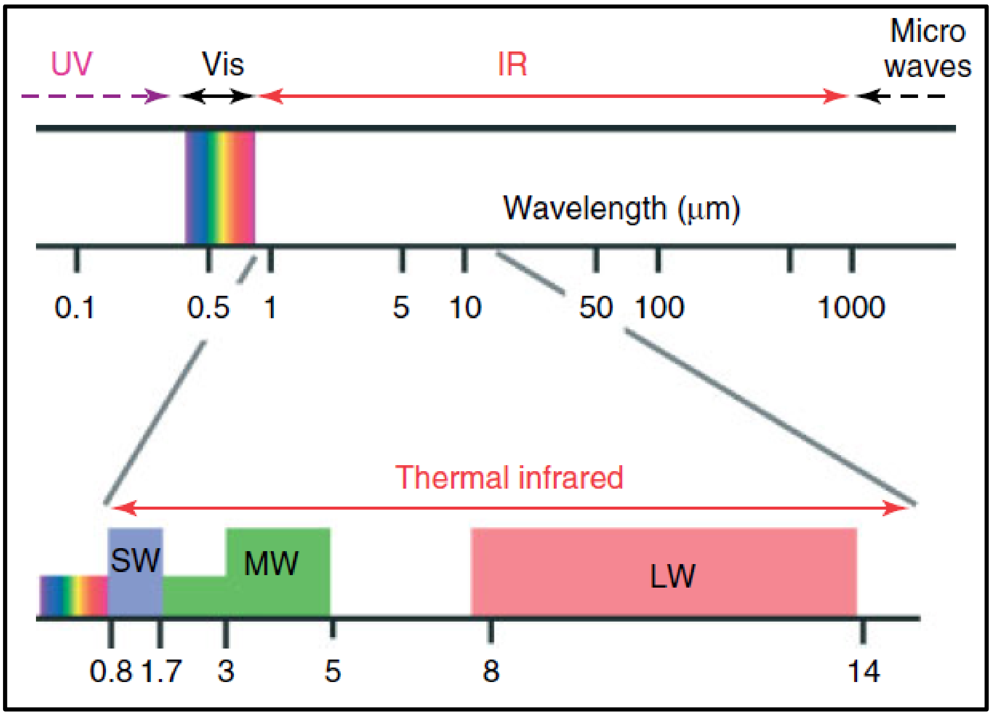

Figure 1: Infrared (IR) and adjacent spectral regions and expanded view of the so-called thermal infrared. This is the region where IR imaging systems for short-wave (SW), mid-wave (MW), or long-wave (LW) cameras exist. Special systems have extended MW or SW ranges (Vollmer and Mollmann 2010).

These spectral bands are defined considering the atmosphere transmittance and the wide range 0.8 μm – 14 μm can be further divided into three smaller sub-groups:

- Short wave infrared (SWIR) of 0.8 μm – 2.5 μm
- Medium wave infrared (MWIR) of 3 μm – 5 μm
- Long wave infrared (LWIR) of 8 μm - 14 μm

According to thermal radiation theory, blackbody is considered as an ideal object that absorbs all incident radiation and radiates a continuous spectrum according to the Planck's law. When integrating the Planck's law for all frequencies, it is possible to obtain the Stefan–Boltzman's law (Equation 1), which describes the total emissive power from a blackbody (Modest 2003; Lahiri et al 2012):

$$E = \sigma T^4$$

Equation 1

where E is the total emissive power (W/m^2), σ is the Stefan Boltzman's constant (σ = 5.67 10^{-8} W/m^2 K^4) and T is the absolute temperature (K). For real surfaces the Stefan Boltzman's law is modified and integrated to the following equation (Equation 2):

$$E = \varepsilon \sigma T^4$$

Equation 2

where ε is the emissivity of the emitting surface at a fixed wavelength and absolute temperature T. For a perfect blackbody emissivity is unity (i.e. = 1), but for real materials emissivity is always less than unity and can vary with the emitted wavelength and temperature itself.

In general, emissivity of a surface is given as the radiant power emitted with respect to the emission radiance from a blackbody at the same temperature (Equation 3):

$$\varepsilon = \frac{W_{real\,body}(T)}{W_{black\,body}(T)}$$

Equation 3

Although the infrared emissions from human skin at 27 °C lies within the wavelength range of 2–20 µm, it peaks around 10 µm. For medical applications a very narrow wavelength band (8–12 µm), termed as body infrared rays, is in general used (Lahiri et al 2012). With the advent of newer generations of detectors, SWIR and MWIR regions are also used in medical thermography (Bagavathiappan et al 2010). Infrared thermal cameras have particular lenses, transparent to this kind of radiation, that focuses the radiation onto a detector consisting of an array of elements (pixels). Those pixels, throughout different type of principle of detection, transform infrared radiation into electrical signal, which is further processed to produce a thermal image where any pixel represents a temperature value, and that can be visualized on a monitor. (Jones 1998; Kennedy et al 2009; Lahiri et al 2012).

1.2.2. Methodological aspects of the application of infrared thermography

Imaging systems with low thermal and spatial resolution has limited in the past the quality of the measurements, affecting the acceptance of the infrared thermography technique in biomedical sciences. New advances in infrared cameras and technology have recently promoted infrared thermography as a powerful measurement tool. A new generation of high-resolution cameras, advanced software and standardized protocols have been developed for biomedical application, resulting in improved diagnostic capability and reliability (Ring and Ammer 2000; Jones and Plassmann 2002; Ring et al 2004; Lahiri et al 2012).

In 1987, the American Medical Association recognized infrared thermography as a feasible diagnostic method. The following thermographic organizations promote the application of biomedical thermal imaging, providing useful standards and guidelines in experimental protocols and settings:

- International Academy of Clinical Thermology
- International Thermographic Society
- American Academy of Medical Infrared Imaging
- European Association of Thermology
- Northern Norwegian Centre for Medical Thermography
- German Society of Thermography and Regulation Medicine

Infrared radiation emitted by a surface depends on the experimental conditions such as moisture, airflow and surrounding temperature. Hence, it is an absolute necessity for thermography experiments, especially in medical applications where temperature changes are within a few degrees, to be performed in controlled environments. For comparing thermographic images, a standard protocol must be followed.

In a paper published by the journal *Thermology International*, Ring and Ammer reported that infrared thermography can produce reliable results in medical application only when certain established standards are followed (Ring and Ammer 2000). They carefully described the standards for experimental protocol, examination room, control of temperature and humidity, subject information processing, imaging system, image acquisition, image processing and data analyses. These standards are a very useful guide for researchers pursuing research in the field of medical infrared thermography.

During thermography experimentations, the subjects have to be kept in a comfortable environment so that the mild thermal stress produced results in vasoconstriction aided cooling of skin, which renders the hot spots due to underlying abnormalities well visible (Jones 1998). It was also reported that, the temperature and the humidity of the examination room should have to be controlled in such a way that the physiology of the subjects is not "stressed into a condition of shivering or perspiring" (Bronzino 2006). It is advisable that the subjects should refrain from exposure to direct sunlight and uses of cosmetics, antiperspirants or deodorants immediately before the thermography examinations.

As recently proposed and discussed, a thermal acclimation time is required for the subjects to achieve thermal equilibrium at rest (Marins et al 2014b). The acclimation may be nude or normal, depending upon the parts of the body to be examined. Figure 2 shows the schematic of a typical experimental set-up. Furthermore, it was shown that the experiment-room must be free from any secondary infrared sources like incandescent lamp or direct sun-light (Bronzino 2006).

Thermographic experiments require an infrared camera, a tripod, a display device and an image processing unit. Nowadays, display and image processing are performed using a personal computer and dedicated software packages. Since from its inception, the infrared cameras have undergone three generations of advancements. In the following paragraph, a synthetic description of development and working principles of various infrared detectors are presented.

The first generation cameras used a single element detector, and two scanning mirrors to produce images. They suffered from white out (i.e. saturation due to high intensity) problems.

The second generation camera were based on two scanning mirrors along with a large linear array or small 2-D array as detectors. A time delay integration algorithm permitted image enhancement.

Third generation cameras are without mirrors and have large focal plane array (FPA) detectors and on-chip image processing, thereby increasing the reliability and sensitivity of such systems (Vainer 2005).

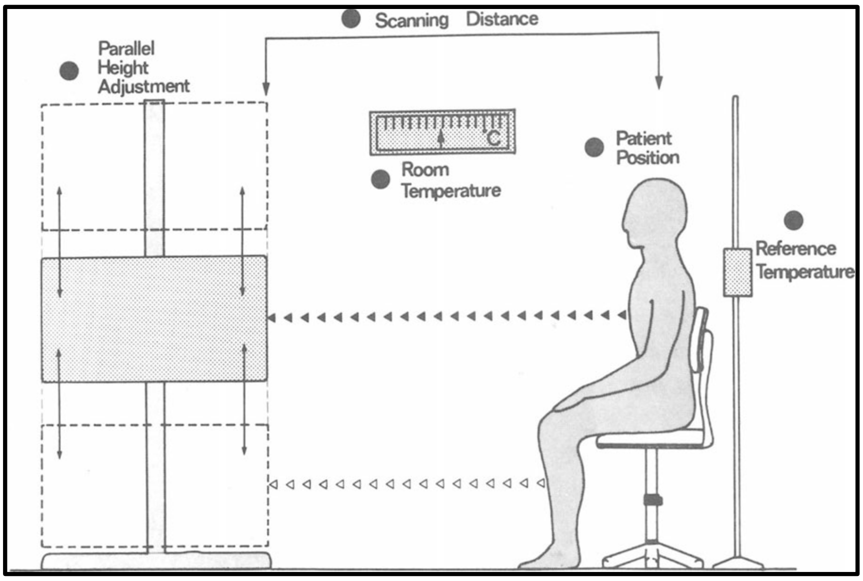

Figure 2: Schema of a typical experimental set-up for biomedical thermography experiment. The temperature and humidity of the experiment-room is maintained within a comfortable limit. The camera is placed in front of the observation surface (Houdas and Ring 2013).

Thermal detectors are classified into two categories: cooled and uncooled. Modern developments in solid state systems have contributed to create the way for production of new types of detectors, which have better accuracy and resolution. Currently, thermal sensitivity of the uncooled cameras is about 0.05 °C compared to 0.01 °C of the cooled ones (Vainer 2005). The uncooled ones have many advantages, such high spatial and temperature resolutions, compactness and portability. Besides, these cameras are light weight, manufactured by silicon wafer technology and are cheap compared to the cooled infrared cameras. Such modern digital uncooled infrared cameras improved medical thermal imaging immensely. FPA based cameras may have spatial resolution less than 2 mm over a range of distances and fields of view (200 x 200 mm to 500 x 500 mm at a distance of 1 m) (Vainer 2005; Lahiri et al 2012).

PART 2: THREE EXPERIMENTAL STUDIES

In this part, three studies will be presented and discussed.

The first one is a methodological study, investigating three different types of images analysis methods. In the first part of this thesis, methodological aspects of the application of thermography in biomedical sciences are presented and discussed: image analysis processing represents a key point in the standardization of the procedures for performing thermographic analysis in the exercise sciences. The paper is entitled "Skin temperature evaluation by infrared thermography: comparison of image analysis methods". Due to such a methodological peculiarity, this paper has been published in a journal covering the entire field of infrared physics and technology: theory, experiment, devices and instrumentation ("*Infrared Physics and Technology*").

The second study presented here is an applicative study, investigating the differences between trained and untrained subjects in the skin temperature dynamics during and after exercise, using infrared thermography. The study involved the expertise sport scientists, physicians, biomedical engineers, resulting in an interdisciplinary project. The paper is entitled "Thermal imaging of exercise-associated skin temperature changes in trained and untrained female subjects" and has been published by the "*Annals of Biomedical Engineering*" in 2013.

It is worth noticing and that a figure from this manuscript has been chosen as cover image of the journal's issue. This choice was justified with these words by the Editor in Chief:

"Of the numerous excellent illustrations in your manuscript, we felt that Figure 1 had the kind of scientific and aesthetic qualities for which our journal strives."

I am particularly proud of this editorial choice, which came in addition to the satisfaction for publishing in a prestigious journal, such as "*Annals of Biomedical Engineering*".

The cover of the Journal with the figure chosen from our manuscript is presented in the following page (Figure 3).

The third study aimed to investigate to investigate the skin temperature response during low intensity resistance training with slow speed as compared to low intensity training with normal speed in squat exercise. It is presented in a preliminary form, since the data analysis is still in progress.

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Figure 3: Cover image of Volume 41, number 4 of the Journal "*Annals of Biomedical Engineering*".

2.1. STUDY1: SKIN TEMPERATURE EVALUATION BY INFRARED THERMOGRAPHY: COMPARISON OF IMAGE ANALYSIS METHODS [1]

2.1.1. Introduction

The first use of Infrared Thermography (IRT) in the biomedical sciences was reported only in 1960 (Ring 2007), although the diagnostic applicability of temperature measurement by infrared technique were already proposed by Hardy in 1934 (Hardy 1934). IRT has been used in the last 50 years to study diseases in which skin temperature is an indicator of inflammation or blood flow changes due to a clinical abnormality (Lahiri et al 2012; Ring and Ammer 2012). In living body measurements, mechanisms of skin heating/cooling are complex due to the combined effects of radiation and local blood flow.

Considerable progress has been achieved over the last 20 years in the knowledge of the physiological mechanism of skin temperature distribution and in the methodology of usability of IRT for the standardization of measurement protocols and the statistical data analysis (Ring 2007; Lahiri et al 2012; Ring and Ammer 2012).

The use of IRT in the measurement of temperature of human skin has the advantage to be completely non-invasive. The advantage of using this technique, compared to alternative methods requiring a contact between the object and the sensor, lies in the fact that with the use of IRT the skin temperature is not influenced by the presence of any probes that could modify the temperature variation of the surface through conduction or through irradiation, avoiding psychological influence too. Thermal Imaging permits also to have a simultaneous temperature map of several parts of the subject analyzed.

In order to perform correct and reliable thermographic measurements on humans, several conditioning factors should be controlled: Ring and Ammer suggest the requirements for the environmental conditions, the experimental set up and the preparation of the subject (Ring and Ammer 2000; Ring and Ammer 2012).

To the authors' knowledge, despite the increasing popularity of the IRT in biomedical field, no papers are currently available investigating the characteristics of the method to calculate the representative temperature value of the anatomical region under inspection.

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[1] Ludwig, N., Formenti, D., Gargano, M., Alberti, G.. Skin temperature evaluation by Infrared Thermography: Comparison of Image Analysis Methods. *Infrared Phys. Tech.* Vol 62, No. 1, pp.1-6. 2014. (see appendices pag. 60).

In the last years IRT has progressively turned into an important technique to record variations of cutaneous temperature linked to physical activity. Gold et al. (Gold et al 2004) measured temperature of the dorsal hand of office workers using a rectangular analysis tool of a thermal camera software, positioned following anatomical features. Merla et al. (Merla et al 2010) studied thermoregulation during and after the exercise in runners, Zontak et al. (Zontak et al 1998) and Torii et al. (Torii et al 1992) for bicycle ergometer, Ludwig et al. (Ludwig et al 2012) for breathing exercise, Formenti et al. (Formenti et al 2013) and Ferreira et al. (Ferreira et al 2008) for resistance exercise in different categories of subjects (trained/untrained and old/young respectively). In such an exercise-associated skin temperature changes studies, researchers began to specify the methods to calculate the representative temperature value of the area under investigation. The analysis of thermographic images is usually performed by a dedicated software selecting a Region of Interest (ROI), ranging from few pixels to some hundred, on a specific body area. In the study concerning breathing exercise, Ludwig et al. (Ludwig et al 2012) considered 10 ROI on the subject trunk. Thermal data of seven ROI was calculated averaging the temperature value of all the pixels included in each ROI. For the others three ROI (left and right pectoral muscles, and navel) thermal data were extrapolated using an alternative method: a larger ROI was selected including all the investigated area (anatomical ROI), then a dedicated software automatically found the warmest pixel in the ROI and averaging the temperature over the 24 neighbouring pixels. This procedure was set up as a consequence of the irregular thermal distribution inside the ROI where subcutaneous circulation was more evident with their irregular shapes. It also permitted to avoid any possible operator dependence, although Ferreira et al. in their experiment found no significant intra-individual variability (Ferreira et al 2008). A similar method was used by Formenti et al. (Formenti et al 2013) for evaluate skin temperature in trained and untrained subjects. They investigated how the training level improves the ability to rapidly elevate skin temperature in response to a localized exercise. To obtain a reliable mean temperature of calves surface, avoiding the operator dependency of on the ROI selection process, a new procedure was set-up. A region was selected by the operator for each calf including all the muscles involved in exercise. Inside this region, the software selected the five hottest pixels with five pixels of minimum distance from each other thus using a total amount of 125 pixels representative of the whole calf surface. This criterion allowed the authors to obtain a more reliable sampling of the warmest areas of the calves. Finally Jones and Plassmann (Jones and Plassmann 2002) presented in their paper different ways to use IRT for diagnostics on human, starting from single static image to very high frequency capture, using the ROI method to calculate the temperature as well .

All the aforesaid papers show a non-homogeneity in the analytical methods to calculate a representative temperature value of a body area. The reason being that, although with similarities, each of the authors uses a specific method to analyze an area of interest.

Skin temperature also depends on anatomical factors, such as the presence of irregularities on the surface and the presence of different kind of subcutaneous tissue (fat tissue and muscular tissue) (Merla et al 2010). These factors can influence distribution of the skin temperature of a specific body area thus; it is frequent to find a non-Gaussian thermal distribution inside a selected ROI in fact analysing cutaneous temperature the authors found abnormal thermal distribution of calves. For example in Figure 4 (left) it is possible to notice that, especially the left calf of the subject, presents a non-regular thermal distribution, while on the right of the same figure there is a subject having a normal thermal distribution of their calves. Accordingly, as demonstrates the irregular thermal distribution characterizing the subject's calves in Figure 4, it may not always be correct the choice to average over the temperature value of all the pixels included in a selected ROI. From this consideration it is clear that the ROI average method can not be used for all skin areas and for all situations. It would be correct to calculate the temperature values using other methods.

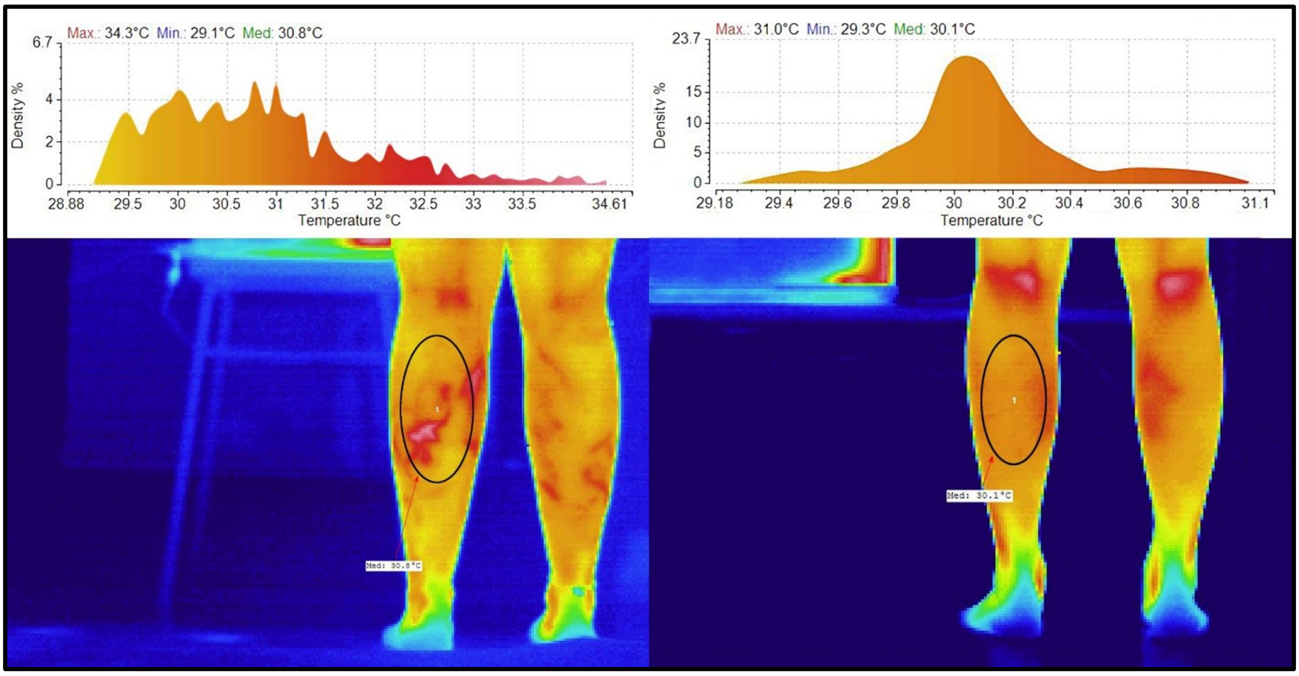

Figure 4: Thermal images of calves of two subjects with irregular (left) and regular normal-like thermal distribution (right).

The aim of this study was to compare three different methods of thermal imaging analysis presented in literature. To perform this comparison the authors evaluated the results obtained through the analysis of thermal images recorded on a well defined muscular area (calves) using different methods (Troi, Tmax, Tot) completely described in the 'Materials and Methods' section.

First, authors hypothesized that the two methods based on wide area calculation, Troi and Ttot, should be equivalent methods, while Tmax, based on threshold criterion, should be slightly different.

Second, the authors sought the best method to check difference in skin temperature between left and right calf of the same subject.

The comparison proposed in this work among the temperature values of the same body area (calves muscular group) obtained by three different methods could be helpful to better understand the advantages and the disadvantages of each method and can contributes to a detailed definition of criticisms and specific skills of these tools for thermal imaging analysis in the field of biomedical applications.

2.1.2. Materials and Methods

Subjects

The subjects taking part in this study were selected from a various heterogeneous population of the Università degli Studi di Milano and from a high level synchronized swimming team. Authors considered subjects from 15 to 45 years old in order to extend as more as possible the sample variety. Thus, authors collected 12 female athletes, 9 male athletes, 10 female sedentaries, 2 male sedentaries. The 33 healthy subjects had a wide range of anthropometric characteristics and level of physical activity (from sedentary to athletes). The choice of different categories of subjects was made in order to test how robust are the methods when there is an high interindividual variability.

They had not assumed drugs or medications with a potential effect on cardiovascular and thermoregulatory functions during the two months prior the tests.

The Ethical Committee of the Università degli Studi di Milano approved this study. After a thorough explanation of the protocol that was going to be used, the subjects, or the parents of the underage ones, accepted informed written consent to participate in this study.

Experimental protocol

Subjects observed the following standard preliminary protocol for infrared thermal imaging measurements, indicated by (Merla et al 2010) and (Ferreira et al 2008) in previous studies: they abstained from assuming alcoholic or caffeine-containing products for a 4-h period prior to the start of the experiment; they removed body hair on legs that were clean and without any cosmetics products to have the most representative thermal images of skin temperature.

The subjects acclimated themselves to the laboratory climatic conditions (temperature 22-23 °C; relative humidity 50 ± 5 %; constant natural and fluorescent lighting and no direct ventilation) for 15 minutes being sit in a rest position on an insolating cushion, then they had to stay in stand anti-gravitational static position for 10 minutes. After this period an operator started recording a series of 3 thermographic images of the calves of subjects (1 image per 20 s). All the measurements were performed in the late morning period in order to limit any effects linked to circadian rhythm variations of temperature (Reilly and Brooks 1986).

Thermographic analysis

The emissivity value was set to 0.97, in keeping with the value of human skin emissivity suggested by (Jones and Plassmann 2002). Thermal images of the surface of the subject's calves were recorded by a 14-bit digital infrared thermo-camera (AVIO, TVS-700, 320 x 240 Microbolometric Array; 8-14 μm spectral range; 0.07 °C thermal resolution; and 35 mm lens). Recordings were made using a digital frame grabber with a rate of one image per 20 seconds. During the measurements the subjects were dressed in swimsuit, and were set 3 m from the thermocamera. in an uniform background with a constant temperature (T=24.35 ± 0.25 °C). Temperature drift due to the heating of the IR detector was corrected by periodic self-calibration and by using a constant temperature reference area in the background (Formenti et al 2013).

Of the 3 thermographic images captured, authors considered the second one, keeping the other two as backup for verification in case of anomalous data.

In order to single out the representative temperature value of the calves area, the authors used three analysis methods called: Troi, Tmax, and Ttot. This imaging process has been performed using dedicated software for thermal images elaboration (GRAYESS® IRT Analyzer, Version 4.8, and a code realized under LabVIEW® platform for standard deviation calculation).

Troi method

A Region of Interest (ROI) per each calf was selected by an operator following physical location based on classical definition of body regions. In this study no landmarks were necessary to select the interested calf area: the criterion followed by the operator was based on the selection of the ROI taking considering the dimension of the leg and the need to maintain the ROI at the same distance from ankle and popliteus.

The final temperature value was calculated as arithmetic mean over the temperature value of all the pixels inside the ROI considered.

Tmax method

One region for each calf including all the surface of the anatomical part under inspection was selected by the operator. Inside this region, the software automatically selected the five hottest pixels having a minimum distance of 5 pixels from each other. The temperature value representative of the whole calf was then obtained by averaging the area of 5x5 pixels around the hottest pixels found by the software (Formenti et al 2013). The results are therefore representative of an overall amount of 125 pixels on a total area of about 1000 pixels.

Ttot method

The operator selected an area larger than the anatomical part involved in. The area started from the popliteus and ended at the ankle including also a large part of the colder background. In this way all the calf surface was included. To consider only calf area and exclude the background, the operator cut off data having temperatures distribution below a threshold corresponding to the lowest value of the leg, in this way has been taken into account only the upper part of the distribution (corresponding to the calf area) (Figure 5).

The final temperature value was calculated as arithmetic mean over the temperature value of all the pixels included in the upper part of this distribution.

Data analysis and statistics

Data obtained by different methods were put in linear correlation (Tmax/Troi; Troi/Tot; Tmax/Ttot) to check the capability of each method to read the actual temperature. To determine the strength of the relationship of the three methods, a correlation coefficient (r) was calculated. Student's t-tests were calculated to detect systematic difference between each couple of data set. The validity of Tmax method with respect to Troi (considered as a standard reference method) was assessed using the Bland and Altman method (Bland and Altman 1986; Bland and Altman 1995). Finally to determine the capability of each method to single out differences between left and right calf it was calculated the difference of temperature between left and right calves (ΔT_{l-r}) in each subject. A Gaussian fit was then applied to ΔT_{l-r} to describe their distribution.

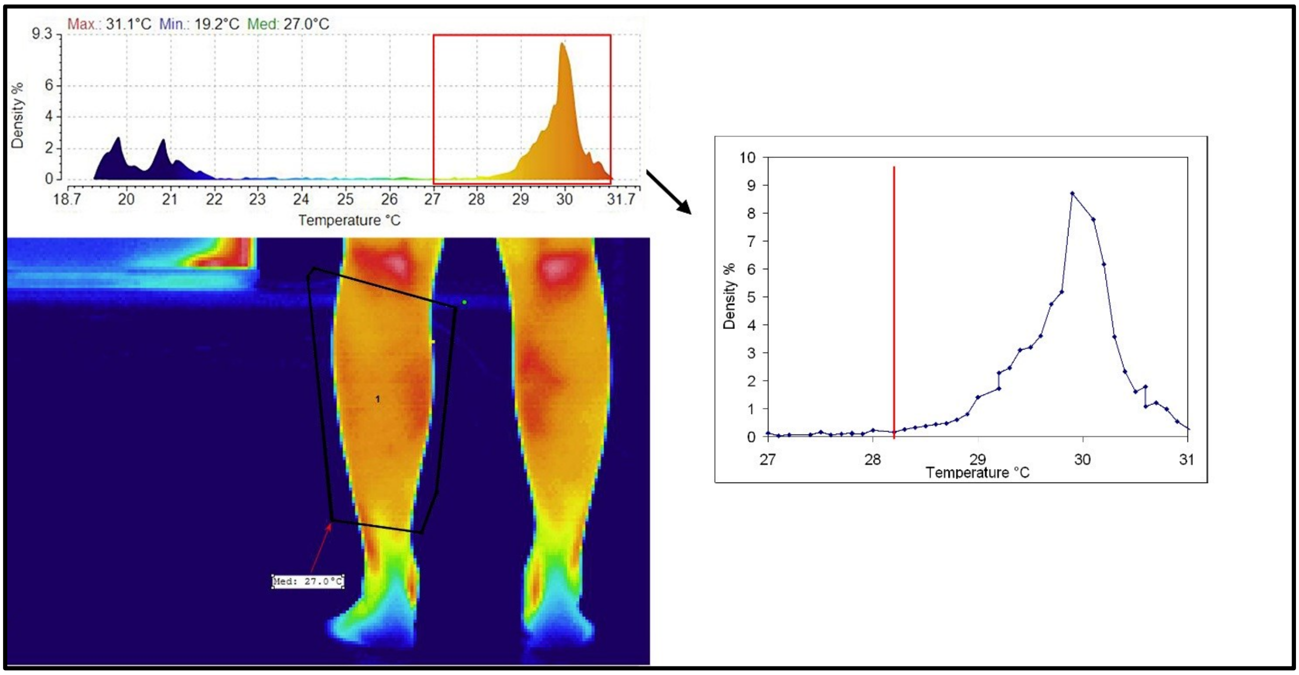

Figure 5: Thermal image and corresponding pixel distribution in a selected area larger then the leg surface. In order to calculate the average value of the calf only the right part of temperature distribution was taken into account. As cut criterion was used the first relative minimum on the left side of the right maxima.

2.1.3. Results

Firstly has been considered the correlation between temperature data obtained by averaging over a ROI (Troi) and over the entire anatomical selected region (Ttot). These results are shown in Figure 6. The very high value of correlation $R^2 = 0.958$ proves that the two methods are equivalent in the seeking of representative value of temperature ($p < 0.05$). Student's t-test has shown that there was no significant difference in mean temperature between Troi and Ttot ($p = 0.9$).

Also temperature data obtained by Tmax method were correlated with the two aforesaid methods both based on the average of the pixels value in a large ROI. Correlations are very high in both cases (Tmax/Troi $R^2 = 0.924$, $p < 0.05$, see Figure 7, and Tmax/Ttot $R^2 = 0.885$, $p < 0.05$). In these cases Student's t-test showed that there were significant differences in mean temperature between both

Tmax/Troi ($p < 0.001$) and Tmax/Ttot ($p < 0.001$). Figure 7 shows the linear correlation between Tmax and Troi. One data red-colored lays far from the linear correlation of all other data, it corresponds to the temperature of the calf shown in Figure 4 (left), presenting an abnormal thermal distribution that was not considered for correlation index calculation.

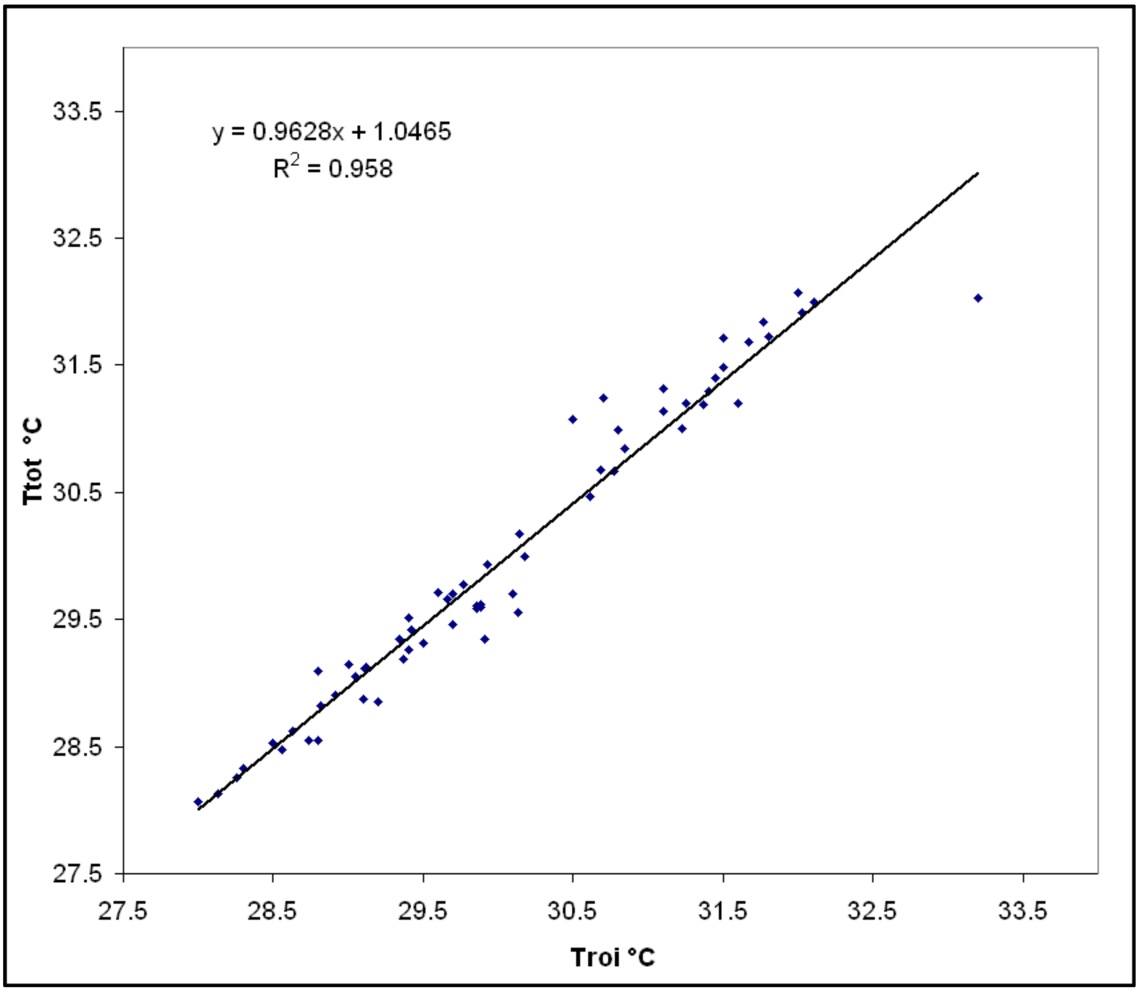

Figure 6: Correlation between temperature averaged on ROI and total anatomical region. Data refer to 64 calves on an heterogeneous population of 32 healthy subjects.

As second step we used these data in the analysis of thermal images in biomedical field, to quantify the difference in temperature between limbs on each subject. Therefore, authors considered the difference in skin temperature of left and right calf to evaluate which is the best method to find controlateral difference. Mean values, their standard deviation (SD) and correlation coefficients between temperature of right and left calves are shown in Table 1 for each method.

The Bland and Altman analysis of Troi/Tmax method has been assessed and is presented in Figure 8. None of the data showed a limit of agreement greater than 95% (±1.96 standard deviation). For the temperature data the correlation value between the difference and the average is -0.0004, demonstrating that there is no correlation.

It is possible to notice that Tmax presents the lower correlation index although mean values are equal. In order to test the hypothesis that difference between the two limbs can be singled out in a different way by the three methods, the Gaussian distribution of the ΔT_{l-r} in each method were analysed too.

The differences between right and left calf found by different methods are plotted in Figure 9a–c. They were fitted with Gaussian function that put in evidence data dispersion and degree of symmetry (Chi-Sqr of ΔT_{l-r}: 0.76; 0.92; 1.32 for Troi, Ttot and Tmax respectively).

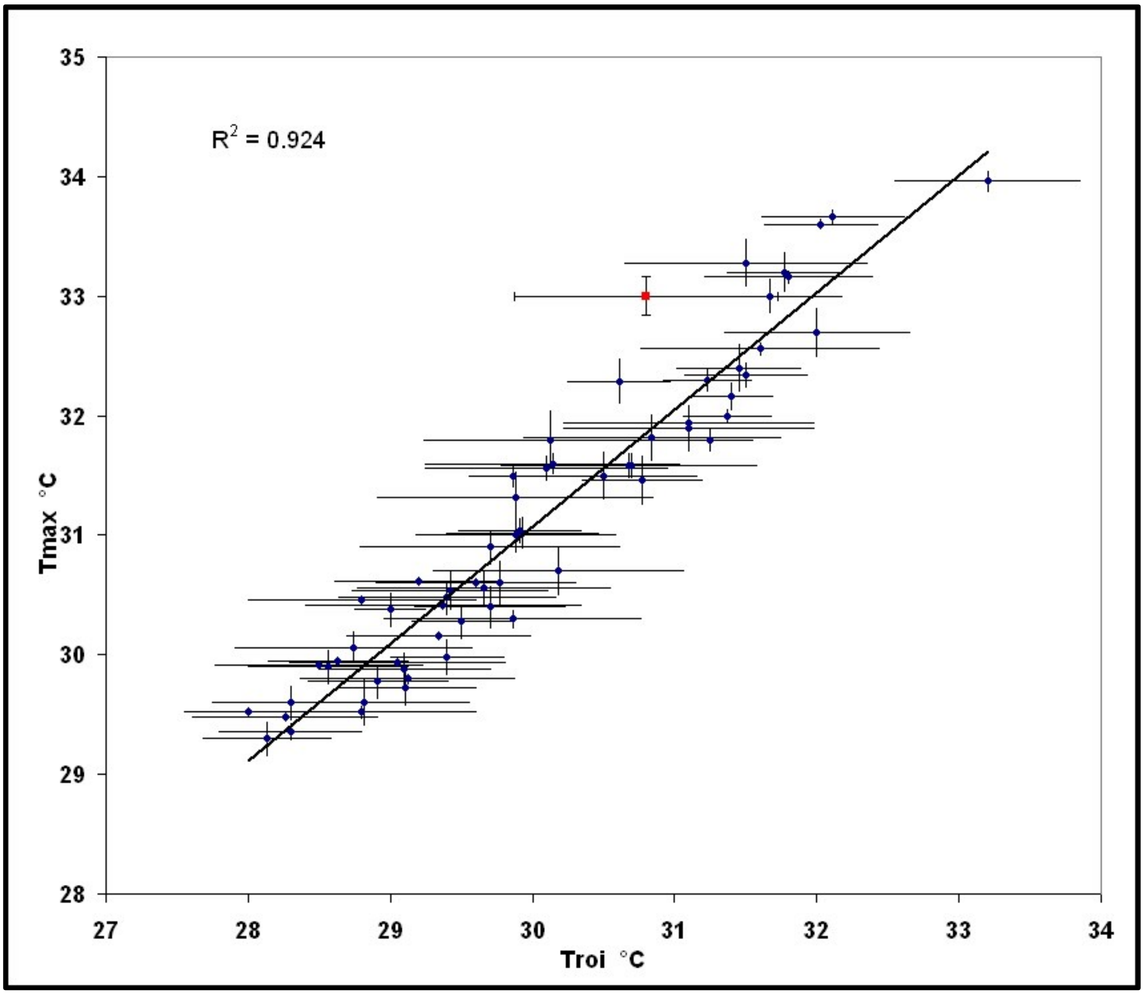

Figure 7: Correlation between temperature averaged on ROI (Troi) and calculated by Tmax method (Tmax). Data refer to 64 calves on an heterogeneous population of 32 healthy subjects. Red dot represents a datum not considered into linear correlation calculation.

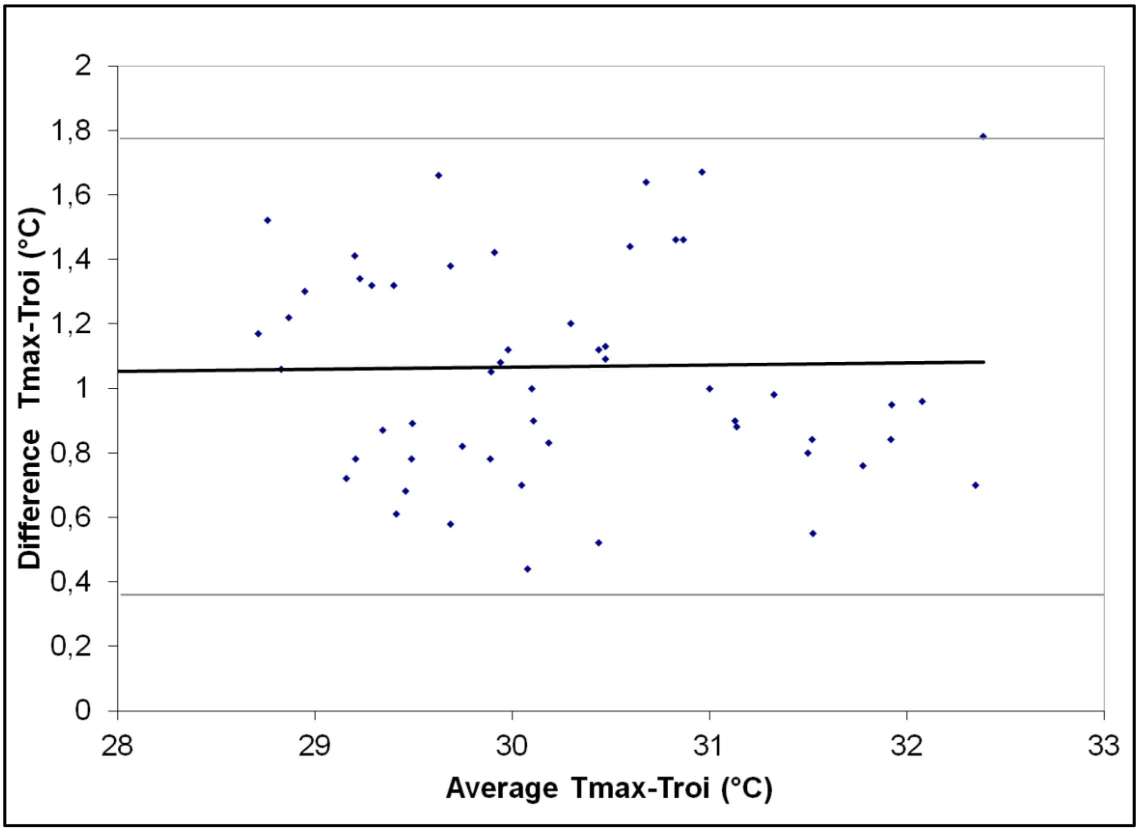

Figure 8: Bland–Altman plot showing relationship between average values of Troi and Tmax (X-axis) and their differences (Troi–Tmax: Y-axis).

Method	Mean value of temperature (°C) ± SD (Right/Left)	Right/Left Linear Correlation Index R^2	Right/Left Pearson's index (r)	Significance (n=32)
Tmax	31.13 ± 1.24 / 31.13 ± 1.26	0.754	0.868	$P<0.05$
Troi	30.04 ± 1.27 / 30.08 ± 1.26	0.828	0.910	$P<0.05$
Ttot	30.07 ± 1.34 / 30.06 ± 1.28	0.881	0.939	$P<0.05$

Table 1: Correlation indices between left and right calves using three different methods.

2.1.4. Discussion

First of all, the high correlation coefficient (0.958, $p < 0.05$) between Troi and Tot confirmed our hypothesis that these two methods can be considered equivalent for evaluation of skin surface temperature. Student's t-test showed no significant difference between the two mean temperature values (p = 0.9). They based the calculation of the representative temperature value as arithmetic average of the temperature of all the pixels inside the selected area. Troi method restricted this calculation to the ROI selected by the operator while Ttot allowed to consider the totality of the investigated body area.

The most utilized method of analysis consists in such average of the temperature values of all the pixels included in the ROI, with the help of markers positioned on the skin. As suggested in the paper by Merla and colleagues (Merla et al 2010), in order to more easily identify these regions, thermal indicators can be positioned on specific points on the body (reperee points of surface anatomy). The procedure to calculate a representative temperature value of a specific body region using a ROI is also reported in other papers. Ferreira et al. (Ferreira et al 2008) used ROI method without any marker on the skin, to avoid any interference such as temperature shift through conduction or irradiation; they calculated the final thermal data averaging the temperature value of each pixel in the ROI, selecting a small ROI (36 x 36 pixels) on the posterior thigh (Ferreira et al 2008). Zontak et al. selected a very small ROI on a finger in an area far from the muscles involved in the exercise (Zontak et al 1998); Bertmaring et al. took into account a small rectangular ROI on the shoulder to minimize the effect of surrounding musculature, further they divided it in six smaller areas in order to verify statistical variation of temperature inside the bigger one (Bertmaring et al 2008). It is interesting to notice that in these papers the authors chose to select small ROI. This is probably due to the necessity to have a localized ROI on the body region under inspection, in order to maintain the standard deviation of temperature as small as possible.

Recording of skin temperature in a specific body area in thermoregulation diagnostic field needs to be reliable and repeatable. Every muscle contraction produces physiological changes reflecting on blood flow that influences skin temperature. Skin temperature depends also on anatomical factors, such as the presence of irregularities on the surface and the presence of different kind of subcutaneous tissue (fat tissue and muscular tissue) (Merla et al 2010). These factors can influence the skin temperature distribution of a specific body area, thus it is frequent to find a non-gaussian thermal distribution inside a selected ROI (Figure 4). From this consideration it is clear that the Troi method can not be used for all skin areas and in all situations.

Troi method seems to be more able to follow different feature of the anatomical part on a population of heterogeneous subjects despite most part of authors used it by setting size and shape of the ROI. This constraint appears to be quite severe in using IRT for moving subjects or in case of an high variability in anthropometric characteristics (Ludwig et al 2010b).

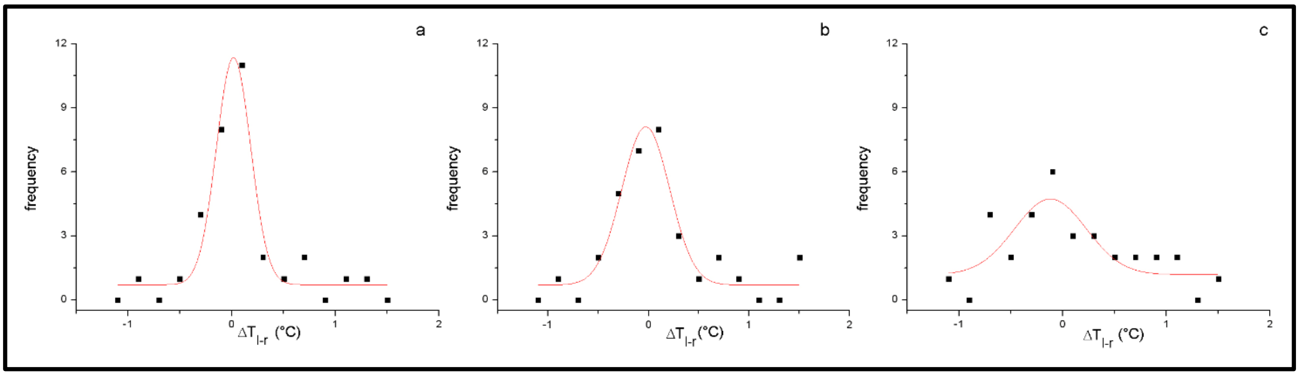

Figure 9: Distribution of $\Delta T_{l\text{-}r}$ for Troi, Ttot, Tmax (a-c) with a Gaussian fit.

Could be interesting calculating the representative temperature values using another method. It is clear that Ttot method permits to consider the totality of the interested body area. With respect to Troi method, Ttot method could be indicated for analysis of wide surface area, avoiding the necessity of any markers used for selecting the ROI on the skin. This is a peculiarity of Tmax method too. An other advantage of this last method is that for every thermal image each subject was represented by a temperature value derived from the same number of pixels, free from any anatomical constraint and representative of the most important cutaneous areas for what concerns heat dissipation processes through skin (Ludwig et al 2012).

As previously said no significant difference in mean temperature was found between Troi and Ttot method, on the other hand a systematic difference in temperature was found comparing Troi and Tmax methods. This is due to the choice to select only the highest temperature by Tmax method. In our trials Tmax method recorded temperature values of 1.07 °C higher than Troi ($p < 0.001$) assumed as standard reference for this kind of measurements.

Furthermore, the Bland and Altman analysis demonstrated that bias (Y-axis) does not show any dependence with respect to the average temperature. Accordingly, over all the range of the temperature recorded in this study (28–33 °C) the Troi and Tmax methods can be considered interchangeably in detecting temperature trend. This is probably due to the high stability of the thermocamera detector over a short temperature range.

The comparison of the three methods to single out differences in temperature between right and left calf of the subjects permits further considerations. Our findings prove that Tmax method put in evidence a bigger difference in temperature between the two calves at rest condition with respect to the other methods (Troi and Ttot).

Distributions of ΔT_{l-r} show a Gaussian-like trend with different correlation index (Figure 9). This could be attributed to the capability of the three methods to read the right vs. left calf asymmetry. This is confirmed by the largest values of both data standard deviation and full width half maximum of the Tmax Gaussian fit.

Temperature data of Tmax right/left calf show the lowest linear correlation (Pearson's corr. coeff. = 0.754) and this could indicate that some data lay far from the linear fit. Therefore, this method of thermal imaging analysis can help in single out in a population those with remarkable asymmetry. In fact, considering the skin temperature as indicator of the blood flow (Wright et al 2006), an explanation of this fact could be found in different capillarization of the two legs due to several phenomena, such as level of training (Jensen et al 2004), injuries and pathological conditions (Eliason et al 2010). Finally, right and left calves may differ depending on the kind of activity in which they are used to be involved. During some exercises it is necessary an alternate activity of the limbs, for instance during walking and running, while other exercises need a simultaneous activity of the limbs (squat exercise); but even if with different time sequence the intensity of the activity of the two limbs are equal for both alternate and simultaneous exercises.

2.1.5. Conclusion

This work contributes to characterize three different methods of thermal images analysis in biomedical field. The comparison among the temperature values of the same body area obtained by each method could be helpful to better understand their advantages and disadvantages.

The three methods can be considered equivalent in giving representative temperature values even if the final data show systematic shift of some tenth of degree. Temperature obtained through ROI selection on a well-defined area can be considered as the most reliable value of the investigated area, but it presents limits in maintaining the ROI for the same body area (static measurements) or in case of moving subject it is operator dependent. Tmax method singles out the highest values of temperature and can be useful for both non-static shooting and when fast thermal response is considered i.e. when thermal inertia of non-vascularized tissue can hide the thermal signal from the most vascularized ones. For this reason Tmax method seems to be more useful in study concerning asymmetry in training activities or pathology thermal patterns.

2.2. STUDY 2: THERMAL IMAGING OF EXERCISE-ASSOCIATED SKIN TEMPERATURE CHANGES IN TRAINED AND UNTRAINED FEMALE SUBJECTS [2]

2.2.1. Introduction

The thermo-regulatory system of human body has the task to maintain a constant temperature despite the variations caused by environmental conditions and/or physical work. The control of heat exchange between human body and the environment is essential for body temperature regulation. The tegumentary apparatus (skin) has the fundamental role of regulation of heat exchange through conduction, convection, radiation and evaporation (Brengelmann et al 1977; Merla et al 2010). The activation of body compensatory vasoregulation occurs during muscles activity, through reduction of blood flow in the splancnic region and tegumentary apparatus. Intense exercise causes heat production in the core structures and activates muscles (Fritzsche and Coyle 2000), with a consequent massive transfer of warmer blood from the internal to the superficial parts of the body. Thus, the vasoconstriction in these regions increases blood flow in the muscular areas while the muscle blood volume remains constant. The blood volume in the internal and periferical parts of the body decreases. In this condition we can observe an increase of the venous return and the cardiac output (Zontak et al 1998; Merla et al 2010).

The effect of physical exercise on skin blood flow has been previously studied and reviewed by Johnson (Johnson 1992) and Kenney and Johnson (Kenney and Johnson 1992). They found that the modifications of cutaneous blood flow during exercise is linked to the individual grade of vasodilation and vasoconstriction. As a consequeance, the cutaneous blood flow influences the skin temperature depending on modification caused by exercise. Johnson (Johnson 1992) and Robinson (Robinson 1963) observed that at the beginning of the exercise the demand of blood flow to working muscles caused a briefly skin vasoconstriction, but as the body core temperature raises, the thermal regulatory processes predominate and the skin vessels dilate, increasing heat dissipation throw the skin, essentially by irradiation and evaporation (Ludwig et al 2010a). Frietzsche and Coyle (Fritzsche and Coyle 2000) showed that the cutaneous blood flow (CBF) is higher in endurance athletes than in untrained subjects

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[2] Formenti, D., Ludwig, N., Gargano, M., Gondola, M., Dellerma, N., Caumo, A., Alberti, G.. Thermal imaging of exercise-associated skin temperature changes in trained and untrained female subjects. *Ann. Biomed. Eng.* Vol. 41, No. 4, pp.863–871. 2013. (see appendices pag. 61).

during exercise. As a result, a higher fitness level is associated to a higher CBF. It stands to reason to hypothesize that trained subjects have a better thermoregulation than untrained ones.

Usually, cutaneous temperature is estimated by averaging values of the cutaneous temperature recorded in predetermined Regions of Interest (ROI) by contact temperature probes, but since many years several group of research tried to use infrared thermography to measure these values (Zaïdi et al 2007; Ferreira et al 2008; Merla et al 2010). Infrared thermography (IRT) is a non-invasive technique that visually represents the whole process during and after exercise. This device allows quantitative and precise evaluation of the spatial distribution and time evolution of cutaneous temperature, allowing data recording rate up to 100 Hz. IRT is widely used for medical diagnostic analysis (Lahiri et al 2012) and is becoming increasingly used to record variations of cutaneous temperature linked to physical exercise.

Using this technique Merla et al.(Merla et al 2010) studied thermoregulation during and after the exercise in runners as well as Torii et al. (Torii et al 1992) and Zontak et al. (Zontak et al 1998) for bicycle ergometer, and Ludwig et al. (Ludwig et al 2012) for breathing exercise. Some differences of heating in different parts of the body with relation to specific swimming style were found by Zaïdi et al. (Zaïdi et al 2007). The above-mentioned studies used IRT to investigate the alterations of skin temperature across various parts of the body following various modalities of exercise (such as running or cycling) involving various muscle groups. Only few studies, such as those by Ferreira et al. (Ferreira et al 2008) and Bertmaring et al. (Bertmaring et al 2008), have addressed the evaluation of thermoregulation during localized exercise. Apart from a preliminary report by Merla et al. (Merla et al 2005), none have assessed the time profiles of skin temperature before, during and after exercise in trained as compared to untrained subjects.

Aim of this study was to investigate the influence of physical fitness on exercise-associated skin temperature changes. Trained and sedentary female subjects were studied. We focused on a specific muscle area (muscle-tendon unit) involved in a steady-load localized exercise (2-min standing heels raise). Since we were interested in obtaining a detailed portrait of the skin temperature dynamics, IRT images were taken prior the initiation of the exercise, during the exercise as well as for 7 minutes after the cessation of the exercise. Our hypothesis was that exercise can induce differences in the trends of skin temperature between trained and untrained subjects.

2.2.2. Material and Methods

Subjects

We considered two groups of female subjects: one of trained and one of sedentary subjects. The group of trained was composed by seven athletes, selected from a synchronized swimming team that took part in Spring Italian Synchronized Swimming National Championship [age=18.43 ± 0.75 years; body weight=54.57 ± 2.29 kg; height=1.62 ± 0.01 m; body mass index (BMI)=20.76 ± 0.68 kg/m^2]. They trained for at least five sessions per week, 2.30 h per session. The choice of a team of synchronized swimming, almost exclusively a female sport, permitted to have an high interindividual homogenity of anthropometric caracteristichs and training conditions.

Seven sedentary female subjects were randomly selected from a list of volunteers belonging to the student population of the State University of Milan (age=20.14 ± 0.46 years; body weight=54.29 ± 2.17 kg; height=1.66 ± 0.03 m; BMI=19.64 ± 0.7 kg/m^2). No statistical difference was found between the age, height, weight, BMI of the two groups. All participants were non smokers, and without cardiovascular or pulmonary diseases. They had not assumed drugs or medications with a potential effect on cardiovascular and thermoregulatory functions during the two months prior the tests.

The Ethical Committee of the Università degli Studi di Milano approved this study. After a thorough explanation of the protocol that was going to be used, the subjects, or the parents of the underage ones, accepted informed written consent to participate in this study.

Experimental Protocol

In a preliminary test aimed to find out the appropriate exercise duration, the subjects performed their maximum number of repetitions of heel raise exercise without overload (using a metronome to standardize the pacing). We chose heels raise exercise because both group weren't used to perform it and thus it could be considered as an unspecific exercise for both groups. Sedentaries and athletes performed the exercise in about 2-2.30 minutes. Hinging on this preliminary evaluation, we decided to set the duration of the exercise to 2 minutes in order to allow completion of the exercise to all subjects.

Subjects observed this standard preliminary protocol for infrared thermal imaging measurements: they abstained from assuming alcoholic or caffeine-containing products for a 4-h period prior to the start of the experiment; they removed body hair on legs that were clean and without cosmetics products before the measurements in order to obtain the most representative thermal images of skin temperature.

After being acclimated to the room climate conditions (temperature 22-23 °C; relative humidity 60 ± 5 %; no direct ventilation and constant intensity of light) for 15 minutes before the exercise, the subjects performed heel-raise exercise without overload for 2 minutes.

The pace of movement was set with a metronome in order to standardize the number of repetitions: in 1 second the subjects raised up heels as high as possible, and during the following second they lowered heels back to the starting position (Figure 10).

All the thermal images were shot (frame rate 0.05 Hz) at the same instant during the exercise, i.e, when the subjects heels re-achieved the starting position with heels on the floor.

All the tests were performed in the late morning period in order to limit possible effects due to circadian rhythm variations.

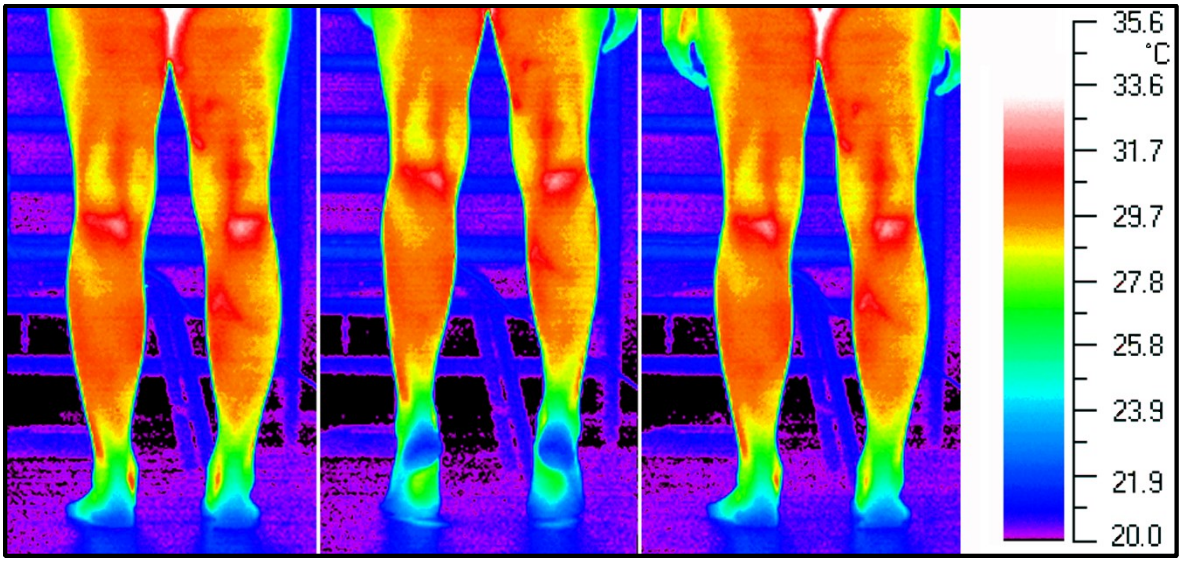

Figure 10: Thermal images of one repetition of standing calf raise exercise performed by an athlete.

Thermographic Analysis

Thermal imaging records temperature of objects without any contact with their surface by detecting infrared radiation emitted following the Stefan-Boltzmann law. It can be written for a real body with an emissivity value ε:

$$E = \varepsilon_{\lambda,\theta,T}\, \sigma T^4 \qquad \text{Equation 4}$$

where E is the energy emitted per unit area and per unit time [Wm^{-2}], ε is the emissivity that depends on the wavelength λ, the angle θ and on temperature T [K], and $\sigma = 5.6693 \times 10^{-8}\, W\, m^{-2}\, K^{-4}$ is the Stefan-Boltzmann constant.

The emissivity value was set to 0.97, in keeping with the value of human skin emissivity reported in literature (Jones and Plassmann 2002). We wish to stress that, when applying infrared thermography to the human body, the noise due to environmental irradiation in far IR band (10 microns) is in general low because of both the reflectance value of skin (R=3%) and the temperature of subjects, about ten degrees above the environmental one.

Finally, it is worth emphasizing that non-contact measurements avoid any local alterations of heat exchanges mechanism through skin, its surrounding and body core, and it free the subjects from physical and psychological constrictions. Source of artificial or natural heat by radiation was avoided in order to reduce IR radiation noise.

Thermal images were recorded during 1 minute of pre-exercise to determinate basal temperature, 2 minutes of exercise and 7 minutes of recovery-time. Thermal image sequences of the surface of the subject's calves were recorded by a 14-bit digital infrared thermo-camera (AVIO, TVS-700, 320 x 240 Microbolometric Array; 8-14 µm spectral range; 0.07 °C thermal resolution; and 35 mm lens). Recordings were made using a digital frame grabber with a rate of one image per 20 seconds. During the trials the subjects were dressed in swimsuit, and were set 3 m from the thermocamera to permit the complete exposure of the posterior region of the legs and of consequence the best recording of the temperature calves. As regarding to the calibration procedure, the subjects were placed in front of a uniform background with a constant temperature (T=24.86 ± 0.20 °C).

The images of the sequence were then corrected from temperature shift due to the periodic self-calibration of the sensor using an area with a constant temperature (in the background) and analyzed with a dedicated software for thermal images elaboration (GRAYESS® IRT Analyzer, Version 4.8). In order to avoid the operator dependency of the ROI selection process, the following procedure was set-up to obtain a reliable temperature of the calves surface. One region including all the muscles involved in exercise was selected for each calf. For each calf inside this area, the software selected the five hottest pixels (the software was instructed to select pixels having a minimum distance of 5 pixels from each other). The temperature value was obtained by averaging over an area of 5x5 pixels around them. In this way the results are representative of an overall amount of 125 pixels on each calf. This allowed us to obtain a more representative sample of the warmest areas of the calves (Figure 11). Finally, we averaged left and right calf temperature values (no significant difference was found between left and right), thus obtaining one representative temperature value in each subject. In this way, for every thermal image, each subject was represented by a temperature value derived from the same number of pixels representative of the most important cutaneous areas for heat dissipation.

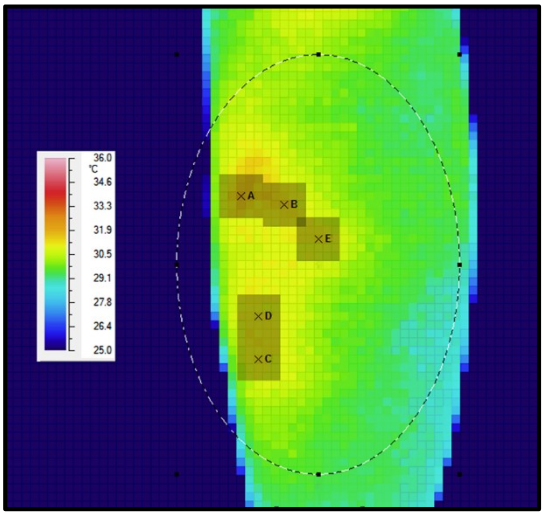

Figure 11: Method to detect representative temperature of the calf. Five hottest spots and relatives 24 pixels around them were taken in account inside a wider area selected by the operator and including all the calf.

Data Analysis

The time course of skin temperature showed a nonlinear profile displaying three phases (Figure 12 shows a representative subject). The first phase was the 1-min time window prior to the exercise. During this phase, the skin temperature remained approximately constant. The basal temperature in each subject was calculated as the mean of three consecutive skin temperatures prior to the initiation of the exercise. The second phase was the 2-min time window during which the subjects performed standing calf raise exercise. During this phase, the skin temperature showed a mixed behavior: in some subjects it began to rise almost immediately following the initiation of the exercise, while in some other subjects the skin temperature decreased a little, achieving a nadir approximately 60 seconds after the initiation of the exercise, and then began to rise. The former behavior of skin temperature was typical of trained subjects, while the latter was typical of untrained subjects. The third phase was the 7-min time window following the end of the exercise. In that period, skin temperature continued to rise, achieved a maximum value and then began to slowly decline.

Data analysis of skin temperature data was aimed to quantify key parameters descriptive of the skin temperature time course. The definition of each parameter (together with its abbreviation and units) is reported in Table 2. Calculation of parameters $Time_{50\%}$ and $Time_{delay}$ was accomplished by linearly interpolating the skin temperature data measured at two consecutive time points. Calculation of parameter T_{ir} (i.e., the temperature increase rate immediately following the time delay) was performed by taking the slope of the regression line estimated from the four consecutive temperature data measured after the time delay.

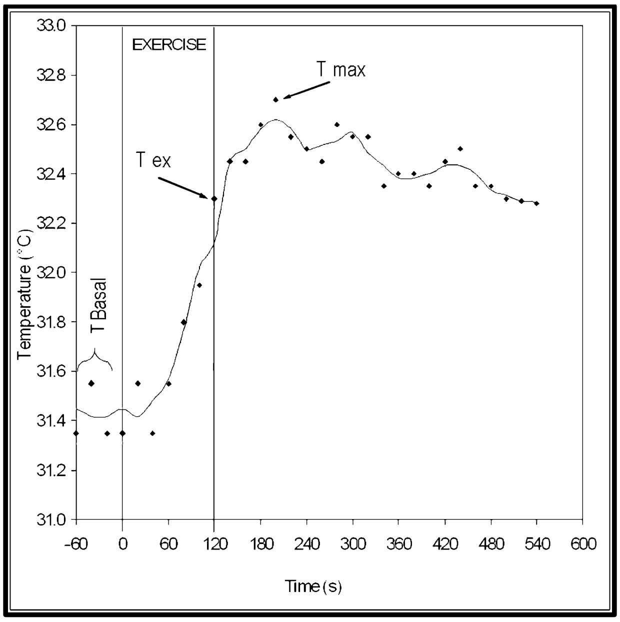

Figure 12: Time course of cutaneous temperature during the experiment in a representative subject (athete). The dots are the experimental data and the continuous line is the smoothed profile provided by a low-pass filter over three points for each homogeneous range (basal, exercise, recover). The figure also illustrates how parameters T_{basal}, T_{ex}, and T_{max} are calculated from the experimental data. The description of such indices in reported in Table 2.

Statistical Analysis

Data were expressed as means ± SEM. The normality of the distribution of age, anthropometric variables, as well as the parameters described in Table 2 was checked by graphical methods and by the Shapiro-Wilk's test.

Age and the anthropometric variables had normal distributions, so that the homogeneity of the sample characteristics (trained vs. untrained subjects) was tested using unpaired pooled Student's t test.

To test whether the pattern of temperature change during the experiment was influenced by the training level, we used two-factor (i.e., subjects' group and time) analysis of variance (ANOVA) with repeated measures on one factor (time). Three crucial time points during the experiment were selected to provide the three repeated measures of temperature: 0 s (basal), 120 s (end of the exercise), 420 s (time when temperature has attained a quasi-steady state in both groups). We were particularly interested in testing for a significant interaction effect between group (trained vs. untrained subjects) and time. Although no violation of the assumption of sphericicity was revealed by application of the Mauchly's test of sphericity, we nonetheless preferred to be on the conservative side and perform the F test for the interaction effect using the Greenhouse-Geisser correction.

Index abbreviation	**Definition**	**Units**
Basal temperature (T_{basal})	Mean temperature value prior to the exercise.	°C
End-of-exercise temperature (T_{ex})	Temperature value achieved at the end of exercise.	°C
Maximum temperature (T_{max})	Maximum value of temperature recorded.	°C
End-of-exercise delta temperature (ΔT_{ex})	T_{ex} - T_{basal}.	°C
Delta max temperature (ΔT_{max})	T_{max} - T_{basal}.	°C
Time to 50% ΔT max ($Time_{50\%}$)	Time when temperature is 50% of ΔT_{max}.	s
Time to T max ($Time_{Tmax}$)	Time when T_{max} is recorded.	s
Time delay	Time between the initiation of exercise and the rise in temperature above T_{basal}.	s
Temperature increase rate (T_{ir})	Initial temperature increase rate after the Time delay.	°C/s

Table 2: Parameters used to characterize the skin temperature time profile.

As for the parameters describing the skin temperature profiles, it was found that the parameters describing key time points of the skin temperature profile (i.e., $Time_{50\%}$, $Time_{Tmax}$, $Time_{delay}$) had non-normal distributions. Thus, statistical comparison between the parameters obtained in the two groups (athletes vs. sedentaries) was performed using either the Student's t test (for parameters T_{basal}, T_{ex}, T_{max}, ΔT_{ex}, ΔT_{max}, T_{ir}) or the Mann-Withney nonparametric test (for parameters $Time_{50\%}$, $Time_{Tmax}$, $Time_{delay}$).

Statistical analysis was carried out with STATA 10 software (StataCorp., College Station, TX, USA). A p-value lower than 0.05 was considered statistically significant.

2.2.3. Results

Figure 13 shows the average skin temperature data recorded in athletes and sedentary controls during the experiment. The temperature profile of the athletes group was characterized by a very short latency period (10 s) followed by an almost linear heating-up attaining an average temperature of 31.8 °C at the end of the exercise. During the post-exercise recovery time, skin temperature reached an average maximum value of 32.2 °C at 265.7 s and then began to decline very slowly. Contrary to athletes, sedentary controls sedentary showed a prolonged latency period (75 s). After that, temperature began to increase more gradually than in athletes group reaching an average temperature of 30.9 °C at the end of exercise. During the post-exercise recovery time, skin temperature reached an average maximum value of 31.6 °C at 440 s and then exhibited a quasi-steady-state.

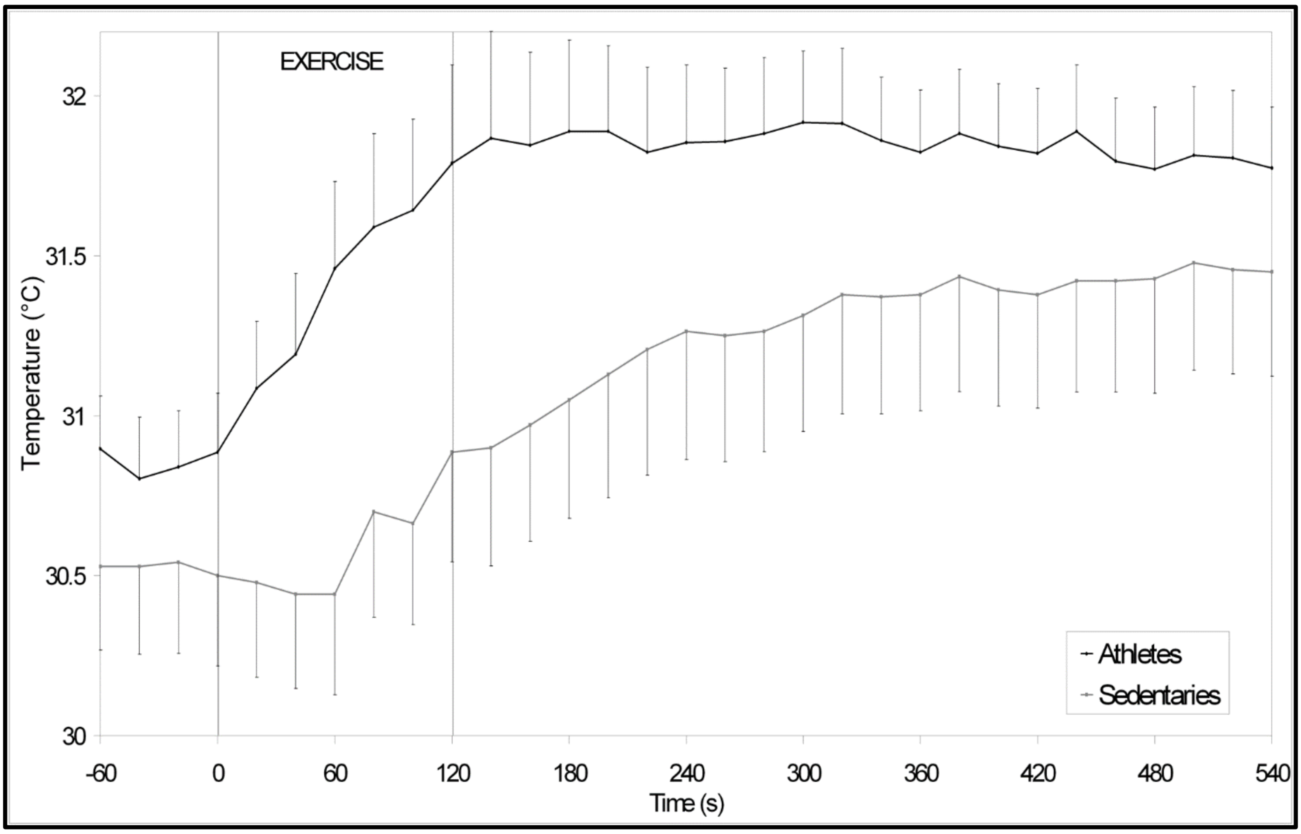

Figure 13: Average skin temperature data recorded in athletes (n=7) and sedentaries (n=7) during the experiment (mean±SEM). To improve the readability of the graphs, discrete experimental data were connected by linear segments, yielding piece-wise linear functions.

Results of the two-way ANOVA with repeated measures provided evidence of a statistically significant interaction between group and time (p=0.03), thus indicating that athletic subjects increased their skin temperature differently with respect to sedentary subjects.

Table 3 reports the mean parameter values together with the results of the statistical comparisons between the athletes and the sedentary groups.

Parameter	Athletes (n=7)	Sedentaries (n=7)	p-value
Basal temperature (T_{basal}, in °C)	30.8 ± 0.2	30.5 ± 0.3	0.37
End-of-exercise temperature (T_{ex}, in °C)	31.8 ± 0.3	30.9 ± 0.3	0.07
Maximum temperature (T_{max}, in °C)	32.2 ± 0.3	31.6 ± 0.3	0.20
End-of-exercise delta temperature (ΔT_{ex}, in °C)	1.0 ± 0.2	0.4 ± 0.1	0.013
Delta max temperature (ΔT_{max}, in °C)	1.3 ± 0.2	1.0 ± 0.2	0.25
Time to 50% ΔT max ($Time_{50\%}$, in s)	80.2 ± 13.9	205.1 ± 28.0	0.007
Time to T max ($Time_{Tmax}$, in s)	265.7 ± 51.6	440.0 ± 39.5	0.018
Time delay (s)	10.0 ± 6.7	75.3 ± 22.1	0.010
Temperature increase rate (T_{ir}, in °C/s)	0.009 ± 0.002	0.004 ± 0.001	0.041

Table 3: Statistical comparison between the two groups (see the Statistics Analysis section for details) was performed using either the Student's t test (for parameters T_{basal}, T_{ex}, T_{max}, ΔT_{ex}, ΔT_{max}, T_{ir}) or the Mann-Withney test (for parameters $Time_{50\%}$, $Time_{Tmax}$, $Time_{delay}$).

2.2.4. Discussion

The main finding of the present study is that, during steady-load localized exercise, the skin temperature of female athletes increases more quickly than that of female sedentary controls. Many lines of evidence point to such a conclusion. First of all, visual inspection of the average time courses of skin temperature during and after the exercise (Figure 13) suggests that the rise in skin temperature that follows the initiation of exercise occurs more quickly in athletes as compared to sedentary subjects. This visual impression is objectively corroborated by the results of the two-way ANOVA for repeated measures which showed a significant interaction between group and time, thus indicating that athletes increase their skin temperature differently with respect to sedentary subjects. Further evidence in this direction is provided by the parameters used to quantify crucial signposts (temperatures as well as time points) of the skin temperature profile (Table 2). The parametric portraits obtained in the two groups (Table 3) confirms that the skin temperature increases more quickly in trained subjects than in sedentary controls.

One initial observation is that the initiation of exercise was not followed by an immediate rise in skin temperature above the baseline. Rather, many subjects, and especially sedentary controls, responded to the initiation of exercise displaying a latency period, during which skin temperature slightly fell below the baseline, achieved a nadir, and finally resumed above the baseline and began to rise. In order to describe this phenomenon in quantitative terms, we introduced the notion of a time delay. We found that the time delay of the athletes was much shorter than that of the sedentaries.

The presence of a latency period at the onset of the exercise has been previously observed by other investigators (Johnson 1992; Kenney and Johnson 1992; Torii et al 1992; Zontak et al 1998; Merla et al 2005; Merla et al 2010). As reviewed by Johnson (Johnson 1992) and Kenney and Johnson (Kenney and Johnson 1992), at the beginning of exercise there is an initial decrease in temperature, followed by a subsequent increase. Torii et al. (Torii et al 1992) found a fall in seven skin temperatures (forehead, arm, hand, foot, leg thigh, and trunk) during the initial stage (9 min) of muscular work on a bicycle ergometer. Zontak et al. (Zontak et al 1998) measured the hand skin temperature during both graded- and constant-load exercise. They found that hand skin temperature continuously decreased throughout the graded-load exercise, but displayed a biphasic pattern during steady-load exercise, with an initial descending trend (reflecting vasoconstriction) followed by an ascending trend (reflecting vasodilation). Merla et al. (Merla et al 2010) monitored the entire cutaneous anterior surface of the body in trained runners during graded-load exercise on a treadmill. Skin temperature continuosly decreased throughout the exercise period (12 minutes) and began its resumption to the basal level during the recovery phase. The findings that, during graded-load exercise involving the whole body, the temperature of either the hand (Zontak et al 1998) or various other surfaces of the body (Torii et al 1992; Merla et al 2010) decreased throughout the exercise period was probably due to a continuous skin vasoconstrictor response, linked to an increase in catecholamine and other vasoconstrictor hormones as the exercise intensity increased (Rowell 1991). In the present study, subjects performed standing heels raise exercise for only 2 minutes, thus producing a little amount of heat confined to a single muscle-tendon unit. In this way, even though the local muscle apparatus was properly loaded, no global phenomena of thermoregulation such as perspiration were activated. Therefore, one possible interpretation of the latency period observed in our study is that the demand of blood flow to working muscles at the beginning of the exercise led to skin vasoconstriction. As the body temperature increased, the skin vessels dilated, increasing heat loss through the skin. Our observation that the latency period was much shorter in athletes than in sedentary controls suggests that the dynamics of initial muscle vasoconstriction and subsequent muscle vasodilation is faster in athletes than in sedentary controls.

After the latency period, skin temperature increased in both groups. Such temperature increase is in accordance with previous work by Bertmaring et al. (Bertmaring et al 2008) and Ferreira et al. (Ferreira et al 2008). In the paper by Bertmaring et al. (Bertmaring et al 2008), IRT was used to assess the changes of skin temperature of the anterior deltoid during static exertion until exhaustion in four different conditions: at two shoulder angles (90° and 115°), and at two work loads (15% and 30 % maximum voluntary contraction). The time course of thermal readings showed a skin temperature increase for all the four conditions. In particular, the condition (90°/30%), which showed the fastest rate of skin temperature change, exhibited a temperature increase of approximately 0.8 °C at the end of the observation period (189 s, on average). In the paper by Ferreira et al. (Ferreira et al 2008), IRT was used to investigate temperature changes in response to a localized exercise (consisting in a 3-minutes knee flexion) in young and elderly subjects. The temperatures of the skin over the knee flexors in the exercised limb and in the controlateral limb were assessed. Infrared thermographic images were taken before the beginning of the exercise and in the post-exercise phase (for 10 minat 2–min intervals). Temperature variation in the exercised limb and in the contralateral limb showed different profiles. When compared with the pre-exercise temperature, the exercised limb showed a positive temperature variation immediately post-exercise, whereas the contralateral limb showed a negative temperature variation immediately post-exercise. In particular, temperature in the exercised limb tended to increase, albeit not significantly, in both young and elderly subjects when compared with the pre-exercise temperature (from 30.4 ±1.5 to 30.8 ±1.5 °C in young subjects and from 28.9±1.8 to 29.0 ±1.8 °C in elderly subjects). The skin temperature changes observed in the present study were larger than those observed by Ferreira et al.. We speculate that this may related with the fact that the type of exercise and the load used in the present study (2-min repetitions of heel raise exercise without overload) was fairly different from the one adopted by Ferreira and colleagues (3-min isotonic exercise of knee extension and flexion with a 1 kg weight resistance placed above the ankle).

In the present study, skin temperature increased approximately to the same extent in the two groups, but the speed of rise of skin temperature resulted higher in athletes than sedentary controls. In fact, the temperature rate of change, T_{ir}, in athletes resulted twice as high as that of sedentary controls. Another evidence that athletes responded to the exercise stimulus more promptly than untrained controls is given by comparing parameters ΔT_{ex} and $Time_{50\%}$ in the two groups. ΔT_{ex} was higher in athletes than in sedentary controls, indicating that, when exercise was stopped, athletes had already increased their skin temperature more than sedentary controls. $Time_{50\%}$ was lower in athletes than in sedentary controls, indicating that athletes were faster than sedentary controls in achieving 50% of the respective skin temperature excursion. It is worth noticing, however, that $Time_{50\%}$ comprises the duration of the latency period. Thus, one may wonder whether the reduced $Time_{50\%}$ observed in athletes simply reflects a shorter time delay or is indeed an evidence of a more rapid increase in skin temperature. To address this issue, we calculated the difference $Time_{50\%}$ - $Time_{delay}$ in the two groups. In this way, we were able to rule out the influence of the different length of the latency period and arrived at a more fair comparison between the two groups. We found that $Time_{50\%}$ - $Time_{delay}$ was significantly lower in athletes than in sedentary controls (70.2 ± 12.8 vs. 129.9 ± 16.2, p=0.018), thus confirming that the speed of rise of skin temperature was higher in athletes than in sedentary controls.

The evidence that trained and untrained subjects exhibit different dynamics of skin- temperature variation is in keeping with the results of a preliminary report by Merla et al. (Merla et al 2005). Our data are not sufficient to establish whether such different time courses in skin temperature are also reflective of different time courses of heat dissipation in the two groups. In fact, heat dissipation not only depends on skin temperature change, but also on local blood flow, which was not evaluated in our study. Fritzsche and Coyle reported that during exercise, trained subjects have an higher cutaneous blood flow (CBF) and have a better efficiency in dissipating heat than untrained ones (Fritzsche and Coyle 2000). It is of interest to notice that the different patterns of skin temperature increase observed in the present study in trained and untrained female subjects are reminiscent of the patterns of CBF increase reported by Fritzsche and Coyle in their study (see Fig. 3 in their paper). On the other hand, the type of exercise that the subjects of the study by Fritzsche and Coyle underwent was rather different from calf rise exercise of our study. In that study, ten trained men and ten untrained men underwent three 20-minutes cycling exercise bouts at 50, 70 and 90% peak oxygen uptake in this order with 30 minutes rest in between. In addition, whole-body sweating took place over the entire experiment, especially in trained subjects. In contrast, in our study the trial consisted of a localized muscle work, lasted only two minutes, and sweating did not occur. Thus, the evidence derived by Fritzsche and Coyle that trained subjects are more efficient than untrained one in dissipating heat cannot be safely extrapolated to our study tout court.

Finally, we put in evidence that our conclusions cannot be surely extended also to male subjects. Heat dissipation is influenced by many variables. Among them, variables such as body surface, presence of body hair, fat distribution are different in male and female subjects. Thus, further studies will be addressed to compare exercise-associated skin temperature changes in male and female subjects.

In conclusion, we found evidence that the level of physical training influences how fast skin temperature increases in response to localized exercise in female subjects. This work contributes to improve the knowledge about the potential of infrared thermography to provide a detailed description of the changes of skin temperature in response to localized exercise. This methodology may be useful for investigating the physiopathology of cutaneous temperature changes and may also present implications to studies aimed to investigate athletic performance.

2.3. STUDY 3: THERMOGRAPHIC SKIN TEMPERATURE RESPONSE TO DIFFERENT MOVEMENT VELOCITY OF SQUAT EXERCISE UNTIL EXHAUSTION: A PRELIMINARY REPORT [3]

2.3.1. Introduction

The cutaneous circulation is controlled by two types of efferent neural systems: a noradrenergic active vasoconstrictor and a cholinergic active vasodilator system (Kenney and Johnson 1992).

The response of cutaneous circulation to dynamic exercise is characterized by initially drive for redistributing of blood flow from inactive tissues (including the skin) to active muscles involved in exercise. During exercise, the increase in metabolism caused by the contraction of the muscles produces heat that must be dissipated; this dissipation inside the body occurs essentially by conduction and by convection through blood vessels. Since the skin blood flow participates in such heat dissipation, there is a competition between cutaneous active vasoconstriction and active vasodilation in which skin blood flow is involved (Kellogg et al 1991; Johnson 1992; Kenney and Johnson 1992).

The increased cardiac output associated with dynamic exercise is divided between two physiological needs: to the muscles involved in exercise (to satisfy the increased metabolic requests) and to the superficial part of the body in order to facilitate the heat transfer from the inner part of the body toward the skin, with the aim to dissipate heat in excess by superficial irradiation.

Thus, exercise creates not only a cutaneous vasodilation but also a cutaneous vasoconstriction, the latter one characterizing the onset of the exercise (Kenney and Johnson 1992; Kellogg 2006).

An initial cutaneous vasoconstriction (decrease of the skin blood flow) accompanies the onset of dynamic exercise. As such exercise continues, core temperature begins to rise until reaching a threshold, beyond which the skin blood flow increases linearly with the core temperature. Reaching a threshold (usually about 38°C), the core temperature continues to rise while the skin blood flow remains approximately constant (Kellogg 2006; Johnson and Kellogg 2010; Simmons et al 2011).

To this aim, one of the main function of the cutaneous circulation is heat regulation. Thermal measurements of skin by using infrared thermography (IRT) can be considered an indirect valid

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[3] Formenti, D., Trecroci, A., Ludwig, N., Gargano, M., Caumo, A., Alberti, G., Thermographic skin temperature response to different movement velocity of squat exercise until exhaustion: a preliminary report. In Book of Abstracts of the 19. Annual Congress of the European College of Sport Science - ISBN 978-94-622-8477-7. 2014. (see appendices pag. 62).

assessment of the skin blood flow (Swain and Grant 1989). IRT is a non-invasive technique that visually represents a two-dimensional map of temperature on a well defined area on the skin, allowing quantitative and precise measurement of the spatial distribution and time evolution of such temperature. IRT has a huge field of application for medical diagnostic analysis (Lahiri et al 2012), but in the last years its use has progressively increased also for evaluating skin temperature variations during and after physical exercise (Zontak et al 1998; Gold et al 2004; Ferreira et al 2008; Merla et al 2010; Ludwig et al 2012; Formenti et al 2013).

There is an increasing interest in proposing low-intensity resistance training to diminish both the mechanical stress on joints and the risk of injuries in sedentary, active subjects and athletes (Alberti et al 2013). Several studies have addressed the effects of low-intensity resistance training with slow movement and tonic force generation (Tanimoto and Ishii 2006; Tanimoto et al 2008; Tanimoto et al 2009b; Tanimoto et al 2009a), demonstrating that such modality of resistance training contributes to create muscular hypertrophy and strength gain as well as high intensity with normal speed exercise, using low and moderate load intensity and reducing the mechanical stress and of consequence the risk of injuries. This method of resistance training has been called slow movement and tonic force generation (LST).

During this modality of training, slow muscular contraction creates a moderate vascular occlusion, resulting in an important muscle deoxygenation. Performing such resistance exercise under ischemia condition promotes muscle hypertrophy and strength. In fact continuous muscular contractions at moderate intensity (about 50% of 1 maximal repetition (1RM)) with slow movement has been shown to suppress both inflow and outflow from the muscle due to increment in intramuscular pressure (Tanimoto et al 2009b). Such blood flow restriction inside the muscles involved in LST would have implications in the regulation of skin blood flow, with important consequence for the blood involved in the heat dissipation process through the skin.

Thus, the aim of this study was to investigate the skin temperature response during low intensity resistance training with slow speed as compared to low intensity training with normal speed in squat exercise. In particular, we investigated the skin temperature time profile of the muscle quadriceps directly involved in the squat exercise by using infrared thermography in young healthy subjects. We hypothesized that low intensity resistance training with slow movement results in a skin temperature response slower than the one of the normal speed exercise with the same intensity.

2.3.2. Material and Methods

Subjects

Thirteen male active subjects volunteered to take part in this study. They belonged to the student population of the Faculty of Exercise Science of the Università degli Studi di Milano. Their mean age, weight, height and body mass index were 25.0 ± 2.0 yr, 70.1 ± 6.3 kg, 179.1 ± 8.3 cm and 21.8 ± 2.1 kg m^{-2}, respectively. They were habitually physically active but none of them were used to train with specific resistance exercise. All participants were non smokers, and without cardiovascular or pulmonary diseases. They had not assumed drugs or medications with a potential effect on cardiovascular and thermoregulatory functions during the two months prior the tests. The Ethical Committee of the Università degli Studi di Milano approved this study. After a thorough explanation of the protocol that was going to be used, the subjects accepted informed written consent to participate in this study.

Experimental Protocol

The experiment was structured in three different days, each one separated by five days with the following one. The subjects were instructed to refrain from strenuous physical activity the two day prior the trials and abstained from assuming alcoholic or caffeine-containing products for a 4-h period prior to the start of the experiment.

A preliminary session was aimed to perform anthropometric measurements, to find out the load of maximum repetition (1-RM) in parallel squat exercise and to familiarize with the testing procedure. During the first 30 min, standardized instructions as to the proper lifting technique were given to the subjects. As they became familiar with the technique, a weight to lift for the 1-RM test was selected. Before the 1-RM test, they performed a good number of warm-up repetitions with a light weight. If the subjects was successful for the first trial of the 1-RM test, 2.5 kg to 10 kg was added for the next attempt. This step was repeated until the subject wasn't able to lift the weight; thus the maximum weight lifted successfully was considered as the 1-RM. This protocol was performed in three to five attempts, with three to five min rest between each attempt. The 1-RM test was performed accordingly to the guidelines established by the National Strength and Conditioning Association (O'Shea 1985). In the following two sessions infrared camera was used for measuring skin temperature of the thighs during two different speed-execution of squat exercise with 50% of 1-RM. The first session involved a squat exercise performed until exhaustion with 1 s eccentric phase and 1 s concentric phase. The last session involved the same exercise with 5 s eccentric phase and 5 s concentric phase. The pace of movement was set with a metronome.

The day before the test sessions, subjects observed a standard preliminary protocol for preparing the skin of the thighs to infrared thermal imaging measurements: they removed body hair on legs that were clean and without cosmetics products.

Before the trials, the subjects performed a standardized warm-up: 5 min of walking on treadmill and 2 min of squat exercise without overload. After being acclimated to the room climate conditions (temperature 22-24 °C; relative humidity 50±5 %; no direct ventilation and constant intensity of light) for 15 minutes before the test at rest condition (Marins et al 2014b), the subjects performed the squat exercise with 50% of 1-RM.

All the thermal images were recorded (frame rate 0.05 Hz) at the same instant during the exercise (i.e, when the subjects re-achieved the starting position at the end of the concentric phase). The same procedure was executed in both 1 s and 5 s trials.

All the tests were performed in the late morning period in order to limit possible effects due to circadian rhythm variations (Reilly and Brooks 1986).

Thermographic analysis

Thermal images were recorded during 2 min of pre-exercise to determine basal temperature, during exercise (prolonged until exhaustion of each subject) and 10 min of recovery-time.

Thermal image sequences of the subject's thighs were recorded by a 14-bit digital infrared thermal camera (AVIO, TVS-700, 320 x 240 Microbolometric Array; 8-14 μm spectral range; 0.07 °C thermal resolution; and 35 mm lens). Recordings were made using a digital frame grabber with a rate of one image per 20 s. During the trials the subjects swimsuit dressed were set 3 m from the thermal camera to permit the complete exposure of the anterior region of the under limbs and of consequence the best recording of the temperature thighs. The emissivity value was set to 0.97, in keeping with the value of human skin emissivity reported by Jones and Plassmann (Jones and Plassmann 2002).

A uniform background with a constant temperature (T=24.86 ± 0.20 °C) was placed behind the subjects to permit the calibration procedure. The images of the sequence were analyzed using a specific software for thermal images elaboration (GRAYESS® IRT Analyzer, Version 4.8).

As already performed in the paper by Formenti and colleagues (Formenti et al 2013) and more recently proposed in the paper by Ludwig and colleagues (Ludwig et al 2014) in order to avoid possible operator dependency, a procedure was set to obtain a reliable temperature value of the thighs surface. One region including all the muscles involved in exercise was selected for each thigh. The software selected the five hottest pixels inside each area (the software was instructed to select pixels having a minimum distance of 5 pixels from each other). The final temperature value was obtained by averaging over an area of 5x5 pixels around them. In this way the results are representative of an overall amount of 125 pixels on each thigh (Formenti et al 2013). This procedure allowed us to obtain a representative sample of the warmest areas of the thighs. Finally, we averaged left and right thighs temperature values (no significant difference was found between left and right thigh), thus obtaining one representative temperature value for each subject.

2.3.3. Data Analysis, Statistics, and Preliminary Results

Figure 14 shows the average skin temperature data recorded in 1 s and 5 s exercise during the experiments (n=13).

Each subject responded to exercise modifying his temperature. The direction of the skin temperature changes (decrease or increase) was not the same for all the subjects: it was possible to identify two subgroups. The majority of the subjects (n=9) showed a temperature decrease in both 1 s and 5 s exercises (down-group; Figure 15). Indeed, the other subjects (n=4) showed a temperature increment in both 1 s and 5 s exercises (up-group; Figure 16).

The next step will be the quantification of key parameters descriptive of the skin temperature time courses in 1 s and 5 s for each subjects. These parameters will be further compared in each group separately, allowing a straightforward comparison between the skin temperature behaviour of 1 s and 5 s exercise.

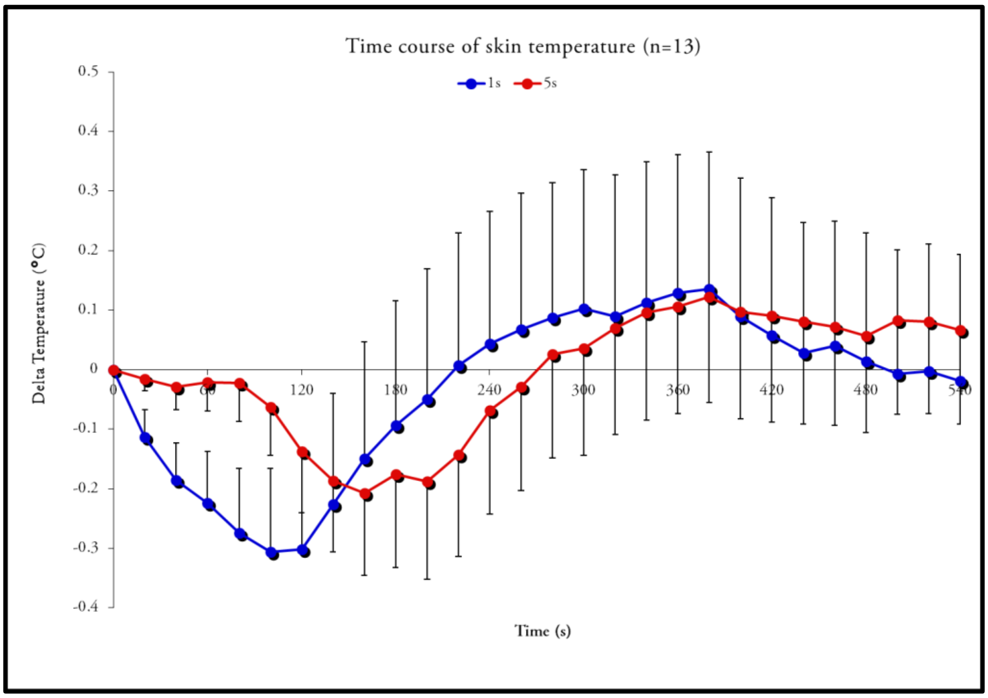

Figure 14: Average skin temperature data recorded during the experiment 1 s and 5 s (n=13; mean±SEM). To improve the readability of the graphs, discrete experimental data were connected by linear segments, yielding piece-wise linear functions.

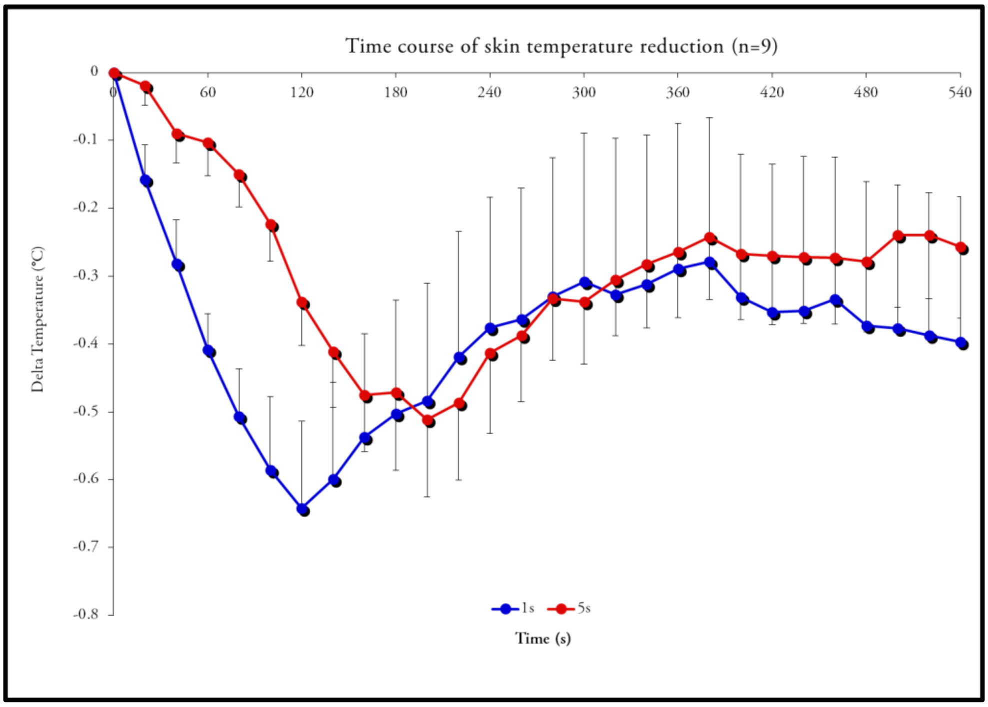

Figure 15: Average skin temperature data recorded during the experiment 1 s and 5 s (n=9; down-group; mean±SEM). To improve the readability of the graphs, discrete experimental data were connected by linear segments, yielding piece-wise linear functions.

2.3.4. Discussion

This study examined the variations in skin temperature response to steady-load localized squat exercise until exhaustion performed with normal speed (1 s exercise) and slow speed (5 s exercise) of movement. The main findings of the present study are as follows: a) the direction of the skin temperature changes in response to exercise was not the same for all the subjects (9 subjects decreased skin temperature during both exercises, 4 subjects increased skin temperature during both exercises); b) the skin temperature changes during 1s exercise seemed to occur more rapidly than that during 5s exercise, in spite of the same amount of temperature modification. It is worth noticing that the statistical analysis has not been performed yet. Therefore, only visual observation of skin temperature behaviors can be done.

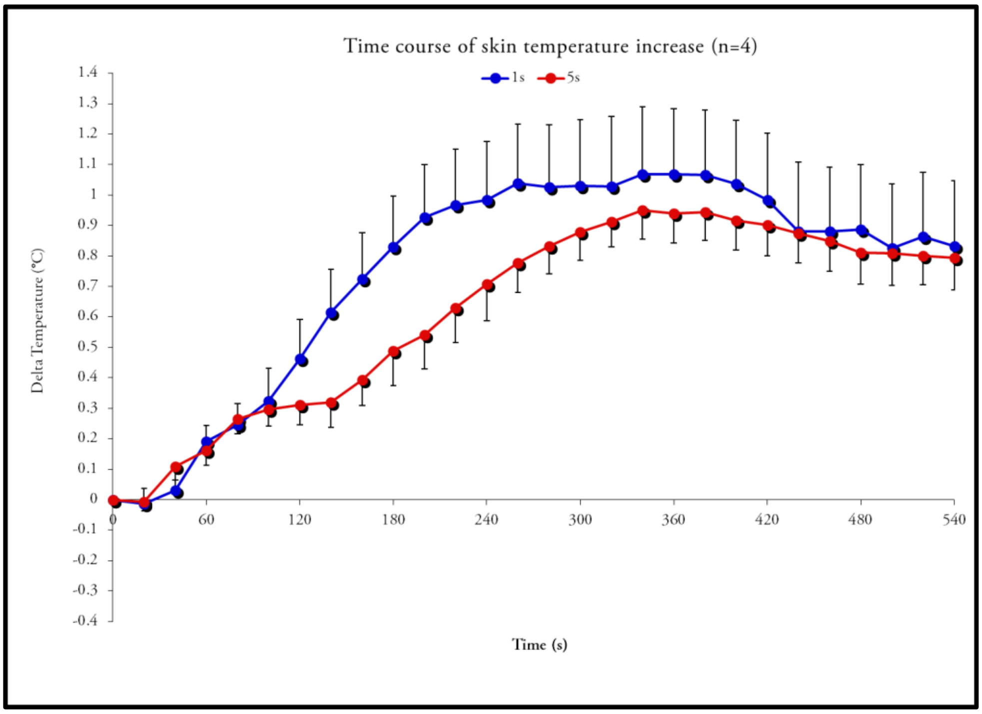

Figure 16: Average skin temperature data recorded during the experiment 1 s and 5 s (n=4; up-group; mean±SEM). To improve the readability of the graphs, discrete experimental data were connected by linear segments, yielding piece-wise linear functions.

The visual impression that the subjects responded to 1 s exercise more rapidly than 5 s exercise seems to be more evident in the subjects that decreased their skin temperature (down-group), but there is a tendency also in the subjects that increased it (up-group).

When averaging all the subjects time course skin temperature values (n=13), visual inspection of the Figure 14 suggests that the change in skin temperature that follows the beginning of the exercises occurred more quickly during 1 s exercise as compared to 5 s exercise. It is worth noticing that the wide error bars in the Figure 14, representing standard error of the mean, are the result of an average over the subjects that decreased their skin temperature and those that increased it. Considering the down-group and the up-group together could be imprecise and misleading, since the total average is not representative neither of the down-group nor of the up-group. Therefore, we chose to consider separately the subjects that decreased skin temperature and those that increased it for comparing skin temperature modifications during 1 s and 5 s exercises.

The fact that the direction of the skin temperature changes (decrease or increase) was not the same for all the subjects was quite unexpected. We had the possibility to consider all the subjects together, but in this case we would not respect such an important individual difference in response to exercise.

A possible explanation to the individual difference in the direction of the skin temperature response to exercise can be found in the competition between vasoconstriction and vasodilation (Kellogg et al 1991). We speculate that the subjects that decreased their temperature (down-group) were characterized by a predominance of vasoconstriction over vasodilation. In opposition, the subjects that responded to exercise increasing their temperature showed a predominance of vasodilation over vasoconstrictor system.

APPENDICES

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Skin temperature evaluation by infrared thermography: Comparison of image analysis methods

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HIGHLIGHTS

- Analysis of calf temperature distribution by infrared thermography.
- Comparison among a well established method of image analysis and two new methods.
- Temperature mean value obtained with different method give comparable results.
- Method based on maxima values shows less operator dependencies.
- New Tmax method seems to be able to detect asymmetry on human body.

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ABSTRACT

Body temperature in medicine is a parameter indicating abnormal activity of human tissues; it is used to diagnose specific pathologies or as an indicator of the muscle activity during physical exercise.

Temperature measurements through infrared thermography have the advantages to be non-invasive and to record temperature data simultaneously from different points on a wide area of the body.

The difference between the values of temperature traditionally measured with contact probes or standard technique and the ones measured by thermal imaging lies in the fact that the first produces a scalar value, while the second gives a distribution over a surface. The analysis of thermographic images, with the goal of obtaining a temperature value representative of a specific area, is usually performed by different methods of averaging temperature values inside a selected Region of Interest (Troi and Tot). In this paper the authors present a critical comparison between the methods mainly used in literature in the specific case of a muscular group of calves on a population of 33 healthy subjects. Here, the authors describe an alternative method (Tmax) to obtain a temperature value of a specific area based on maximal temperature detection instead of considering the average temperature on the selected area. No meaningful difference in mean temperature between Troi and Ttot was found ($p = 0.9$), while temperature values calculated using Tmax were higher than the above methods ($p < 0.001$). The high correlation among the compared methods prove that they can equally represent temperature trends in cutaneous thermographic analyses.

1. Introduction

The first use of infrared thermography (IRT) in the biomedical sciences was reported only in 1960 [1], although the diagnostic applicability of temperature measurement by infrared technique were already proposed by Hardy in 1934 [2]. IRT has been used in the last 50 years to study diseases in which skin temperature is an indicator of inflammation or blood flow changes due to a clinical abnormality [3,4]. In living body measurements, mechanisms of skin heating/cooling are complex due to the combined effects of radiation and local blood flow.

Considerable progress has been achieved over the last 20 years in the knowledge of the physiological mechanism of skin temperature distribution and in the methodology of usability of IRT for the standardization of measurement protocols and the statistical data analysis [1,3,4].

The use of IRT in the measurement of temperature of human skin has the advantage to be completely non-invasive. The advantage of using this technique, compared to alternative methods requiring a contact between the object and the sensor, lies in the fact that with the use of IRT the skin temperature is not influenced by the presence of any probes that could modify the temperature

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Thermal Imaging of Exercise-Associated Skin Temperature Changes in Trained and Untrained Female Subjects

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Abstract—Heat dissipation during sport exercise is an important physiological mechanism that may influence athletic performance. Our aim was to test the hypothesis that differences exist in the dynamics of exercise-associated skin temperature changes between trained and untrained subjects. We investigated thermoregulation of a local muscle area (muscle–tendon unit) involved in a localized steady-load exercise (standing heels raise) using infrared thermography. Seven trained female subjects and seven untrained female controls were studied. Each subject performed standing heels raise exercise for 2 min. Thermal images were recorded prior to exercise (1 min), during exercise (2 min), and after exercise (7 min). The analysis of thermal images provided the skin temperature time course, which was characterized by a set of descriptive parameters. Two-way ANOVA for repeated measures detected a significant interaction ($p = 0.03$) between group and time, thus indicating that athletic subjects increased their skin temperature differently with respect to untrained subjects. This was confirmed by comparing the parameters describing the speed of rise of skin temperature. It was found that trained subjects responded to exercise more quickly than untrained controls ($p < 0.05$). In conclusion, physical training improves the ability to rapidly elevate skin temperature in response to a localized exercise in female subjects.

Keywords—Infrared thermography, Heels raise, Blood flow, Thermoregulation, Sedentaries–athletes.

INTRODUCTION

The thermo-regulatory system of human body has the task to maintain a constant temperature despite the variations caused by environmental conditions and/or physical work. The control of heat exchange between human body and the environment is essential for body temperature regulation. The tegumentary apparatus (skin) has the fundamental role of regulation of heat exchange through conduction, convection, radiation, and evaporation.[2,15] The activation of body compensatory vasoregulation occurs during muscles activity, through reduction of blood flow in the splancnic region and tegumentary apparatus. Intense exercise causes heat production in the core structures and activates muscles,[5] with a consequent massive transfer of warmer blood from the internal to the superficial parts of the body. Thus, the vasoconstriction in these regions increases blood flow in the muscular areas while the muscle blood volume remains constant. The blood volume in the internal and periferical parts of the body decreases. In this condition we can observe an increase of the venous return and the cardiac output.[15,20]

The effect of physical exercise on skin blood flow has been previously studied and reviewed by Johnson[8] and Kenney and Johnson.[10] They found that the modifications of cutaneous blood flow (CBF) during exercise is linked to the individual grade of vasodilation and vasoconstriction. As a consequence, the CBF influences the skin temperature depending on modification caused by exercise. Johnson[8] and Robinson[16] observed that at the beginning of the exercise the demand of blood flow to working muscles caused a briefly skin vasoconstriction, but as the body core temperature raises, the thermal regulatory processes predominate and the skin vessels dilate, increasing heat dissipation through the skin, essentially by irradiation and evaporation.[12] Frietzsche and Coyle[5] showed that the CBF is higher in endurance athletes than in untrained subjects during exercise. As a result, a higher fitness level is associated to a higher CBF. It stands to

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sports and whose average age was 23.8 years. The participants' dominant hand/feet reaction times to voice and light stimuli were recorded. The complex action format was set up by a table tennis robot. It was set up to throw 90 balls per minute of which balls are thrown to different places of the table selecting any of the 3 colors (white-yellow-pink) randomly (total: 120 balls). The participants were asked not to react to white balls, but to touch yellow balls and catch the pink ones after they hit the table by using their dominant hand only. Actions were converted into scores according to a scale. Results and Discussion The dominant hand-voice reaction time was estimated approximately as 170.5 ms in martial arts athletes whereas it was estimated approximately as 176.1 ms in e-athletes and as 196.3 ms in the control group. There was no statically significant difference between the martial arts athletes and e-athletes in terms of reaction times. Nevertheless, both groups have statically significant shorter reaction times than the control group. Although the average of total scores in the complex action format was estimated at 166 for the athletes, 156 for the e-athletes and 138 for the control group. The precision level of the performed action was the highest for martial arts athletes and the lowest for the control group for each color of balls. The shorter reaction time of the martial arts athletes was an expected result. That the reaction times of e-athletes were nearly the same as the ones of martial arts athletes and were expressively shorter than the control groups. That the precision levels of the e-athletes in the complex action format were better than the control group can be regarded as the increase in visual perception and decrease in reaction times. As far as the literature review, the methodology for the complex action format has been designed for the first time and is still in the process of development. Contact: esagdilek@hotmail.com

13:00 - 14:00

Mini-Orals

MO-PM10 Thermoregulation 1

THERMOGRAPHIC SKIN TEMPERATURE RESPONSE TO DIFFERENT MOVEMENT VELOCITY OF SQUAT EXERCISE UNTIL EXHAUSTION: A PRELIMINARY REPORT

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Università degli studi di Milano

INTRODUCTION Blood flow restriction resistance training is an effective training method for improving muscular function using low and moderate load intensity (Alberti et al., 2013). It has implications in the regulation of skin blood flow, with important consequence for the blood involved in heat dissipation through the skin. The aim of this study was to investigate the skin temperature (ST) response by using infrared thermography during slow speed low intensity exercise as compared to normal speed low intensity exercise in squat trial (Tanimoto et al., 2006). We hypothesized that low intensity resistance exercise with slow movement would result in a ST response slower than the one of the normal speed exercise with the same intensity. METHODS 9 active males (23.6±1.1yrs, 69.7±6.8kg, 176±6.2cm) performed 2 sessions of deep squat exercise until exhaustion, with 50% of 1 RM. The pace of movement was set in 1s eccentric / 1s concentric and 5s eccentric / 5s concentric phase in the 1st and in the 2nd session respectively. Thermal images were recorded every 20s before exercise (2min), during exercise (until exhaustion), and after exercise (10min). ΔT (Tpeak-Tbasal) and Time50% (time to reach 50% of ΔT) were identified and compared in 1s vs 5s trials by using paired t-test. RESULTS Surprisingly, a different behaviour of ST during and after exercise was observed among subjects: a decrease in ST in 5 subjects (down group) and an increase in the other 4 (up group). Thus, statistics was performed in each group separately. The ΔT of the up group in 1s (1.1±0.42°C) and 5s (1.0±0.50°C) were approximately twice that of the down group in both 1s (-0.50±0.15°C) and 5s (-0.42±0.28°C). The ΔT in 1s was similar to ΔT in 5s in both groups. The ST changes (Time50%) in the down group occurred slowly ($p<0.01$) in 1s (30.1±17.2s) vs 5s (107.3±25.3s) as well as in the up group (139.1±17.2s in 1s vs 184.9±58s in 5s; $p>0.05$). The ST changes during 1s and 5s trials occurred more rapidly in the down group than in the up group. DISCUSSION It was shown that the response of cutaneous circulation to dynamic exercise is characterized by a initial vasoconstriction to dissipate heat from the core through the skin followed by vasodilation driving the blood flow from inactive tissue (including the skin) to active muscles involved in exercise (Kellogg D.L., 2006). We speculate that the unexpected different behaviour of the ST response in the 2 groups was probably due to a time-dependent predominance of vasoconstriction over vasodilation or viceversa. REFERENCES Alberti G. et al. (2013) SCJ Kellogg D.L. (2006) JAP Tanimoto M. et al. (2006) JAP

VASTUS LATERALIS REPRESENTS THE ASSOCIATION BETWEEN NEUROMUSCULAR ACTIVATION AND THERMORREGULATION IN CYCLING

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Introduction Fitness level improves the efficiency of the thermoregulatory system. However, it is still unknown how neuromuscular activation affects skin temperature. Therefore, this study assessed the relationship between neuromuscular activation and skin temperature during cycling exercise. Methods Ten male physically active participants underwent an incremental cycling test to exhaustion while muscle activation was recorded from rectus femoris (RF), vastus lateralis (VL), biceps femoris (BF) and gastrocnemius medialis (GM). Muscle recruitment was assessed via frequency band analyses of muscle activation signals. Thermography images were recorded before and immediately after exercise at four body regions of interest corresponding to the muscles where EMG activity was recorded. Results Significant inverse relationship between variation in skin temperature and variation in overall neuromuscular activation ($p<0.04, r>0.5$) and significant positive relationship between skin temperature and low frequency components of neuromuscular activation ($p<0.01, r>0.7$) were observed for vastus lateralis. Discussion A recent study (Abate et al. 2013) showed differences in thermoregulation due to level of physical fitness. Higher maximum overall activation is associated with better fitness level (Häkkinen et al. 1998) and our participants used large motor units rather than small (less low frequency component) to sustain the increases in workload (Gregory and Bickel 2005). For this reason, larger increases in skin temperature for participants presenting increased low frequencies and limited overall activation of vastus lateralis during maximal aerobic exercise could be associated to their reduced fitness level. In conclusion, participants with higher overall muscular activation and lower frequency content in activation for vastus lateralis presented a better adaptive response of their

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