

# Augmentation of Brachial Reactive Hyperemia by Local Forearm Heating: A plethysmography-Based Comparison with the Standard Room-Temperature Protocol

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## Abstract

**Introduction:** Reactive hyperemia (RH) is widely used to assess microvascular endothelial function, but its magnitude may be limited when performed on a normally perfused, non-heated forearm. Local skin heating to 43.5°C produces sustained nitric oxide-dependent vasodilation and may augment the RH response.

**Material and methods:** Twenty healthy adult males (non-smokers, medication-free) underwent standard RH induced by 5-min brachial artery occlusion followed by 3 min reperfusion. ECG and bilateral fingertip plethysmography digital pulse waves were recorded to derive pulse transit time (PTT) and finger-to-finger pulse-arrival-time difference (f-f  $\Delta T$ ). In 11 of these participants, the forearm was then heated with an electric heat pad to  $43.5 \pm 0.4^\circ\text{C}$  for 25–30 min, and RH was repeated. Indices were calculated from 60–80 cardiac cycles during the first two minutes of reperfusion.

**Results:** At room temperature, RH increased PTT by about 5%–6% and markedly increased f-f  $\Delta T$ , consistent with improved distal perfusion. When RH was repeated after local heating, the reactive hyperemia index was significantly higher (about 1.5-fold,  $p < 0.007$ ), indicating that prior heating enhanced the hyperemic response. In contrast, heated RH did not produce further increases in PTT or f-f  $\Delta T$  compared with RH at room temperature, suggesting dissociation between enhanced microvascular recruitment and conduit-level stiffness changes.

**Conclusions:** Performing RH after local forearm heating at 43.5°C amplifies plethysmography-derived hyperemia indices compared with the conventional room-temperature test, mainly by enhancing the endothelial and microvascular component rather than further reducing arterial stiffness. Thermal priming may improve the sensitivity of plethysmography-based endothelial assessment in physiological studies.

**Keywords:** microvascular endothelial function; photoplethysmography; pulse transit time; reactive hyperemia

## Introduction

RH following a brief arterial occlusion is a classical, non-invasive maneuver used to assess microvascular endothelial function because it creates a transient, high-shear environment distal to the occlusion, leading to nitric-oxide (NO)-mediated vasodilation and a short-lived increase in flow<sup>1,2</sup>. In clinical laboratories, this response is often quantified by ultrasound flow-mediated dilation (FMD) of the brachial artery or by photoplethysmography (PPG)-derived timing and amplitude indices that track the arrival of the pulse wave in the digits<sup>3</sup>. PPG-based indices are attractive in situations where ultrasound is unavailable, because they are inexpensive, easy to repeat, and sensitive to both vasodilation and changes in arterial tone<sup>4,5</sup>. However, when RH is performed on a ther-

monneutral forearm, increases in pulse transit time are modest (~6%) and decreases in peripheral PWV are similarly modest (~8%), even in young, healthy adults<sup>6,7</sup>. This limited response may be explained by the fact that, under thermoneutral conditions, cutaneous blood flow is primarily maintained by sympathetic vasoconstrictor activity, resulting in relatively low vascular conductance and a restricted ability of the microcirculation to dilate after cuff release<sup>8,9</sup>. Moreover, indices dominated by conduit-artery behavior (e.g., PWV) often correlate only weakly with RH or FMD, emphasizing that conduit stiffness and microvascular reactivity are related but partly independent domains<sup>10,11</sup>. Local skin heating of the forearm to ~42–43°C is a well-validated means of inducing a sustained, NO-dependent, high-shear state in the downstream vasculature<sup>12</sup>. After an initial axon-reflex peak, skin blood flow reach-

es a plateau after about 20–30 min, largely explained by endothelial NO release<sup>9</sup>. When local skin heating is combined with an ischemia–reperfusion stimulus, the resulting vasodilatory response is amplified compared with heating alone, reflecting the synergistic activation of endothelial nitric oxide and sensory nerve pathways<sup>13</sup>.

To our knowledge, very few studies have compared PG-derived RH performed in thermoneutral conditions with RH performed on a thermally primed forearm in the same subjects. Most prior studies on cutaneous heating have focused on laser-Doppler or plethysmographic assessments of skin blood flow, without directly reporting finger-to-finger pulse-arrival-time difference ( $f-f \Delta T$ )<sup>14</sup>. Therefore, the aim of this paper is to present a comparison between standard RH at RT and RH after 30 min of local forearm heating at 43.5°C in the same cohort.

Our primary hypothesis was that thermal priming would increase RHI compared with RH at RT. The secondary hypothesis was that the sequence of ischemia–reperfusion followed by local heating can alter the timing and amplitude of the PPG-derived indices ( $f-f \Delta T$  and PTT), reflecting the influence of prior ischemic stress on subsequent microvascular responsiveness<sup>15</sup>. Therefore, this study aimed to compare standard reactive hyperemia with thermally augmented reactive hyperemia using digital plethysmography, and to explore whether local heating improves the clinical usefulness of RH as a simple, non-invasive marker of microvascular endothelial function.

## Participants and Methods

### Study design and ethical approval

This was a single-center, prospective physiological study conducted in the Department of Physiology, College of Medicine, Al-Mustansiriyah University (Baghdad, Iraq). The protocol complied with the Declaration of Helsinki (2013) and was approved by the Departmental Ethics Committee of the College of Medicine. All participants provided informed verbal consent before enrolment.

### Participants

Twenty healthy adult young males were recruited. Inclusion criteria: age  $\geq 18$  years, non-smoker, no cardiovascular, metabolic, or inflammatory disease, no vasoactive or antihypertensive medication, and abstinence from caffeine or strenuous exercise  $\geq 12$  h before testing. Exclusion criteria: fever, forearm skin lesions, intolerance to heating or poor PG signal quality. Anthropometric parameters (height, weight, BMI) and resting brachial artery blood pressure (SBP, DBP, MBP) and heart rate (HR) were assessed after 10 min rest in semi-supine position using an automated device (Rossmax, Switzerland).

### Instrumentation and signals

Participants were instructed to breathe spontaneously and remain silent throughout the examination. Digital pulse wave

(DPW) signals were recorded from the middle fingers of both hands using piezoelectric finger pulse transducers. The electrocardiogram (ECG) signals were simultaneously acquired using Lead II. All signals were sampled at 1 kHz with a 26T data acquisition unit and LabChart Pro 7.2 software (ADInstruments, Australia) was used for recording and offline signals analysis. This arrangement allowed beat-to-beat extraction of morphological and timing indices.

### RH protocol at RT

Participants rested in semi-supine position in a room maintained at 20–25°C. Baseline ECG and DPW were recorded for 2 min. A pneumatic cuff was placed on the left upper arm above the brachial artery, inflated rapidly to 230 mmHg, and maintained for 5 min to ensure complete arterial occlusion. After 5 min, the cuff was released and recordings continued for 3 min. Pulse wave indices were average over 60–80 cardiac cycles ( $\sim 1$  min) after release, when PTT show the greatest deviation from baseline.

### Local forearm heating

After the first RH test, participants rested  $\sim 10$  min. In 11 volunteers (subset of the original 20), an electric heating pad was wrapped around the left (non-dominant) forearm and set to  $43.5 \pm 0.4^\circ\text{C}$  at the pad surface—producing a skin temperature  $\sim 40$ – $41^\circ\text{C}$  (measured by Non-contact infrared thermometer, Unaan Model YNA-800), known to elicit near-maximal NO-dependent vasodilation (**Figure 1**). DPW and ECG were recorded for 1 min every 5 min over 30 min to characterize the active-hyperemia plateau. During the last 5 min of continuous heating at 43.5°C, the standard 5-min brachial artery occlusion followed by 3-min reperfusion was repeated, yielding RH on top of AH (thermally augmented RH).



**Figure 1.** The setup for forearm local heating pad.

### Derived indices

From each cardiac cycle, the following indices were derived:

1. PTT (ms): The interval from the R-wave of the ECG to the first peak of the first derivative of the DPW signal in the test hand<sup>3,4</sup>.

2.  $f-f \Delta T$  : The delay between DPW arrival times in the two middle fingers; larger positive values indicate prolonged wave propagation (an increase in PTT) in the reperfused arm<sup>16</sup>. PTT and  $f-f \Delta T$  were calculated during the 1st min of RH. This is based on the finding that this is the time at which the maximum change in PTT is occurred<sup>6</sup>.
3. Reactive Hyperemia Index (RHI): It is calculated as follows: the ratio of the occluded arm's means DPW amplitude (left middle finger signal) at each min post occlusion (A) to the mean DPW amplitude from baseline readings of the same arm (B). The result is further divided by the same ratio from the control non-occluded arm (C/D) (right middle finger signal), which allows the device to account for systemic vascular changes during testing. RHI was calculated during the 2nd min of RH. This is based on the finding that this is the time at which the maximum change in RH is occurred<sup>6</sup>.

### Statistical analysis

Variables were checked for normality (Shapiro–Wilk). Values are mean  $\pm$  SD. Paired  $t$ -tests were applied to normally distributed variables; otherwise Wilcoxon signed-rank tests were used. For the whole cohort ( $n = 20$ ) descriptive statistics summarized direction and magnitude of change. A  $p$  value  $< 0.05$  was considered significant.

## Results

### Participants

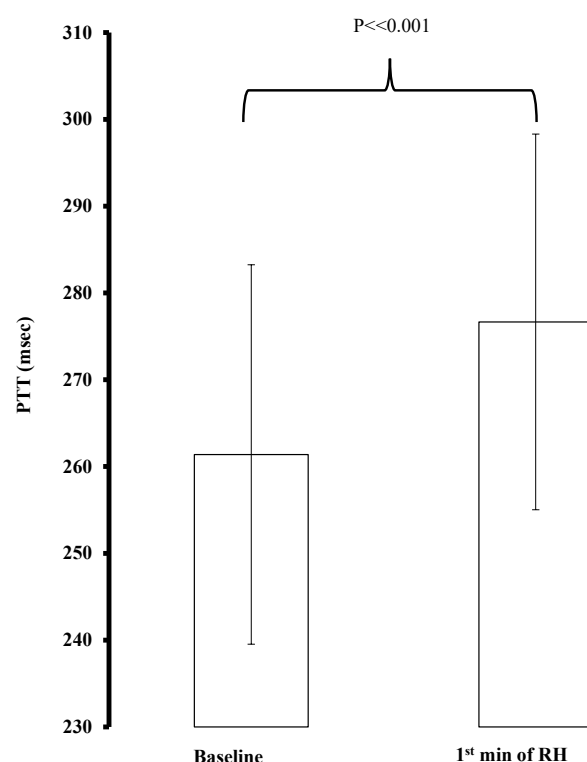
**Table 1** summarizes the participants' anthropometric characteristics. The mean BMI places the cohort in the overweight range.

**Table 1: Anthropometric characteristic of the participants.**  
N = 20.

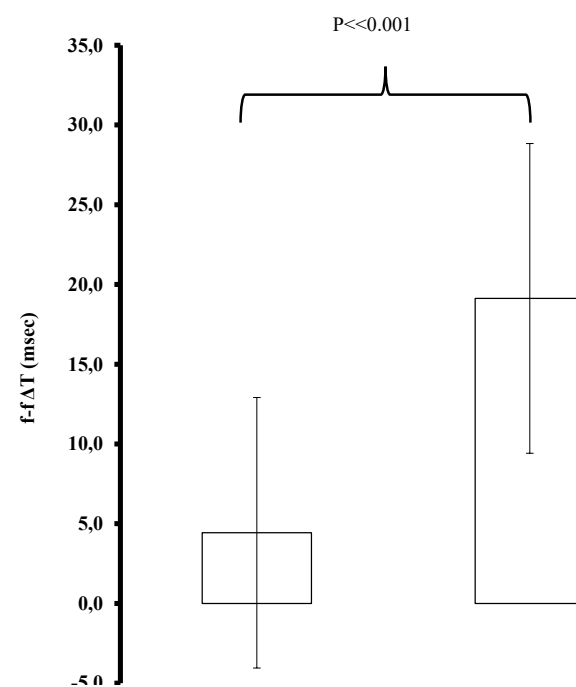
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|--------------------------|-----------------|
| Age (years)              | 24.3 $\pm$ 3.6  |
| BMI (Kg/m <sup>2</sup> ) | 27.1 $\pm$ 5.6  |
| SBP (mm Hg)              | 120.0 $\pm$ 7.7 |
| DBP (mm Hg)              | 72.2 $\pm$ 9.4  |
| MBP (mm Hg)              | 88.1 $\pm$ 7.7  |
| HR (beat/min)            | 69.6 $\pm$ 8.0  |

Twenty healthy adult males completed the RH protocol; eleven also completed the heated-RH protocol. All recordings were analyzable and no adverse events or heat-related discomforts were reported. During RH at RT, cuff release produced a rapid rise in PTT (~5–6 % above baseline) (**Figure 2**), and a large positive shift in  $f-f \Delta T$  (by 375%) (**Figure 3**).

In contrast to RH at RT (20–25°C), the forearm heating protocol (AH) at heating pad temperature of 43.5°C produced no significant changes in any of the two vascular-stiffness measures. PTT and  $f-f \Delta T$  remained unchanged over the 25 minutes of active forearm heating relative to the preheated baseline values (263  $\pm$  18 ms, and 2.9  $\pm$  11.2 ms, respectively). Forearm heating at 43.5°C for 25 min is associated with an increase in

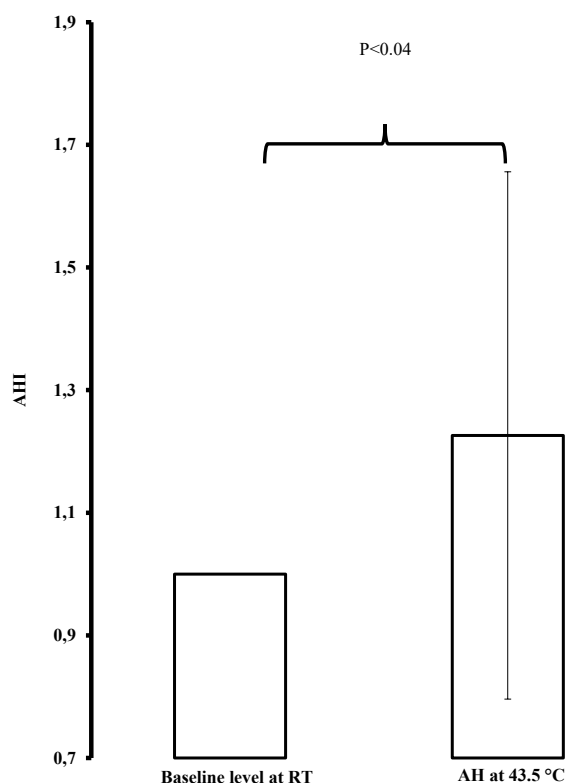


**Figure 2.** Pulse transit time (PTT) during baseline recording and during 1<sup>st</sup> min of reactive hyperemia at room temperature.  $n = 20$ .



**Figure 3.** Pulse arrival time ( $f-f \Delta T$ ) during baseline recording and during 1<sup>st</sup> min of reactive hyperemia at room temperature.  $n = 20$ .

AHI by 22% relative to pre-heated baseline value of 1 (pre-heating blood flow magnitude was considered as 1) (**Figure 4**). At room temperature (20–25 °C), RHI nearly doubled during RH (2.0 $\pm$ 1.1) (**Figure 5**). The baseline value was set to 1, representing resting (pre-occlusion) flow. Thus, the RHI increase at room temperature (~100%; **Figure 5**) was far greater than the AHI increase during heating (~22% at 43.5 °C; **Figure 4**),

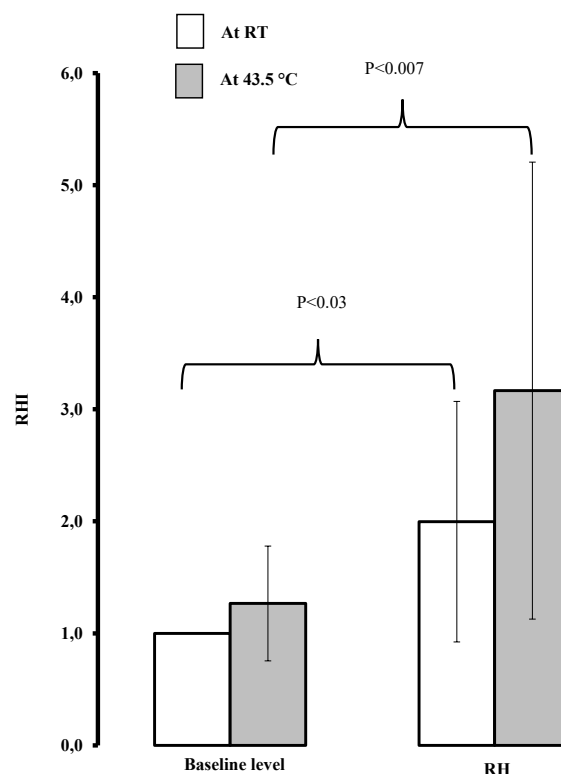


**Figure 4.** Active hyperemia index (AHI) after 25 minutes of heating at 43.5 °C (active hyperemia, AH); the preheated baseline hyperemia index was set to 1 (n = 20).

indicating that the RH response is roughly three times larger than the AH response. After 25 min of heating, the forearm already demonstrated active hyperemia (AHI  $\sim 1.3 \pm 0.5$ ). This value was considered as a baseline value for the subsequent measurement of RH after 25 min at 43.5°C. When RH was imposed on this background, RHI rose to  $\sim 146\%$  over the baseline value ( $p < 0.007$ ) (**Figure 5**), confirming that local heating amplified the hyperemic response. PTT and f-f  $\Delta T$  increased significantly by 5%, and 307%, respectively relative to baseline values ( $266 \pm 18$  ms and  $5.7 \pm 6.7$  respectively). These increases were essentially the same as those observed during RH at room temperature, indicating that the vascular stiffness indices (PTT and f-f  $\Delta T$ ) did not change during RH even after the forearm was heated to 43.5°C.

## Discussion

This study demonstrates that performing reactive hyperemia (RH) on a forearm pre-heated to approximately 43.5°C yields a greater hyperemic response than RH at RT, even when both tests are performed on the same individual and under identical occlusion conditions. The finding aligns with experimental physiology showing that the combination of local heating and ischemia-reperfusion enhances cutaneous blood flow more than either stimulus alone<sup>17,15</sup>. The possible mechanism is that local heating attenuates sympathetic vasoconstrictor tone<sup>18</sup> and increases shear stress in the resistance vessels, which primes endothelial nitric-oxide synthase activity and promotes nitric-oxide (NO) release<sup>12,17</sup>. Consequently, when the



**Figure 5.** Reactive hyperemia index (RHI) during post-occlusion reactive hyperemia (RH) at room temperature (RT) and at 43.5 °C. n = 11.

cuff is released, the reperfusion phase occurs on an already dilated microvascular bed<sup>12</sup>, enabling a larger volume of blood to perfuse the tissue and producing higher RHI and longer PTT<sup>5</sup>.

Despite larger increases in PTT and f-f  $\Delta T$  after RH, these indices did not rise further during RH at 43.5°C. Although forearm heating to 43.5°C robustly dilates cutaneous vessels<sup>19</sup>, this does not necessarily yield detectable changes in heart-to-finger PTT derived from fingertip PPG. The most likely explanation are (i) local forearm heating primarily affects the skin and does not substantially alter conduit- or brachial-artery stiffness<sup>14</sup>. Because heat penetration to deeper tissues is limited, skin temperature rises markedly while intramuscular temperature increases only modestly, leaving the mechanics of deeper arteries largely unchanged<sup>18</sup>. Consequently, a surface pad at 43.5°C raises the local “core” temperature only to about 38°C—insufficient to evoke major endothelial NO release in deeper vessels. (ii) Heating primarily reduces vascular resistance rather than stiffness, and since stiffness depends on pressure, PTT may remain unchanged when local heating increases skin blood flow without altering mean arterial pressure<sup>20</sup>. (iii) Furthermore, the heated segment represents only a small portion of the overall heart-to-finger path, so localized effects are diluted; PWV interpretation thus requires well-defined arterial segments<sup>21</sup>. Previous work likewise reported that flow-mediated or RH-related vasodilation can improve without a parallel reduction in PWV, confirming partial functional independence between these vascular domains<sup>22,23</sup>. Our observations therefore reinforce the concept that microvascular endothelial function and conduit stiffness respond differently to thermal and ischemic stimuli. The PTT prolonga-

tion (~5–6%) observed during room-temperature RH closely match prior PPG-based RH studies<sup>3,4</sup>. Similarly, the addition of occlusion–reperfusion to local heating has been shown to amplify vasodilatory responses<sup>15,17</sup>.

The clinical importance of RH lies in its ability to assess endothelial and microvascular reserve non-invasively. Impaired RH responses reflect endothelial dysfunction, which is considered an early vascular abnormality and has been linked to cardiovascular risk and future cardiovascular events. Therefore, the present finding that local heating enhanced RHI suggests that thermally augmented RH may increase the sensitivity of plethysmography-based vascular testing, especially when standard RH responses are modest. This approach may provide a simple physiological tool for early assessment of peripheral microvascular dysfunction, although its value in patient populations requires further validation.

**Strengths:** The study's within-subject design, dual-finger normalization, and simultaneous ECG/DPW recording enhance internal validity. All tests were ethically approved and non-invasive, providing a reproducible model for thermal augmentation of RH assessment.

**Limitation:** The paired comparison included only 11 subjects and exclusively males, which may limit generalizability. Heating was slightly higher (43.5°C at pad surface) than the classical 42°C skin protocol. PTT and f-f  $\Delta T$  was derived from PG rather than applanation tonometry, and measurements were

confined to a 5-min occlusion paradigm. Nevertheless, the results offer valuable insight into how local thermal priming enhances microvascular reactivity without proportionally affecting conduit stiffness.

## Conclusion

Reactive hyperemia measured by digital plethysmography is visibly augmented when the test is performed on a forearm locally heated for 25–30 min to 43.5°C. Thermal priming increases the hyperemia index and preserves the typical PTT response pattern and does not cause an additional increase in PTT, confirming that thermal and ischemic stimuli primarily enhance the microvascular / endothelial component. This simple modification may improve the sensitivity of PG-based endothelial testing for research and clinical physiology applications.

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