

Effects of whole body heat stress on sublingual microcirculation in healthy humans

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Abstract

Purpose To assess the effect of whole body heat stress on sublingual microcirculation.

Methods Fourteen apparently healthy subjects participated in the study. Passive body heating was performed by immersing the subjects up to the waist in a water bath at 44 °C continuously until a rectally obtained core temperature of 39.5 °C was reached. Systemic hemodynamic parameters and sublingual microcirculation were evaluated and recorded before heating, immediately after heating, and 1 h after heating.

Results The subjects showed very high physiological stress and significantly increased noradrenaline and prolactin concentrations in the blood. Whole body heating

resulted in significantly increased oxygen uptake, heart rate, and cardiac output. One hour after heating, heart rate remained increased, but cardiac output almost returned to baseline. Mean arterial pressure significantly decreased after heating and remained decreased for at least 1 h. There was no significant difference in the microvascular flow index and proportion of perfused vessels of small vessels at the end of heating and 1 h after heating, in comparison with baseline variables. However, functional capillary density and total vessel density of small vessels significantly increased at the end of heating (10.8 ± 2.4 vs. 11.7 ± 2.0 1/mm and 19.5 ± 3.5 vs. 22.2 ± 3.3 mm/mm², $p < 0.05$, respectively) and remained increased 1 h after heating.

Conclusion Whole body heat stress increases sublingual functional capillary density, oxygen consumption, and cardiac output.

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Keywords Microcirculation · SDF videomicroscopy · Passive body heating · Hyperthermia

Abbreviations

| | |
|-----------------|--------------------------------|
| MAP | Mean arterial blood pressure |
| HR | Heart rate |
| CO | Cardiac output |
| MFI | Microvascular flow index |
| PPV | Proportion of perfused vessels |
| PVD | Perfused vessel density |
| FCD | Functional capillary density |
| TVD | Total vessel density |
| VO ₂ | Oxygen consumption |

Introduction

Whole body heat stress can be life threatening. During the past decade, the number of deaths caused by overheating

appears to have increased, especially in athletes, military personnel, and occupational workers (Luterbacher et al. 2004; Valdes et al. 2014). Mueller and Colgate (2012) reported 32 deaths caused by exertional heat stroke during football practice at high schools between 2002 and 2012. During prolonged intense physical exercise (e.g., marathon running and football), rectal temperature can increase from a resting temperature of 37 °C to 39.5 °C and higher, at which point cells can be damaged and the functions of organs and central nervous system can be impaired (Maughan et al. 1985; Shapiro and Seidman. 1990). Sohar et al. (1976) demonstrated that as many as 22 % of participants in a Finnish sauna with temperatures of 80–90 °C and relative humidity of 30–40 % had rectal temperatures greater than 39 °C over 20 min (or until they suffered discomfort). In such circumstances, heat illnesses are associated with fundamental pathophysiological consequences, such as homeostasis disturbance, systemic inflammatory response syndrome, and changes in blood flow. Microcirculation is a vital part of blood flow and is the main prerequisite for adequate tissue oxygenation and thus organ function (Ince 2005). However, in the case of overheating, it is unclear how microcirculation is altered.

It has been established that individuals who undergo heat exposure have increased blood flow to the skin (Edholm et al. 1956; Pearson et al. 2011; Peters et al. 2000). Methods, such as laser Doppler monitoring and plethysmography, used to investigate skin blood flow reflect flow in variable volumes of tissue, but they are unable to detect it in individual vessels, and do not represent true capillary flow in humans (Swain and Grant 1989). A previous study with rats using videomicroscopy showed that heat stress increased the density of flow active capillaries in the subepidermal plexus (Westphal and Richardson 1992).

Thus, cutaneous vasodilatation increases blood flow to the skin and ‘recruits’ subepidermal capillaries, thereby increasing convective transfer of heat from the core to the periphery. This occurs in conjunction with sweating. This increase of skin perfusion often requires increased cardiac output and the redistribution of blood flow from the central circulation to the skin (Heinonen et al. 2011; Rowell et al. 1969, 1970).

In contrast with blood flow to the skin, it is unclear how heat stress influences capillary perfusion in organs other than skin in humans. Improved microcirculatory imaging techniques, such as sidestream dark field (SDF) imaging in hand-held devices, have allowed the direct observation of microcirculation at the bedside. This technique visualizes the blood flow at the individual capillary level in available thin mucosa (often sublingual) and helps to assess flow and density in humans (Goedhart et al. 2007). Clinical data have shown that the sublingual region is clinically relevant

in detecting microcirculatory alterations in critically ill patients (De Backer et al. 2002; Pranskunas et al. 2013). Studies with these patients showed that decreased capillary density and flow in sublingual mucosa are associated with increased mortality (De Backer et al. 2002; Sakr et al. 2004). In addition, improvement in microvascular perfusion in response to treatment is associated with an improvement in organ function (Trzeciak et al. 2008). However, it is unclear whether physiological stress, such as heat stress, causes detectible sublingual microcirculatory changes in healthy humans. Therefore, the aim of our study was to assess the impact of whole body heat stress on sublingual microcirculation.

Materials and methods

Subjects

Fourteen healthy subjects (9 men and 5 women) with no history of heat injury were recruited to this study. The subjects were moderately physically active (<2 h week⁻¹), but did not participate in any formal physical exercise or sports program. They had not been involved in any temperature-manipulation program or procedure for at least 3 months. Each subject volunteered to participate in the study after being informed of the purpose, experimental procedures, and known risks of the study. Each subject read and signed a written informed consent form that was consistent with the principles outlined in the Declaration of Helsinki. The local ethics committee approved this study.

Preliminary measurements

The anthropometric characteristics of the subjects are presented in Table 1. The subjects’ weight (kg), body fat (%), body mass index (TBF-300, Tanita, UK Ltd. Philpots Close, UK) and height (cm) were measured. Body surface area (m²) was estimated by $128.1 \times \text{Weight}^{0.44} \times \text{Height}^{0.60}$ (Tikuisis et al. 2001). Skinfold thickness (mm) was

Table 1 Anthropometric characteristics of subjects

| | |
|--------------------------------------|-------------|
| Age (years) | 22.1 ± 3.5 |
| Height (cm) | 180.4 ± 9.1 |
| Mass (kg) | 74.9 ± 12.9 |
| Body surface area (m ²) | 1.9 ± 0.2 |
| Mean subcutaneous fat (mm) | 12.1 ± 4.2 |
| Body fat (%) | 19.9 ± 7.1 |
| Body mass index (kg/m ²) | 22.9 ± 3.1 |

Values are mean ± SD

measured (Skinfold caliper SH5020, Saehan, Masan, Korea) at 10 sites: chin, subscapular, chest, side, suprailium, abdomen, triceps, thigh, knee, and calf (McArdle et al. 2009), and mean subcutaneous fat thickness was calculated (Allen et al. 1956).

Experimental protocol

The experiment was conducted indoors at the same time of day (07:00–11:00). The subjects fasted for at least 12 h before the experiment. To standardize the state of hydration and the feeling of thirst, the subjects were allowed to drink water as desired until 60 min before the experiment. The measurements were performed at a room temperature of 23 °C and a relative humidity of 60 %.

Upon arrival at the laboratory, subjects were weighed nude and then asked to rest in a semi-recumbent posture for 10 min, dressed in a T-shirt, swim shorts, and socks. Resting pulmonary gas exchange was recorded for the next 20 min in the same semi-recumbent posture. Rectal (T_{re}) temperature and systemic hemodynamic stabilization were assessed. T_{re} was measured throughout the experimental trial using a thermocouple (Rectal Probe, Ellab, Hvidovre, Denmark; accuracy ± 0.01 °C) inserted to a depth of 12 cm past the anal sphincter. The rectal thermistor sensor was placed by each subject.

Control T_{re} and mean arterial blood pressure (MAP), heart rate (HR), and cardiac output (BioZ, CardioDynamics, San Diego, CA, USA) were measured, and sublingual evaluation of microcirculation was performed. After a blood sample was drawn from a vein and stored for later analysis, the subjects began the water immersion heating protocol. The water-bath temperature was 44 °C, and the subjects were immersed to the waistline (Brazaitis and Skurvydas. 2010; Brazaitis et al. 2012). Pulmonary gas exchange was recorded during water immersion. The procedure continued until the T_{re} increased to 39.5 °C, and the exposure time until this T_{re} was achieved and recorded. At the end of heating, sublingual evaluation of the microcirculation was performed and a blood sample was drawn from a vein. Within about 1 min after leaving the bath, subjects were towel dried and clothed in a long-sleeved sweater, trousers, and socks, and were positioned in a semi-recumbent posture. At that time and 1 h after the heating, the subject's systemic hemodynamics were measured. Evaluation of the sublingual microcirculation was also performed 1 h after the heating. Nude body mass was recorded within 45 min after exit from the water bath (Brazaitis and Skurvydas. 2010). Skin temperature (T_{sk}) was measured before and at the end of the water immersion using thermistors taped at three sites: back, thigh, and forearm (DM852, Ellab; accuracy ± 0.01 °C).

Physiological stress index (PSI)

The method used to measure PSI has been described elsewhere (Moran et al. 1998). Thus, the following normalized physiological stress index was applied:

$$PSI = \frac{5(T_{ref} - T_{re0})}{(39.5 - T_{re0})^{-1} + 5(HR_t - HR_0)} \times (180 - HR_0)^{-1}$$

The measurements for PSI were taken before (T_{re0} and HR_0) and at the end of passive heating (T_{ref} and HR_t). T_{re} and HR were assigned with the same weight using a constant of 5. Thus, the index was scaled to a range of 0–10: 1–2 (no/little heat stress); 3–4 (low heat stress); 5–6 (moderate heat stress); 7–8 (high heat stress); and 9–10 (very high heat stress), within the limits of the following values: $36.5 \leq T_{re} \leq 39.5$ °C and $60 \leq HR \leq 180$ beats min^{-1} .

Blood variables

Blood samples for the evaluation of cytokines and catecholamines were obtained before and immediately after heating. Blood samples for the measurement of dopamine, epinephrine and norepinephrine concentrations were collected in vacuum tubes using EDTA as an anticoagulant (EDTA-K3, 3 ml), mixed gently by inverting 8–10 times and kept at 2–8 °C until centrifugation. The blood samples were centrifuged at $1,200 \times g$ for 15 min within 30 min of blood collection. The plasma samples were separated as quickly as possible (maximum 10–15 min) from the red blood cells after centrifugation, and kept at -70 °C until analysis. The epinephrine and norepinephrine concentrations were measured using an ELISA kit (Gemini Analyser, Stratec Biomedical GmbH, Birkenfeld, Germany).

Blood samples for the measurement of cortisol, interleukin 6 (IL-6) and tumor necrosis factor- α (TNF- α) concentrations were collected by venepuncture into vacuum tubes for serum separation, using a gel separator (5 ml). Blood samples were allowed to clot, and the serum was separated by centrifugation at $1,200 \times g$ for 15 min. The serum samples were aliquoted and stored at -70 °C until analysis. Concentrations of IL-6 and TNF- α were measured by ELISA (Gemini Analyser, Stratec Biomedical), and cortisol concentrations were measured using an automated enzyme immunoassay analyser (AIA-2000, Tosoh Corp., Tokyo, Japan).

Videomicroscopic measurements and analysis

Images of the sublingual microcirculation were obtained before heating, at the end of heating, and 1 h after heating, using SDF videomicroscopy (Microscan[®], MicroVision Medical, Amsterdam, The Netherlands). SDF videomicroscopy is based on the principle that emitted green light (wavelength 530 nm) is absorbed by the hemoglobin

content in red blood cells (Goedhart et al. 2007). Thus, red blood cells are seen as black or gray bodies during imaging. The vessel walls are not visualized, so vessels can only be detected by the presence of red blood cells. This method can be used only for tissues covered by a thin epithelial layer, that is, mostly mucosal surfaces, such as the sublingual mucosa.

After gentle removal of saliva and other secretions using isotonic saline-drenched gauze, the SDF device was applied to the sublingual region, avoiding pressure artifacts by establishing a threshold image. Sequences of 20 s from at least three areas were recorded on a hard disc using a personal computer and AVA software (version 3.0) (MicroVision Medical, Amsterdam, The Netherlands). Video clips were analyzed offline by two investigators blind to the intervention in random order to prevent coupling.

Each image was divided into four equal quadrants. Quantification of flow (no flow: 0; intermittent flow: 1; sluggish flow: 2; continuous flow: 3) was scored per quadrant, for each vessel diameter cohort (small: 10–20 μm ; medium: 21–50 μm ; large: 51–100 μm). The MFI was calculated as the sum of each quadrant score divided by the number of quadrants in which the vessel type was visible. The final MFI was averaged over a minimum of 12 quadrants (three regions, four quadrants per region) derived from the overall flow impressions of all vessels with a particular range of diameter in a given quadrant.

Calculation of total vessel density (TVD) of small vessels was performed with the AVA software package (MicroVision Medical, Amsterdam, The Netherlands), as described and validated recently (Dobbe et al. 2008), using a cut-off diameter for small vessels (mostly capillaries) of $<20 \mu\text{m}$. We defined the perfused vessel density of small vessels, an estimate of functional capillary density (FCD), and the proportion of perfused vessels (PPV) of small vessels in terms of the number and percentage of crossings with perfused small vessels per total length of three equidistant horizontal and three equidistant vertical lines. This method has been described elsewhere by De Backer et al. and is in accordance with reports of a round table conference (De Backer et al. 2007).

Spirometry

A mobile spirometry system (Oxycon Mobile, Jaeger/VIASYS Healthcare, Hoechberg, Germany) was used to measure pulmonary gas exchange at rest and during water immersion. This system uses a tightly fitting face mask that covers the nose and mouth with a lightweight integrated flow meter (Triple V volume sensor; 45 g) with a dead space of 30 ml. The system monitors ventilatory parameters, oxygen uptake and VCO_2 production on a breath-by-breath basis. The processing, recording, and

battery system comprise two units attached to a belt, which was hung as closely as possible to the subject's nose and mouth during immersion. The data were stored on memory cards and PC hardware. This instrument was calibrated before recording, as indicated in the manufacturer's manual, using the automatic volume- and gas-calibration functions. A flow-volume sensor calibration procedure assures that the Oxycon quantification system (including the amplifier, Triple V sensors and pressure transducer) is functioning correctly. The gas analyzer and delay time calibrations were also automatic, as provided by the manufacturer: a calibration gas at 180 kPa (15.2 % O_2 , 5.02 % CO_2 and 79.78 % N_2) was introduced to the Oxycon to attain gain, offset and delay times within 1 %. The values of VO_2 (in ml/min/kg and l/min) and VCO_2 (l/min) were registered before and at the end of heating.

Statistics

Primary outcomes were the sublingual FCD and TVD of small vessels. The Statistical Package for Social Sciences was used to conduct the statistical analysis. Data were checked for normality using the Shapiro–Wilk test. Because no deviation from normality was detected, data are reported as mean \pm SD. These data were analyzed with parametric tests and Pearson rho for correlations. A one-way repeated-measures ANOVA model with Bonferroni correction was used to compare multiple sets of related values. A $p < 0.05$ was considered significant.

Results

Rectal temperatures of 39.5 $^{\circ}\text{C}$ in the subjects were achieved in 72 ± 24 min. During heating, the subjects lost 1.5 ± 0.5 kg of body weight. Whole body heating resulted in a significantly increased heart rate and cardiac output by comparison with baseline variables (Table 2). Because of the heating, the subjects showed very high physiological stress. This was confirmed by a mean physiological stress index of 8.2 in the case of significantly increased noradrenaline and prolactin concentration in the blood (Table 3). One hour after heating, heart rates remained increased, but cardiac output returned to baseline values. Mean arterial pressure significantly decreased at the end of the heating and remained decreased 1 h after heating. Table 4 shows the sublingual microcirculation response associated with the whole body heating. There was no significant difference in MFI and PPV of small vessels at the end of the heating and 1 h after heating, in comparison with baseline variables. However, the FCD and TVD of small vessels were significantly increased at the end of heating and remained increased 1 h after heating (Table 4).

Table 2 Systemic hemodynamic response to heating

| | Baseline | End-heating | 60 min after heating |
|---|--------------|---------------|----------------------|
| Rectal temperature (°C) | 36.9 ± 0.2 | 39.5 ± 0.2* | 38.3 ± 0.5* |
| Heart rate (beats/min) | 68 ± 11 | 100 ± 15* | 81 ± 17* |
| MAP (mmHg) | 90 ± 6 | 82 ± 10* | 85 ± 8* |
| Cardiac output (L/min) | 7.6 ± 0.8 | 9.9 ± 1.9* | 8.3 ± 0.9 |
| SV (L/min) | 117.8 ± 20.4 | 100.5 ± 22.6* | 105.6 ± 22.7* |
| SVRI (dyn s/cm ⁵ /m ²) | 2004 ± 222 | 1,480 ± 438* | 1,771 ± 333* |

Values are mean ± SD

MAP mean arterial pressure, SV stroke volume, SVRI systemic vascular resistance index

Significantly different from baseline: * $p < 0.05$

Table 3 Blood variable responses to whole body heat stress

| | Baseline | End-heating | p |
|-----------------------|---------------|-------------------|-------|
| Cortisol (nmol/L) | 590.7 ± 79.9 | 651.0 ± 122.7 | 0.327 |
| Adrenaline (ng/mL) | 1.9 ± 0.9 | 3.3 ± 5.8 | 0.779 |
| Noradrenaline (ng/mL) | 7.5 ± 3.9 | 15.3 ± 4.4* | 0.018 |
| Dopamine (ng/mL) | 28.4 ± 21.6 | 41.5 ± 33.7 | 0.463 |
| IL-6 (pg/mL) | 17.6 ± 36.7 | 22.9 ± 45.5 | 0.249 |
| IL-1beta (pg/mL) | 4.4 ± 3.0 | 4.5 ± 2.8 | 0.715 |
| TNF alfa (pg/mL) | 5.2 ± 1.0 | 5.1 ± 0.8 | 0.799 |
| Neopterin (nmol/L) | 5.0 ± 1.1 | 6.1 ± 2.1 | 0.091 |
| Prolactin (mU/L) | 470.4 ± 250.5 | 1,638.1 ± 1282.1* | 0.012 |

Values are mean ± SD

Significantly different from baseline: * $p < 0.05$

Table 4 Sublingual microcirculatory response to heating

| | Baseline | End-heating | 60 min after heating |
|-----------------------------|-------------|-------------|----------------------|
| MFI s | 2.98 ± 0.05 | 2.96 ± 0.09 | 2.98 ± 0.05 |
| PPV s (%) | 98.7 ± 1.0 | 98.4 ± 2.1 | 97.6 ± 2.7 |
| FCD (1/mm) | 10.8 ± 2.4 | 11.7 ± 2.0* | 11.8 ± 1.8* |
| TVD s (mm/mm ²) | 19.5 ± 3.5 | 22.2 ± 3.3* | 21.9 ± 3.3* |

Values are mean ± SD

MFI s microvascular flow index of small vessels, PPV s percentage of perfused small vessels, TVD s total vessel density of small vessels, FCD functional capillary density

Significantly different from baseline: * $p < 0.05$

At the end of heating, we observed significant increases in VO_2 and VCO_2 (Table 5). A statistically significant relationship could be demonstrated between the change in FCD and change in VO_2 during heating (Fig. 1). There was no correlation between changes in the FCD or TVD of small

vessels and changes in cardiac output or rectal to peripheral temperature gradients.

Digital microphotographs of microcirculation during heating are presented in Fig. 2.

Discussion

To our knowledge, this is the first study to investigate the sublingual microcirculatory response to heat stress. Our results show that whole body heating until rectal temperature reached 39.5 °C induced very high physiological stress and increased FCD in the sublingual mucosa in healthy humans.

Most previously published studies have investigated the microcirculation of the skin or muscles during heating. Studies using laser Doppler showed increased skin blood flow during both local and whole body heating (Bormyr et al. 1997; Pearson et al. 2011). However, laser Doppler flowmetry did not distinguish between capillaries and blood flow in other vessels (Lima and Bakker 2005). Westphal and Richardson (1992) used skin videomicroscopy in rats to demonstrate that heat stress also ‘recruits’ subepidermal capillaries. These responses occurred through a combination of locally and neurally mediated cutaneous vasodilatation, increased cardiac output, and the redistribution of blood away from the central vascular beds to the cutaneous circulation (Brothers et al. 2009; Crandall and Gonzalez-Alonso 2010; Cui et al. 2004; Rowell et al. 1969, 1970). Widmer et al. (2007) found that local heating induced capillary ‘recruitment’ in the Pallid bat wing independently of sensorineural and nitric oxide-dependent mechanisms.

There is currently ongoing debate challenging the phenomenon of recruitment of new capillaries. It is estimated that although 100 % of capillaries in skeletal muscle at rest are perfused by plasma, only around 80 % contain erythrocytes (Poole et al. 2013). In our study, vessels were measured by the detection of flowing erythrocytes as distinct from the visualization of vessel walls; therefore, capillaries without erythrocytes would remain undetected during preheating. With the influx of erythrocyte flow into these capillaries during heating, they would then become visible, hence increasing FCD and TVD of small vessels in case of normal convective flow, which represents PPV and MFI of small vessels. It is interesting to note that if severe heat stress has caused maximal ‘recruitment’ of all sublingual capillaries, results here match well with estimates of capillary flow in muscle, with our starting resting values ~80 % of our final heated values. According to our results, FCD increased from mean of 10.8 to 11.7 vessels per mm and matched approximately 80 % to 87 %. This possibly indicates that increase in capillary density is functional with remained reserve. For capillary proliferation and increase

Table 5 Metabolic variables and temperature gradients during heating

| | Baseline | End-heating | <i>p</i> |
|------------------------------------|--------------|--------------|----------|
| VO ₂ (ml/kg/min) | 2.9 ± 2.0 | 5.2 ± 2 | 0.005 |
| VO ₂ (ml/min) | 197.6 ± 82.8 | 339.8 ± 60.8 | 0.003 |
| VCO ₂ (ml/min) | 175.8 ± 72.1 | 311.5 ± 60.3 | 0.003 |
| Metabolic rate (W/m ²) | 33.4 ± 11 | 59.0 ± 6.7 | 0.003 |
| Rectal-to-back (°C) | 3.4 ± 0.9 | 4.1 ± 0.9 | 0.158 |
| Rectal-to-forearm (°C) | 5.3 ± 0.7 | 4.7 ± 1.0 | 0.195 |
| Rectal-to-thigh (°C) | 6.1 ± 0.8 | 0.34 ± 0.9 | 0.002 |

Values are mean ± SD

VO₂ oxygen uptake, VCO₂ carbon dioxide production

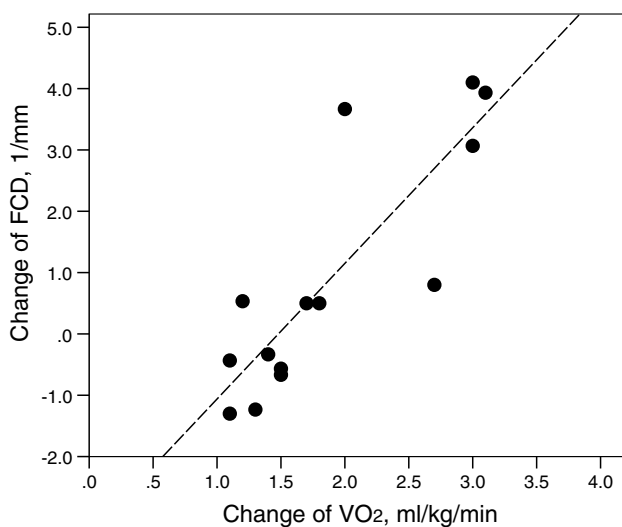


Fig. 1 Relationship between change of functional capillary density (FCD) and change of oxygen consumption (VO₂) during heating: $y = 2.21 \times x + -3.27$, $R^2 = 0.72$, $p < 0.001$

of new capillaries more time is needed. Gong et al. (2006) showed that whole body hyperthermia at 42 °C for 15 min in rats significantly increased vascular endothelial growth factor (VEGF) expression as early as 4 h post-heating and maximum expression at 12 and 24 h post-heating in the left and right ventricles, respectively. Multiple areas of extensive capillary proliferation in the heart occurred at 48 and 72 h post-heating.

Erythrocyte flux is controlled by arterioles, and not by precapillary sphincters (Poole et al. 2013). According to Poiseuille's Law, blood flow through a vessel is directly proportional to the driving pressure along the vessel and its radius to the fourth power, and inversely proportional to the length of the vessel and the dynamic blood viscosity. Theoretically, vasodilatation and/or increased cardiac output should be able to increase blood flow at the entrance of the capillaries and increase influx of the red blood cells. In our study, we observed increased cardiac output and decreased systemic vascular resistance at the end of heating and decreased systemic vascular resistance 60 min after heating.

Earlier studies have shown increased blood flow in the forearm skin, but not in forearm muscles during both local and indirect whole body heat stress (Detry et al. 1972; Edholm et al. 1956). However, using positron emission tomography, Heinonen et al. (2011) demonstrated that local heating, but not whole body heating, which increased core temperature by 1 °C, increased calf muscle blood flow in humans. These findings could be attributed to a combination of indirect whole body heating, which induced insufficient increases in muscle temperature, causing slight increases in muscle blood flow, coupled with sympathetically mediated vasoconstriction (Heinonen et al. 2011; Keller et al. 2010; Pearson et al. 2011). Pearson et al. (2011) demonstrated that heat stress, induced by increases in leg

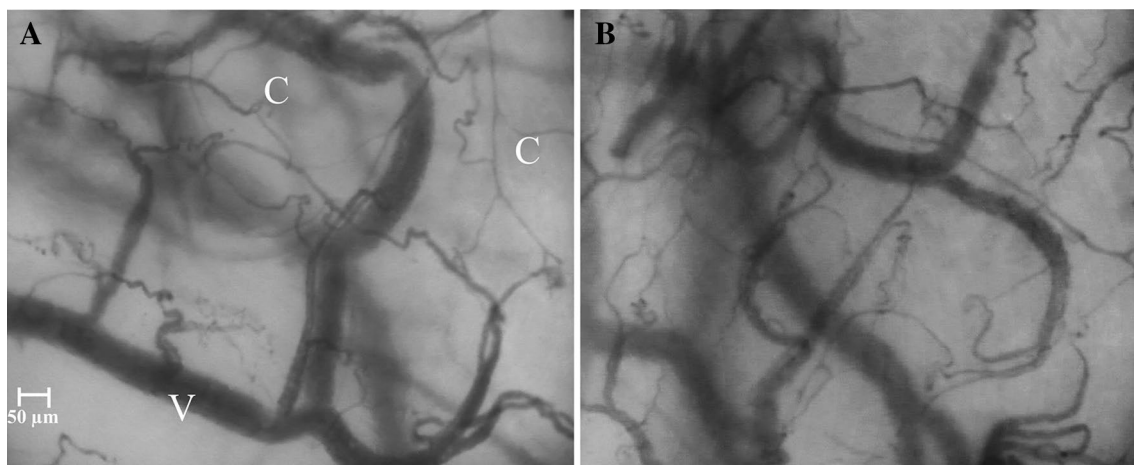


Fig. 2 Digital microphotographs of microcirculation before (a) and immediately after (b) heating. C capillaries, V venules

muscle blood flow measured via duplex Doppler ultrasound, has a strong relationship with increases in muscle tissue temperature. They observed increases in plasma noradrenaline by contrast with normal plasma adrenaline values and suggested that the increase in plasma noradrenaline was largely caused by a spillover of noradrenaline into the circulation from sympathetic nerve terminals. These data support our results, which showed increased noradrenaline and normal adrenaline concentrations in the blood. We also observed significant increases in prolactin levels, as a marker of hyperthermia. Previous studies showed that exercise-induced and passive hyperthermia-induced identical core temperatures yielded similar prolactin responses despite the different cardio-vascular responses to these two types of body heating (Low et al. 2005).

Previous studies showed that muscle sympathetic nerve activity is elevated with exposure to heat stress (Keller et al. 2010; Niimi et al. 1997). The increases in skeletal muscle blood flow during heat stress increased muscle sympathetic vasoconstrictor activity, which indicated the involvement of vasodilators known as functional sympatholytics, such as ATP (Pearson et al. 2011) and perhaps NO (Thomas and Victor 1998). These are capable of overriding the augmented neural vasoconstrictor reflexes on the muscle microvasculature. Interestingly, erythrocyte ATP release is sensitive to physiological increases in temperature independent of oxygenation (Kalsi and Gonzalez-Alonso 2012). Perhaps a similar mechanism overwhelms sympathetic vasoconstriction and acts in sublingual mucosa during heating.

In our study, we observed increased cardiac output, increased heart rate, and reduced mean arterial pressure immediately after heating was ended. These results are consistent with previous studies (Heinonen et al. 2011; Pearson et al. 2011). These results might have been caused by dehydration and a mismatch between increased cardiac output and peripheral vasodilatation during heat stress (Montain and Coyle 1992). Because of dehydration, the subjects in our study lost on average 1.5 kg of body weight during heating. Previous studies demonstrated that significant reductions in mean arterial pressure were observed at each of the 0.5 °C elevations in body temperature during heating in euhydration and hypohydration conditions and support the notion of a mismatch between increased cardiac output and peripheral vasodilatation (Fan et al. 2008).

We found that heat stress significantly increased VO_2 . Some studies demonstrated that passive body heating at rest has no impact on VO_2 (Cabanac and White 1995). However, other study showed significant increase of VO_2 with longer heating time to higher core target temperature (Fan et al. 2008). Causes of VO_2 increase during heat stress while subjects are at rest are not clear and may be associated with increased metabolic demand due to greater

thermal load which thereby altering greater hyperpnea and causes more intensive work of respiratory muscles. Moreover, we found a significant relationship between the change of FCD and change of VO_2 during heating. Previous studies showed an increase in both skeletal muscle capillary supply and peak oxygen consumption after exercise. Only some of these studies suggested a significant relationship between capillarity and peak VO_2 (Hepple et al. 1997), which might be because systemic hemodynamics are more responsible for VO_2 than is skeletal muscle. Although there is a significant correlation between whole body VO_2 and sublingual microcirculation, it does not necessarily imply cause and effect. Without measuring VO_2 at the sublingual mucosa, it is not possible to make this claim.

Our study has some limitations. We observed the microcirculation in the sublingual mucosa, but not in the tissue in deeper layers, such as muscles. This was because of the technical limitations of the SDF imaging method and the easy accessibility of organs with a thin epithelial layer in healthy humans. We did not measure sublingual temperature and VO_2 or evaluate long-term microcirculatory effects after heating. We did not exceed a rectal temperature of 39.5 °C because of ethical considerations. In primates and heat-stroke patients, circulating endotoxin was detected at rectal temperatures of approximately 42 °C and remained elevated despite cooling (Bouchama et al. 1991; Garibaldi 1972). Moreover, splanchnic blood flow showed an initial decrease at 40 °C (Kregel and Moseley 1996). Observation time and achieved temperature levels could be crucial factors in our inability to detect increased cytokine levels and decreased microcirculatory perfusion, as found in sepsis. Baseline hemodynamic variables were measured before the water immersion. Water immersion is known to increase venous return and hence stroke volume should decrease HR (Schmid et al. 2007), but these responses in our study were out of scope and not evaluated.

In conclusion, whole body heat stress increases sublingual FCD in the case of increased systemic oxygen consumption and increased cardiac output. The sublingual mucosa was suitable for detecting changes in capillary density during physiological stress in healthy humans.

Conflict of interest The authors declare that they have no conflicts of interest.

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