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Lydia Tsoutsoubi

*FAME Laboratory, School of Exercise Science, University of Thessaly, Trikala, Greece.,
lydiatsoutsoubi@gmail.com*

Leonidas G. Ioannou

*FAME Laboratory, Department of Exercise Science, University of Thessaly, Trikala, Greece.,
ioannoulg@gmail.com*

Stephen S. Cheung

Department of Kinesiology, Brock University, St. Catharines, Ontario, Canada, scheung@brocku.ca

Andreas D. Flouris

*FAME Laboratory, School of Exercise Science, University of Thessaly, Trikala, Greece.,
andreasflouris@gmail.com*

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Influence of Core Temperature Changes During Whole-Body Warming and Cooling on Cutaneous Vascular Reactivity

Lydia Tsoutsoubi¹, Leonidas G. Ioannou¹, Stephen S. Cheung², and Andreas D. Flouris¹

¹FAME Laboratory, Department of Physical Education and Sport Science, University of Thessaly, Trikala, Greece

²Department of Kinesiology, Brock University, St. Catharines, Ontario, Canada

Abstract

Objective: Endothelial function, the ability of cells of the vascular endothelial wall to secrete compounds, is linked with metabolic and cardiovascular disease risks. One of the most well-known noninvasive tests used to assess skin vascular reactivity as a measure of endothelial function is the reactive hyperemic response test (RHRT). However, there is lack of consensus regarding the impact of thermoregulation on endothelial (dys)function and the results from the RHRT. Thus, the aim of the present study was to investigate the impact of core temperature on cutaneous vascular reactivity, as assessed via the finger RHRT. **Approach:** Following a 15-minute baseline period, seven adults entered a water tank maintained at 42°C and passively rested in a semi-supine position. Thereafter, they entered a water tank maintained at 12°C. They were immersed until their rectal temperature (T_{re}) increased or decreased about 0.5°C above and below the baseline T_{re} respectively. This procedure was repeated twice and an occlusion was conducted during the baseline period and at the second repetition of water immersions. **Main results:** During the post-occlusion phase, skin blood flow (SkBF) was greater, comparing to pre- and occlusion phases, across all T_{re} levels (five levels: baseline, mild hyperthermia 1, mild hypothermia 1, mild hyperthermia 2, mild hypothermia 2). Also, SkBF throughout pre-occlusion, occlusion, and post-occlusion was greater during mild hyperthermia 2. **Significance:** We found a significant impact of core temperature on SkBF and cutaneous vascular reactivity which affects the diagnostic indicators obtained from the RHRT and can impact the final outcome.

Keywords: cutaneous circulation, reactive hyperemia, endothelial function, rectal temperature, skin blood flow, water immersion

Introduction

Endothelial function, the ability of cells of the vascular endothelial wall to secrete compounds, is linked with metabolic (Minson, 2010) as well as ischemic cardiovascular (hypertension, coronary artery disease, chronic heart failure) disease risk (Hasdai et al., 1997; Rubinshtein et al., 2010; Zeiher et al., 1995). Also, coronary endothelial dysfunction is related to ischemic cardiovascular outcome and stroke (Halcox et al., 2002; Rubinshtein et al., 2010; Schächinger et al., 2000; Suwaidi et al., 2000; Targonski et al., 2003). Despite its important prognostic value, coronary artery endothelial (dys)function is relatively difficult to evaluate using a gold standard method such as the intracoronary acetylcholine challenge and, most often, it is assessed as a measure of vascular reactivity at the cutaneous circulation (Holowatz et al., 2008; Khan et al., 2008; Minson, 2010; Rubinshtein et al., 2010). Changes in the cutaneous vascular reactivity (i.e., measured at the skin) often occur prior to the emergence of clinical symptoms of coronary artery endothelial dysfunction (Ijzerman et al., 2003; Khan et al., 2000, 2005, 2008), supporting its prognostic and diagnostic value.

One of the most well-known noninvasive tests used to assess skin vascular reactivity as a measure of endothelial function is the reactive hyperemic response test (RHRT) (Bonetti et al., 2004; Komura et al., 2016) which evaluates the transient increase in blood flow at the skin surface that occurs following a period of arterial occlusion (Lorenzo & Minson, 2007; Skrha et al., 2001). The RHRT is the most reproducible test of endothelial function with a day-to-day coefficient of variation of 19.4% when performed in a thermoneutral environment (Agarwal et al., 2010). When used in clinical practice, the RHRT generates diagnostic indicators that can detect angiogram-positive disease with high accuracy (Hummel et al., 1978; Ouriel et al., 1982) and there is a strong correlation between reactive and post-exercise (walk for five minutes, or until forced to stop due to claudication) hyperemia (Hummel et al., 1978).

Given the diagnostic value of the RHRT, particularly during exposure to extreme environments, it is important to explore the effect of body temperature on its accuracy. This consideration stems from the abundance of temperature-sensitive transient receptor potential ion channels in cells of the vascular endothelial wall (Earley et al., 2007; Flouris & Schlader, 2015) suggesting that core temperature may influence the results of the RHRT. This hypothesis has been confirmed in adult

pigs, showing that mild hypothermia (33–35°C) diminishes the hyperemic response recorded after a period of arterial occlusion (Olivecrona et al., 2007). In humans, a large epidemiological comparison using the Framingham Offspring Cohort data demonstrated that the hyperemic response during the RHRT was augmented at higher outdoor and room temperatures (Widlansky et al., 2007). Despite measuring a large sample of 1973 participants, the design of the aforementioned study was limited due to substantial age differences in the participants tested at different seasons as well as the fact that environmental temperature data were collected from a local weather station (Widlansky et al., 2007). Another human study with 274 participants reported opposing results, showing that higher outdoor temperatures in the surrounding region (assessed via weather-station data) were associated with attenuated reactive hyperemic responses (Nawrot et al., 2005). These conflicting results highlight the lack of consensus regarding the impact of thermoregulation on endothelial (dys)function and the results from the RHRT.

To address the abovementioned lack of consensus, we investigated the impact of core temperature change on cutaneous vascular reactivity, as assessed via the finger RHRT. To provide a potent thermal stimulus, we sequentially immersed participants in hot (42°C) and cold (12°C) water while their arms and hands remained outside the water tank, in a thermoneutral environment (25°C). This procedure was conducted twice (hot–cold–hot–cold). The first hot–cold set of immersions aimed at exploring the impact of core temperature on skin blood flow (SkBF), while the second hot–cold set of immersions aimed at assessing the impact of core temperature on the RHRT.

Materials and Methods

Participants and Ethics

Seven nonsmoking adults (two males; five females; age: 25.5 ± 6.1 years; body mass index: 22.7 ± 1.6 kg/m²; body fat: $15.8 \pm 6.7\%$; body surface area: 1.8 ± 0.1 m²) were recruited from a university and local communities via posters and word-of-mouth. Participants were screened for Raynaud's phenomenon and they were not under any prescription medication such as antidepressants, diuretics, antihypertensives, or antihistamines during the past year. Female participants were tested during the early follicular phase (days 1–6) of their menstrual cycle. The experimental protocol conformed to the standards set by the Declaration of Helsinki and was approved by the appropriate ethical review board. Participants were given a full explanation of all the procedures and signed a written informed consent.

Power analysis was conducted and the minimum required sample size was determined using data from a recent study (Hansen et al., 2017) that investigated changes

in reactive hyperemia index (RHI; 0.46 ± 0.28) and the natural logarithm of RHI (LnRHI; 0.23 ± 0.13) during the same day (i.e., two RHRTs separated by 1.5 h) collected from 20 healthy participants. Sample size calculations were conducted using G*Power 3.0 (Faul et al., 2007). The “Differences between two dependent means” method was used to calculate the power of the between-effects for two measurements (i.e., two RHRTs). Statistical power and α error probability were set to 0.95 and 0.05, respectively. The minimum required sample size was determined by calculating the effect size “dz.” The aforementioned published data (Hansen et al., 2017) demonstrated effect sizes of 1.64 (for RHI) and 1.77 (for LnRHI). Based on these results (i.e., RHI and LnRHI), the minimum required sample size for our study was six participants. Thus, seven volunteers were recruited to participate in this study.

Experimental Protocol

The study included a familiarization session and an experimental session conducted at least three days apart, to ensure that the participants were provided with enough time to consider all potential risks and discomforts. During the familiarization session, participants were exposed to all data collection procedures and underwent anthropometric measurements. The experimental session took place in a thermoneutral environment (air temperature: 25°C; relative humidity: 40%). Upon arrival, participants dressed down to a bathing suit (males wore a one-piece swimming suit; females opted for a one- or two-piece swimming suit). Thereafter, instrumentation took place. Once all sensors were applied, participants were requested to relax seated in a semi-supine position for 15 minutes to ensure that blood flow and arterial pressure were adjusted to body posture and room temperature. During this baseline period, participants' upper body was partly covered with a thin blanket to minimize heat loss due to lack of clothing and, therefore, to avoid a possible reduction in body temperature prior to the immersions.

After the baseline period, participants entered a water tank maintained at 42°C water temperature until their rectal temperature (T_{re}) increased by 0.5°C above baseline. Thereafter, they entered a water tank maintained at 12°C until their T_{re} decreased by 0.5°C below baseline. This procedure was conducted twice (hot–cold–hot–cold) and the average \pm standard deviation (SD) duration of each immersion was 10.3 ± 3.7 , 31.3 ± 10.8 , 39.4 ± 4.8 , and 28.2 ± 8.3 minutes, respectively, yet no specific immersion duration was set, as the goal was to reach a specific level of T_{re} change. During water immersions, participants remained relaxed in a semi-supine position and were immersed up to the clavicular level; their hands were supported at the level of the heart and were not immersed at any time. Arterial blood pressure was recorded every 10 minutes throughout the protocol via manual auscultation.

tion, and the collected data were linearly extrapolated to generate 1-minute values. Participants were required to abstain from caffeine and alcohol for 12 hours before the experimental session. Also, they were instructed to consume a light breakfast and ≥ 0.5 L of water within 60 minutes prior to their arrival to ensure that they would start the measurements under a hydrated and satiated state. During the experiment, they were allowed to consume *ad libitum* water to prevent dehydration. Furthermore, they were advised to not exercise and avoid exposure to extreme environmental conditions at least 48 hours before the experimental session and especially between awakening and arriving in the laboratory to avoid possible interference with body temperature and circulatory function.

Reactive Hyperemic Response Test (RHRT)

The first hot–cold set of immersions aimed at exploring the impact of T_{re} fluctuations on SkBF, while the second hot–cold set of immersions aimed at assessing the impact of T_{re} fluctuations on the RHRT. As such, a RHRT at the right index finger was conducted during the end of the baseline period, the end of the second hot water immersion, and the end of the second cold water immersion. The RHRT included three 5-minute phases and was conducted based on previous guidelines (Faizi et al., 2009) as follows: (i) the pre-occlusion phase, where no manipulation was applied; (ii) the occlusion phase, where a standard blood pressure cuff was inflated to 200 mmHg to fully occlude the brachial artery; and (iii) the post-occlusion phase, where the cuff was released instantaneously to induce a hyperemic reaction.

Laser Doppler Flowmetry

The SkBF was measured with a laser Doppler flowmeter (LDF; Perimed, Stockholm, Sweden) at the pulp of the right-hand index finger. The probe (PR 407 small straight probe, Perimed) was held in place with a plastic mini holder (diameter: 5 mm; PH 07-5, Perimed) which was fixed to the skin using double-sided adhesive strips (PF 105-3, Perimed) without constricting the finger. SkBF was expressed in perfusion units (PU) and was sampled at 32 Hz which was, subsequently, averaged every 1 second. These 1-second SkBF data were used to calculate the reactive hyperemia ratio (RHR) for each 5-minute phase (pre-occlusion, occlusion, and post occlusion phase) of the RHRT as follows:

$$\text{RHR} = \frac{\text{SkBF in the post occlusion phase}}{\text{SkBF in the pre occlusion phase}}$$

In addition, the natural logarithm of RHR (LnRHR) was also calculated. Finally, cutaneous vascular conductance

(CVC) was calculated every minute as the ratio of LDF to the mean arterial pressure (in PU/mmHg).

Finger Plethysmography

During the RHRT performed at baseline and the second hot water immersion, endothelium-mediated changes in the digital pulse waveform were measured on the fourth (ring) finger of each hand using an Endo-PAT 2000 (Itamar Medical, Israel) according to previous guidelines (Axtell et al., 2010; Itamar Medical, 2015). The Endo-PAT uses plethysmographic probes to automatically compute the RHI (RHI = [SkBF in the post-occlusion phase]/[SkBF in the pre-occlusion phase]). RHI was calculated as a beat-to-beat arterial pulse wave signal, relative to the same ratio in the control arm, corrected for baseline vascular tone of the occluded arm (Axtell et al., 2010; Itamar Medical, 2015). LnRHI was also calculated by the Endo-PAT system. Endothelial dysfunction was defined as RHI ≤ 1.67 or LnRHI ≤ 0.51 (Itamar Medical, 2015).

Core and Finger Temperatures

T_{re} was measured with a flexible probe (2 mm in diameter; Mallinkrodt Medical, St. Louis, MO, USA) self-inserted 15 cm beyond the anal sphincter. T_{re} was recorded at 8-second intervals (Smartreader 8 Plus, ACR, Vancouver, Canada) and was used to calculate 1-minute averages. Finger temperature was measured using ceramic chip skin thermistors (MA-100, Thermometrics) attached with surgical tape (3M Canada) to the pad's lower part (below the Doppler probe mini holder) on the second (index) and fourth (ring) fingers of each hand. Mean finger temperature (T_f) was calculated for each hand by averaging the temperature of index and ring finger ($T_f = [\text{index} + \text{ring}]/2$).

Statistical Analysis

To investigate the impact of T_{re} on cutaneous vascular reactivity, we used univariate analysis of variance with *post hoc t*-tests incorporating Bonferroni adjustment to detect the impact of RHRT phase (three levels: pre-occlusion, occlusion, post-occlusion) and T_{re} level (five levels: baseline, mild hyperthermia 1, mild hypothermia 1, mild hyperthermia 2, mild hypothermia 2) on SkBF. To investigate the impact of T_{re} on cutaneous vascular reactivity as assessed via the diagnostic indices from LDF (RHR and LnRHR) and Endo-PAT (RHI and LnRHI), we used paired-sample *t*-tests, effect sizes, 95% limits of agreement, as well as percentage coefficients of variation to assess differences in RHR, LnRHR, RHI, and LnRHI between baseline and mild hyperthermia 2. To investigate the impact of T_{re} on cutaneous vascular reactivity, we used multivariate analysis of variance with *post hoc t*-tests

incorporating Bonferroni adjustment (to reduce the probability of type I error) to detect the impact of RHRT phase (three levels: pre-occlusion, occlusion, post-occlusion) and T_{re} level (five levels: baseline, mild hyperthermia 1, mild hypothermia 1, mild hyperthermia 2, mild hypothermia 2) on T_f of the occluded and the non-occluded arm. The level of significance for all analyses was set at $p \leq 0.05$ except for *post hoc* tests where Bonferroni adjustment was applied. Statistical analyses were conducted using SPSS 25.0 for Windows (IBM, Armonk, NY, USA).

Results

The SkBF was greater during the post-occlusion phase as compared to the pre-occlusion and occlusion phases, across all T_{re} levels (main effect of RHRT phase; $F_{(2, 27385)} = 3625.8$; $p < 0.001$; Table 1). Also, the SkBF across the RHRT phases (pre-occlusion, occlusion, post-occlusion) was greater during mild hyperthermia 2 (main effect of T_{re} level; $F_{(4, 27385)} = 11032.5$; $p < 0.001$; Table 1). Importantly, the changes in SkBF during the RHRT phases were greater during mild hypothermia (45% reduction during occlusion and 106% increase post-occlusion compared to pre-occlusion values) compared to baseline (94% reduction during occlusion and 24% increase post-occlusion compared to pre-occlusion values) and mild hyperthermia (95% reduction during occlusion and 6% increase post-occlusion compared to pre-occlusion values) (interaction effect of RHRT \times T_{re} ; $F_{(8, 27385)} = 2019.5$; $p < 0.001$). This interaction was confirmed via *post hoc* *t*-tests and effect size calculations showing that SkBF was significantly different across the RHRT phases and T_{re} levels (Figure 1).

During mild hyperthermia 1 and 2, the T_f of the non-occluded (main effect of T_{re} level; $F_{(4, 361)} = 379.95$; $p < 0.001$; Table 2) and the occluded (main effect of T_{re} level; $F_{(4, 361)} = 511.74$; $p < 0.001$; Table 2) arm was increased compared to baseline and mild hypothermia 1 and 2. No main effect of RHRT on T_f was demonstrated ($p > 0.05$). Further analysis of T_f during the three RHRT phases for the non-occluded arm demonstrated large effect size (≥ 0.8) for all the T_{re} levels except the baseline. The effect size for the occluded arm was large (≥ 0.8) for mild hyperthermia 1, mild hyperthermia 2, and mild hypothermia 2, and medium (0.5–0.8) for mild hypothermia 1.

Table 1
Skin blood flow (PU) across different T_{re} levels at each RHRT phase.

T_{re} level	Pre-occlusion phase	Occlusion phase	Post-occlusion phase
Baseline	158.6 \pm 133.9	9.4 \pm 8.7	197.1 \pm 143.9
Mild hyperthermia1	220.2 \pm 146.7	375.3 \pm 149.3	356.8 \pm 167.3
Mild hypothermia 1	27.6 \pm 12.5	30.1 \pm 14.0	34.8 \pm 24.9
Mild hyperthermia 2	407.6 \pm 106.8	19.7 \pm 22.0	376.5 \pm 83.7
Mild hypothermia 2	19.2 \pm 9.11	11.4 \pm 5.8	39.4 \pm 24.7

Note. Values are expressed as mean \pm SD.

Comparisons in the diagnostic indices from Endo-PAT and LDF between baseline and mild hyperthermia 2 demonstrated significant differences only for RHR and LnRHR (data recorded via LDF; $p < 0.005$). Further analysis demonstrated large effect size (≥ 0.8) between baseline and mild hyperthermia 2 for RHR, LnRHR, and LnRHI as well as medium effect size (0.5–0.8) for RHI (Figure 2). Also, we found a medium effect size (0.5–0.8) of the differences between baseline and mild hypothermia 2 for RHR and LnRHR. The calculated 95% limits of agreement for the differences between baseline and mild hyperthermia 2 were as follows: RHR, 0.8 ± 1.1 ; LnRHR, 0.5 ± 0.5 ; RHI, 0.4 ± 2.1 ; LnRHI, 0.3 ± 1.1 . The respective percentage coefficients of variation were as follows: RHR, 35.8%; LnRHR, 76.86%; RHI, 64.3%; LnRHI, 128.6%. The 95% limits of agreement for the differences between baseline and mild hypothermia 2 were 0.7 ± 0.7 (RHR) and 0.3 ± 0.3 (LnRHR), while the respective percentage coefficients of variation were 34.1% (RHR) and 42.9% (LnRHR).

As anticipated, CVC was significantly increased during mild hyperthermia 1 and 2, and attenuated during mild hypothermia 1 and 2 compared to baseline (main effect of T_{re} level; $F_{(4, 416)} = 135.0$; $p < 0.001$; Figure 3). Naturally, CVC was significantly decreased during occlusion (main effect of T_{re} level; $F_{(2, 416)} = 52.52$; $p < 0.001$; Figure 3) and increased during post-occlusion, as compared to pre-occlusion (Figure 3).

Discussion

The present study shows that there is a significant impact of core temperature on SkBF and cutaneous vascular reactivity, as assessed via the finger RHRT by both LDF and Endo-PAT. Specifically, we found that (i) SkBF adaptations during the RHRT phases depend on T_{re} , (ii) the reactive hyperemic response is augmented during mild hypothermia, and (iii) the diagnostic indicators obtained from the RHRT vary widely based on the level of T_{re} .

The existing literature presents conflicting results, reporting that reactive hyperemia during the RHRT can be augmented (Widlansky et al., 2007) or attenuated (Nawrot et al., 2005) during exposure to higher outdoor temperature (assessed via weather-station data). Thus, the impact of finger blood flow thermal adaptations on the

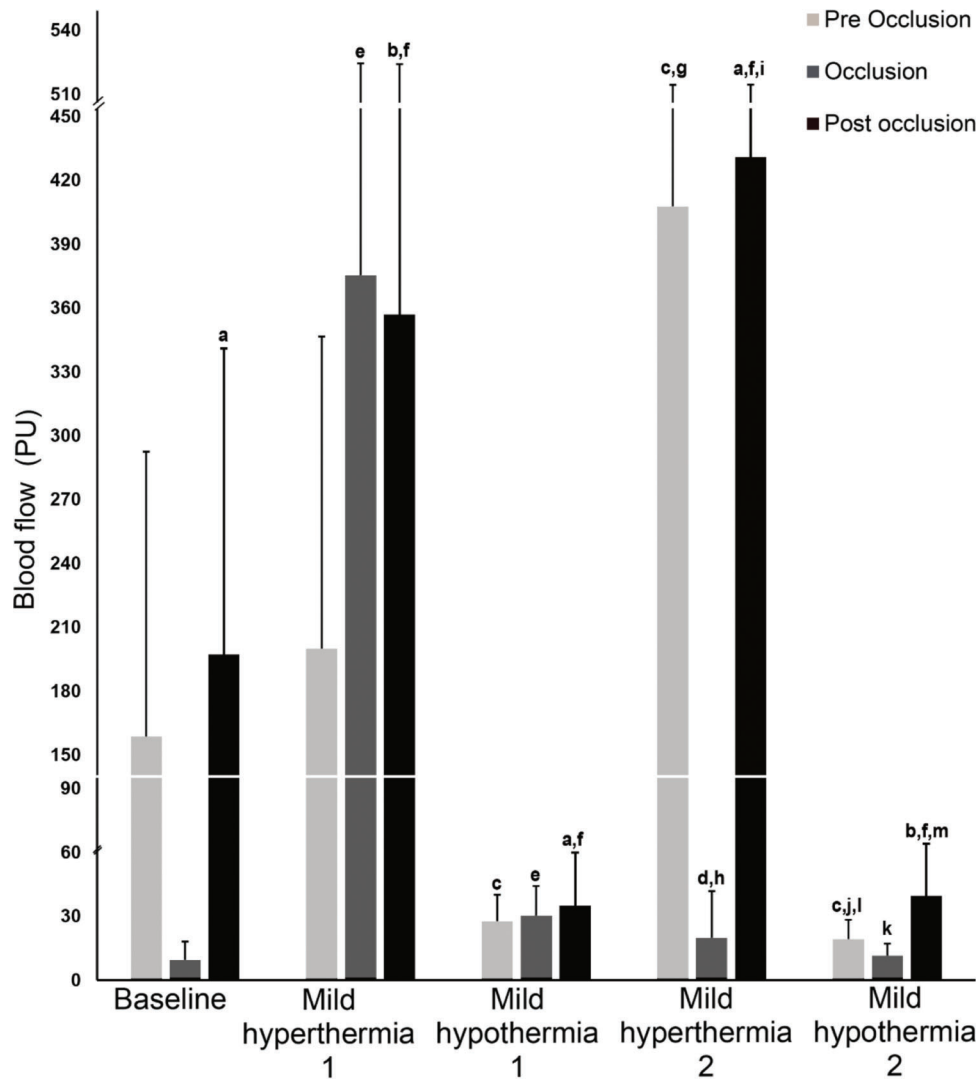


Figure 1. Comparison of skin blood flow (mean \pm SD) as expressed in PU (average of 1-second values for all participants) during the five different rectal temperature levels (i.e., baseline, mild hyperthermia 1, mild hypothermia 1, mild hyperthermia 2, mild hypothermia 2) and during the three different RHRT phases (i.e., pre-occlusion, occlusion, post-occlusion). Note: a, statistically significant differences and small effect sizes between pre-occlusion and post-occlusion phases; b, statistically significant differences and large effect sizes between pre-occlusion and post-occlusion phases; c, statistically significant differences and large effect sizes against baseline for the pre-occlusion phase; d, statistically significant differences and medium effect sizes against baseline for the occlusion phase; e, statistically significant differences and large effect sizes against baseline for the occlusion phase; f, statistically significant differences and large effect sizes against baseline for the post-occlusion phase; g, statistically significant differences and large effect sizes between hyperthermia 1 and hyperthermia 2 for the pre-occlusion phase; h, statistically significant differences and large effect sizes between hyperthermia 1 and hyperthermia 2 for the occlusion phase; i, statistically significant differences and medium effect sizes between hyperthermia 1 and hyperthermia 2 for the post-occlusion phase; j, statistically significant differences and medium effect sizes between hypothermia 1 and hypothermia 2 for the pre-occlusion phase; k, statistically significant differences and large effect sizes between hypothermia 1 and hypothermia 2 for the occlusion phase; l, statistically significant differences and large effect sizes between hyperthermia 2 and hypothermia 2 for the pre-occlusion phase; m, statistically significant differences and large effect sizes between hyperthermia 2 and hypothermia 2 for the post-occlusion phase.

ability of plethysmography to obtain an arterial pulse wave remained previously unknown. Our results help solve this controversy, revealing that the reactive hyperemic response is markedly attenuated during mild hyperthermia of 0.5°C. This is logical to anticipate as increases in blood flow would be limited during mild hyperthermia given the peripheral vasodilation at the baseline phase of the RHRT due to higher body temperature. However, during normothermia or hypothermia, marked increase in blood flow relative to pre-occlusion phase would be expected. In

line with previous studies (Simmons et al., 2011), we found that cutaneous vascular conductance is significantly increased during mild hyperthermia (compared to mild hypothermia). Moreover, we extend the available knowledge by showing that CVC is increased post-occlusion (compared to pre-occlusion).

Our findings demonstrate that mild hyperthermia is associated with blunted cutaneous vascular reactivity. The latter has been previously linked with a higher incidence of cardiac death, myocardial infarction, as well as cardiac

Table 2
Finger temperature (°C) across different T_{re} levels at each RHRT phase.

T_{re} level	Pre-occlusion phase		Occlusion phase		Post-occlusion phase	
	OA	Non-OA	OA	Non-OA	OA	Non-OA
Baseline	31.3 ± 3.0	31.6 ± 3.2	30.1 ± 3.0	31.4 ± 3.2	30.8 ± 2.8	31.4 ± 3.2
Mild hyperthermia 1	30.6 ± 2.9	30.6 ± 3.1	31.9 ± 2.4	31.7 ± 2.4	33.1 ± 1.3	32.8 ± 1.3
Mild hypothermia 1	27.9 ± 2.0	27.5 ± 3.0	27.0 ± 1.8	26.7 ± 2.7	26.2 ± 1.6	26.0 ± 2.4
Mild hyperthermia 2	30.5 ± 1.9	30.5 ± 1.7	32.3 ± 1.5	33.3 ± 1.4	32.3 ± 1.8	34.8 ± 0.9
Mild hypothermia 2	29.7 ± 2.1	30.0 ± 1.9	28.3 ± 1.4	28.6 ± 1.3	27.3 ± 1.0	28.6 ± 0.9

Note. Values are expressed as mean ± SD. OA, occluded arm; non-OA, non-occluded arm.

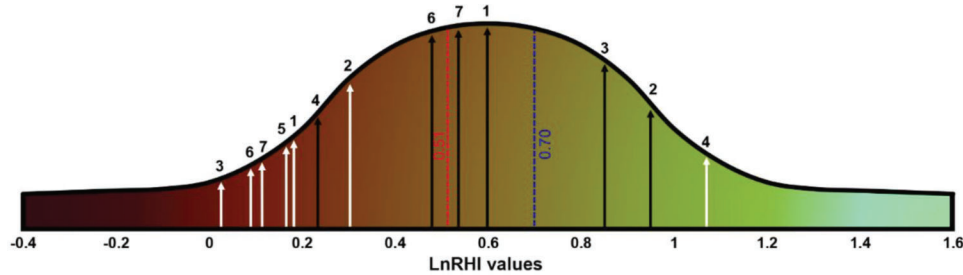


Figure 2. The distribution function of LnRHI in the nonselective population, as typically illustrated in the Endo-PAT report. Lines on the normal distribution curve represent the study participants. Black lines indicate results at the baseline rectal temperature level. White lines indicate results at the mild hyperthermic rectal temperature level. The LnRHI in the population (mean: 0.70 marked in blue) and the dichotomy threshold for normal endothelial function (median: 0.51 marked in red) are shown as vertical lines.

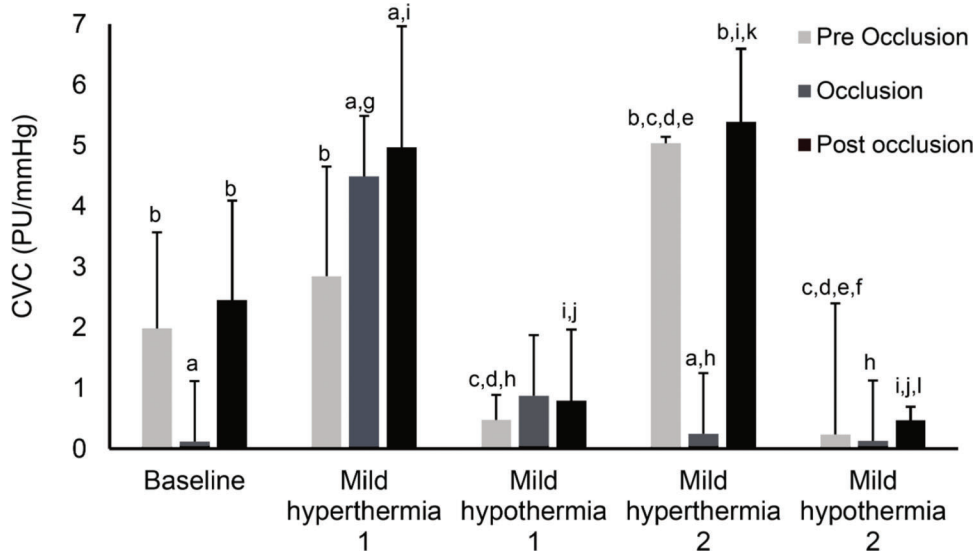


Figure 3. Comparison of CVC (mean ± SD) as expressed in PU/mmHg during the five different rectal temperature levels and during the three different RHRT phases. Note: a, statistically significant differences with pre-occlusion phase; b, statistically significant differences with occlusion phase; c, statistically significant differences for the pre-occlusion phase compared to baseline; d, statistically significant differences for the pre-occlusion phase compared to mild hyperthermia 1; e, statistically significant differences for the pre-occlusion phase compared to mild hypothermia 1; f, statistically significant differences for the pre-occlusion phase compared to mild hyperthermia 2; g, statistically significant differences for the occlusion phase compared to baseline; h, statistically significant differences for the occlusion phase compared to mild hyperthermia 1; i, statistically significant differences for the post-occlusion phase compared to baseline; j, statistically significant differences for the post-occlusion phase compared to mild hyperthermia 1; k, statistically significant differences for the post-occlusion phase compared to mild hypothermia 1; l, statistically significant differences for the post-occlusion phase compared to mild hypothermia 2.

hospitalization (Rubinshtein et al., 2010). Furthermore, a review by Minson (2010) concluded that endothelial dysfunction may be an early marker of atherosclerosis,

while microvascular dysfunction is associated with hypertension, coronary artery disease, and chronic heart failure. Taken together with our findings, these results suggest that

hyperthermia—caused either by extreme heat exposure or by heavy exercise in warm/hot environment—may contribute to a risk for cardiovascular instability. Interestingly, the same finding has been reported by research investigating changes in heart rate variability during extreme heat exposure (Flouris et al., 2014a, 2019) as well as the changes in cardiovascular responses during the induction and decay of heat acclimation (Flouris et al., 2014b).

The impact of heat balance on the RHRT also affects the diagnostic indicators obtained from the test, assessed either via Endo-PAT or via LDF. Specifically, the calculated percentage coefficients of variation ranged from 36% to 129%, suggesting that an RHI index of 0.6 recorded during a RHRT at normal core temperature would be estimated—in the worst-case scenario—as low as 0.1 or as high as 1.1 at mild hyperthermia ($RHI \leq 1.67$ indicates endothelial dysfunction) (Itamar Medical, 2015). Similarly, a LnRHI index of 0.6 recorded during a RHRT at normal core temperature would be estimated—in the worst-case scenario—as low as 0 or as high as 1.4 at mild hyperthermia ($LnRHI \leq 0.51$ indicates endothelial dysfunction). These worst-case estimation limits are significant and can have a major impact in the final outcome and diagnostic accuracy of the RHRT.

As mentioned above, the RHRT is the most reproducible test of endothelial function with a 19.4% day-to-day coefficient of variation when performed in a thermoneutral environment. It is important to note, however, that our data may reflect an impact of repeated iterations of occlusion and reperfusion in the same experimental session. This notion is supported by a recent study (Hansen et al., 2017) that performed a same-day (two RHRTs separated by 1.5 h) and a day-to-day (two RHRTs separated by 24 h) evaluation of endothelial (dys)function via the RHRT. The study authors reported an increase of 15.2% in RHI and 13.0% in LnRHI between the same-day ($RHI: 0.46 \pm 0.28$; $LnRHI: 0.23 \pm 0.13$) and the day-to-day ($RHI: 0.39 \pm 0.30$; $LnRHI: 0.20 \pm 0.15$) measurements.

Conclusion

We found a significant impact of core temperature on SkBF and cutaneous vascular reactivity, as assessed via the finger RHRT. The diagnostic indicators obtained from the RHRT vary by 36–129% based on the level of heat balance and can impact the final outcome of the test. Therefore, clinical tests for SkBF and endothelial function should be performed in a thermoneutral environment, and participants should be observed as not exhibiting any thermoregulatory issues or distress. In addition, our results suggest that hyperthermia—caused either by extreme heat exposure or by heavy exercise in warm/hot environment—may increase the risk for cardiovascular instability. On the whole, the take-home message for practitioners is that the examination for endothelial function must be performed under normal

temperature conditions and the patient's body temperature should be at normal levels (36.5–37.5°C) to obtain accurate results.

Limitations

It is important to note that our study evaluates the endothelial function and SkBF at the pulp of the fingers; thus these results may not be applied at the nonglabrous skin. Also, our results would be strengthened by measuring a larger group of participants and by continuous blood pressure recordings instead of extrapolated values based on measurements taken every 10 minutes. Finally, the long duration of the experimental protocol did not allow us to record Endo-PAT measurements during the second cold water immersion due to data overflow. Future studies could use a noninvasive automated device to continuously measure arterial blood pressure in the finger without affecting the skin blood flow by constricting the forearm.

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