Nitric oxide and neurally mediated regulation of skin blood flow during local heating

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In humans, locally heating nonglabrous skin evokes vasodilation that is mediated by neurogenic reflexes and locally released substances (10, 14, 16). Interactions between the local factors and neural mechanisms involved in the vasodilator response to locally heating the skin are extremely complex and poorly understood. Furthermore, studies investigating the cutaneous microvascular response to local heating have varied with respect to the temperature used, the length of time heat is applied, and the rate at which skin is heated (2, 7, 10, 14, 16, 18, 20, 21, 23). All of these factors may initiate different mechanisms and impact the cutaneous vascular response to the local heating stimulus. However, in general, local heating evokes an initial dilator response that peaks in a few minutes, followed by a brief nadir, and then a secondary dilation to a plateau that can be sustained.

In this context, the skin is known to be innervated by two distinct branches of the sympathetic nervous system: an adrenergic vasoconstrictor system that contributes to resting cutaneous vascular tone and a cholinergic vasodilator system featuring an unknown neurotransmitter coreleased with acetylcholine (11). Release of tonic adrenergic vasoconstrictor tone contributes only minimally to the overall rise in skin blood flow (SkBF) when the skin is directly heated (16). The sympathetic cholinergic nerves are the primary mechanism involved in thermoregulatory vasodilation, but evidence suggests that these nerves are not involved in the vasodilation during local heating (11).

A recent study by Kellogg and colleagues (10) provides the best insight into the SkBF response to local heating to date. These authors determined that nitric oxide (NO) plays a major role in the sustained cutaneous vasodilation (i.e., the secondary plateau) during prolonged local heating but that NO is not the sole vasodilator mechanism involved. They also observed that NO synthase (NOS) inhibition does not impact the vasodilation in subjects who reported feeling “mild pain” for 5–20 s at the initiation of heating the skin to 42°C, suggesting that other mechanisms may have been activated. Along these lines, heat-sensitive nociceptors have been shown to release vasoactive peptides, most likely calcitonin gene-related peptide (CGRP) and substance P, even in the absence of a conscious perception of heat pain (14). Interestingly, pain thresholds are lower for rapidly rising heat stimuli than for slowly rising stimuli and are strongly dependent on the stimulus pattern (14). Taken together, these studies suggest that directly heating the skin can activate neurogenic reflexes and locally released substances but that the interactions between these various mechanisms are still poorly understood.
Using this information as a background, we hypothesized that there are at least two independent mechanisms contributing to the rise in SkBF during a local heating protocol that does not result in a conscious perception of pain. Therefore, our goal in the present study was to examine the interaction between neurally and NO-mediated regulation of vascular tone during local heating.

**METHODS**

**Subjects**

Twenty men (18–33 yr old) and ten women (19–28 yr old) volunteered for the following studies. Most subjects participated in several protocols. All women were studied during menstruation (early follicular phase of the menstrual cycle) because the menstrual cycle is known to alter the SkBF response to local heating (6). All subjects were healthy, normotensive, and nonsmokers. Institutional Review Board approval was obtained, and each subject gave informed consent before participation.

**Instrumentation**

Subjects were instrumented for all protocols as follows. A water-perfused suit was worn to clamp whole body skin temperature between 32 and 33°C, measured by four copper-constantan thermocouples placed on the chest, back, upper arm, and thigh. The water-perfused suit did not cover the face or the forearm being studied and was used to minimize the influence of whole-body skin temperature on reflex changes in SkBF at the local skin sites.

Two microdialysis fibers (MD 2000, Bioanalytical Systems) with 10-mm-long, 20-kDa membranes were placed in the dermal layer of the ventral aspect of the nondominant forearm of each subject. Insertion of the microdialysis fibers was performed by first placing a 25-gauge needle into the dermal layer of the skin. The microdialysis fiber was then threaded through the needle so that the microdialysis membrane was 1 cm from the lumen of the needle. The needle was then partially withdrawn as the microdialysis membrane was pulled into place in the skin. Once the membrane was in place between the insertion and exit points, the needle was completely withdrawn. The microdialysis fibers were taped in place, and Ringer solution was perfused through the fibers at a rate of 2 μl/min. The two microdialysis sites were at least 5 cm apart.

To obtain an index of SkBF, cutaneous red blood cell flux was measured over the two microdialysis sites using a Perimed Periflux System 5000 with integrated laser-Doppler probes. Each probe has one optic fiber emitting a laser light surrounded by seven receiving fibers. The integrated probe provides a greater surface area measured by the laser-Doppler system than standard single receptor laser-Doppler probes. Skin temperature was controlled at the two microdialysis sites with a Peritemp 4005 local heating unit that covers ~700 mm² of tissue. Red blood cell flux was measured directly over the microdialysis membrane in the center of the local heating unit probes. A copper-constantan thermocouple was placed under each heating unit to monitor local skin temperature at the heat probe-skin surface interface.

After placement of the microdialysis fibers, SkBF over the microdialysis sites was monitored to determine that the insertion trauma had resolved before the studies were started (between 75 and 140 min). Once the insertion trauma had resolved, the subjects underwent the individual protocols. To ensure that blood pressure was stable throughout the experimental protocols, blood pressure was continuously measured with a Finapres device on the middle finger of the dominant arm (model 2300, Ohmeda). In all experiments, 50 mM sodium nitroprusside (Nitropres, Ciba Pharmaceuticals) were infused through the microdialysis fibers for 20–30 min at the end of each protocol to maximally vasodilate the skin at both sites. We performed pilot data to determine that an infusion at this dose of sodium nitroprusside resulted in maximal vasodilation of the skin. All data are expressed as a percentage of maximal SkBF (i.e., %SkBF max).

**Protocols**

Three separate studies were performed to address the goals of this project and are presented below and in Table 1. As described above, the subjects were instrumented so that there were two separate microdialysis sites. One of the sites was randomly assigned serve as the control site, and the second site was randomly assigned to undergo one of the remaining studies (I–III). Overall, a total of 40 experimental sites in the 30 subjects were studied in this project.

**Local Heating Protocol**

After the microdialysis probe insertion trauma had resolved, SkBF was recorded for 30 min to serve as a baseline. During the baseline period, the temperature of the local heating units at the microdialysis sites was kept constant at 33°C. After baseline, temperature of the local heating units was increased at a rate of 0.5°C every 5 s to a temperature of 42°C. The local heating units were held constant at 42°C for 50–80 min depending on the protocol for the second site. Subjects did not feel any sensations of pain during the rise in temperature at this rate or with prolonged heating at this temperature. On the basis of preliminary observations, we chose a local heater set temperature below that which results in maximal cutaneous vasodilation (~43°C) to eliminate the possibility of pain sensations in our subjects. It is important to note that, in our study, the set temperature of the local heating units differed from the skin temperatures at the local heater-skin surface interface. At a heater set temperature of 42°C, skin temperature was consistently between 39.0 and 40.0°C in all subjects, as measured with a copper-constantan thermocouple placed under the local heating unit. Not surprisingly, this resulted in an average SkBF well below (87 ± 4%) the maximum vasodilation value determined by sodium...
nitroprusside infusion or heating the skin to >42°C (9, 10, 22).

Study I: Role of NO in the SkBF response to local heating. The rationale for this study was to examine the role of NO in the initial SkBF response to local heating of the skin. Additionally, to examine the interaction between NO and neurally mediated vasodilation, it was necessary to examine the contribution of NO during the plateau phase of the local heating response. To this end, we repeated the local heating study of Kellogg and colleagues (10) using our local heating protocol. To accomplish these objectives, two separate trials (protocols IA and IB) were performed in which NOS was inhibited by perfusion of 10 mM Nω-nitro-l-arginine methyl ester (l-NAME) dissolved in Ringer solution through the microdialysis fibers at a rate of 2 μl/min. We performed pilot work to determine that concentrations of l-NAME >10 mM did not further reduce SkBF under any condition tested. In protocol IA (8 experiments), an infusion of 10 mM l-NAME was started after 35–40 min of local heating. In this protocol, the skin site was continuously heated until SkBF had decreased in response to NOS inhibition and reached a stable SkBF value for at least 10 min. In protocol IB (8 experiments), 10 mM l-NAME were infused for 30 min before and throughout the local heating period.

Study II: Role of the cutaneous nerves in the SkBF response to local heating. The rationale for this study was to examine the initial and prolonged SkBF response to the standard local heating protocol when the lateral antebrachial cutaneous nerve was blocked proximal to the site of blood flow measurement. A secondary goal for this study was to examine the interactions between the cutaneous nerves and the NO-mediated responses. For these protocols, the lateral antebrachial cutaneous nerve was blocked by injection of 5–6 ml of 0.5% bupivacaine at the antebrachial fossa before insertion of the microdialysis fibers. The microdialysis fibers were then placed in the area of skin numbed by the cutaneous nerve block (innervation territory). A maintenance injection of ~2 ml was injected at the same site at the antebrachial fossa halfway through the study. Blockade of the antebrachial cutaneous nerves was verified before and at the end of the study by a lack of sensation to various stimuli (pinprick, heat) and by the lack of a vasoconstrictor response to 3 min of whole body skin cooling, accomplished by circulating 10°C water through the water-perfused suit. In protocol IIA (6 experiments), the local heating protocol was performed in the innervation territory of the blocked nerves. After 30–40 min of heating, 10 mM l-NAME was infused through the microdialysis probe until SkBF fell and reached a stable SkBF value for at least 10 min. In protocol IIB (6 experiments), the standard local heating protocol was performed in the innervation territory with NOS inhibition for 30 min before and throughout the heating period.

Study III: Axon reflex-mediated vasodilation in the SkBF response to local heating. The rationale for this study was to examine the role of the axon reflexes in the SkBF response to local heating. A secondary goal for this study was to examine the interactions between the axon reflex-mediated responses and the NO-mediated responses. After insertion of the microdialysis fibers, local axon reflexes were blocked with the application of 5 g EMLA cream (2.5% lidocaine and 2.5% prilocaine ointment; Astra USA, Westbrook, MA) under a sterile Tagaderm dressing (3M Health Care, St. Paul, MN) for at least 60 min. The Tagaderm dressing was then removed, and a second application of 5 mg EMLA cream was applied under another Tagaderm dressing for an additional 60 min. Care was taken to ensure that the EMLA cream did not come in contact with the entry and exit points of the microdialysis fiber. Blockade of the axon reflexes was verified by lack of sensation or SkBF response to various stimuli within the blocked area (pinprick, stroking the skin) and by lack of a neurogenic inflammatory response (flare response) in the blocked area to a painful heat stimulus applied adjacent to or within the blocked area. In protocol IIIA (6 experiments), the local heating protocol was performed in the axon reflex-blocked area. After 30–40 min of heating, l-NAME (10 mM) was infused through the microdialysis probe until SkBF decreased and reached a stable SkBF value for at least 10 min. In protocol IIIB (6 experiments), the local heating protocol was performed in the axon reflex-blocked area with NOS inhibition for 30 min before and throughout the heating period.

Data Analysis

Data were digitized and stored on a computer at 100 Hz. Data were analyzed off-line with signal-processing software (Windaq, Dataq Instruments, Akron, OH). Baseline, plateau, and SkBF with NOS inhibition were calculated by averaging values over a stable 10-min period. Initial peak and the postpeak drop in SkBF (termed the “nadir”) were calculated by averaging the values over a 1-min period.

Statistical Analysis

All data are presented as means ± SE. Within a given protocol, the vasomotor responses during baseline, initial peak, nadir, plateau, and (when applicable) fall in SkBF with subsequent NOS inhibition were analyzed by repeated-measures ANOVA followed by specific means comparisons. To investigate the role of NO (study I), the cutaneous nerves (study II), and the axon reflexes (study III) in the SkBF response to local heating, values obtained during baseline, the initial peak, nadir, and plateau phase were compared with the control trials by paired t-tests. To compare interactions between NO-mediated responses (study I) and axon reflex-mediated responses (study III), values obtained during the baseline, initial peak, nadir, plateau phase and the decline in SkBF with NOS inhibition were compared by paired t-tests.

RESULTS

Table 1 displays the protocol number and a general description of each study for clarity. Table 2 summarizes the SkBF responses during baseline, local heat-

Table 2. Statistical comparison of data from the various protocols

<table>
<thead>
<tr>
<th>Protocol No.</th>
<th>Baseline</th>
<th>Initial Peak</th>
<th>Nadir</th>
<th>Plateau</th>
<th>NO Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14 ± 2</td>
<td>75 ± 3*</td>
<td>56 ± 3*</td>
<td>87 ± 4*</td>
<td>26 ± 3*</td>
</tr>
<tr>
<td>IA</td>
<td>14 ± 3</td>
<td>73 ± 5*</td>
<td>54 ± 6</td>
<td>86 ± 5</td>
<td>29 ± 3*</td>
</tr>
<tr>
<td>IB</td>
<td>10 ± 2†</td>
<td>56 ± 3*</td>
<td>21 ± 3†</td>
<td>40 ± 5†</td>
<td>40 ± 6†</td>
</tr>
<tr>
<td>IIA</td>
<td>20 ± 3†</td>
<td>75 ± 4*</td>
<td>59 ± 4*</td>
<td>89 ± 3*</td>
<td>29 ± 3*</td>
</tr>
<tr>
<td>IIB</td>
<td>12 ± 4</td>
<td>56 ± 3*</td>
<td>23 ± 4*</td>
<td>40 ± 6*</td>
<td>29 ± 3*</td>
</tr>
<tr>
<td>IIIA</td>
<td>10 ± 2†</td>
<td>32 ± 2†</td>
<td>14 ± 4†</td>
<td>83 ± 4*</td>
<td>35 ± 4*</td>
</tr>
<tr>
<td>IIIIB</td>
<td>8 ± 2†</td>
<td>31 ± 3†</td>
<td>12 ± 2†</td>
<td>42 ± 5†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as a percentage of maximal skin blood flow during infusion with 50 mM sodium nitroprusside. *Significant difference from baseline within a given protocol, P < 0.05. †Significant difference from control for a given phase of the heating protocol (baseline, initial peak, nadir, plateau), P < 0.05.
ing, and NOS inhibition for all of the various protocols and the control trials.

**SkBF Responses to the Local Heating Protocol**

Figure 1A is a representative tracing of the temperature responses to the local heating protocol. At the initiation of heating, skin temperature at the heating probe-skin surface interface rapidly increased to 39–39.5°C and remained stable throughout the heating protocol. Figure 1B is a representative tracing of the SkBF response to the local heating protocol. After the baseline period (33°C), the local heater temperature was increased to 42°C, resulting in a rapid increase in SkBF to an initial peak (average ± SE for all subjects: 75 ± 3% SkBF_{max}). This was followed by a transient drop in SkBF (56 ± 3% SkBF_{max}), followed by a secondary progressive rise to a plateau (87 ± 4% SkBF_{max}). After prolonged heating (>50 min), SkBF began to decline in some, but not all, subjects despite the maintenance of an elevated skin temperature. At the termination of heating (local heater returned to 33°C), a paradoxical increase in SkBF occurred in most subjects before beginning to decline.

**Study I: Role of NO in the SkBF response to local heating.** Figure 2A shows a representative tracing of the SkBF response when L-NAME was infused after 40 min of local heating. L-NAME infusion caused a consistent, gradual decline in SkBF to 26 ± 3% SkBF_{max}. SkBF after NOS inhibition remained significantly higher than baseline SkBF (P < 0.05). Figure 2B shows a representative tracing of the SkBF response to local heating when L-NAME is infused for 30 min before and throughout the heating protocol. Baseline SkBF was significantly decreased from 14 ± 3 to 10 ± 2% SkBF_{max} with NOS inhibition (P < 0.05). The initial peak was significantly lower when NOS was inhibited before the start of the heating from 73 ± 3 to 56 ± 3% SkBF_{max} (P < 0.01), suggesting that NO is required for the full expression of this initial response to heating but plays a greater role during the plateau phase of the local heating response. SkBF then returned to near baseline levels, followed by a gradual increase that in most cases did not reach a plateau after >60 min of heating. Between 40 and 50 min of heating, SkBF averaged 40 ± 5% SkBF_{max}.

**Study II: Role of the cutaneous nerves in the SkBF response to local heating.** Figure 3A is a representative tracing of the SkBF response to local heating in the innervation territory of the blocked sensory and sympathetic cutaneous nerves. Baseline SkBF was significantly higher than in the control trial (20 ± 3 vs. 14 ± 3%; P < 0.05). However, the initial peak, nadir, and plateau phase did not differ from the control trial. Figure 3B is a representative tracing of the SkBF response when NOS was inhibited before local heating in the blocked area. There were no differences observed in the SkBF response to local heating between this protocol and protocol IB (when NOS was inhibited before the local heating). Combined, these results suggest that the sympathetic cutaneous nerves do not contribute to any portion of the local heating response (except for contributing to cutaneous vascular tone at rest) or to the NO-mediated mechanisms of this response.
Study III: Axon-reflex mediated vasodilation in the SkBF response to local heating.

Figure 4A is a representative tracing of the SkBF response to local heating when the SkBF response is normalized to the maximal response to sodium nitroprusside (50 mM). The initial peak in SkBF to local heating was significantly reduced compared with the control condition (32 ± 2 vs. 75 ± 3%). However, the plateau phase and the decline in SkBF to 10 mM L-NAME were similar to the responses in the control trial. When L-NAME was infused prior to local heating in the axon reflex-blocked area of skin (Fig. 4B), the initial peak was similar to the initial peak during the axon reflex block only in Fig. 4A (31 ± 3 vs. 32 ± 2%; P > 0.10). This suggests that NO does not contribute to the initial peak response to local heating when the axon-reflexes are blocked.

DISCUSSION

The goal of this study was to explore the mechanisms involved in the cutaneous vasodilator response to local heating. To this end, we performed a series of separate studies to systematically examine the role of the axon reflexes, the cutaneous nerves, and NO and their interactions on the SkBF response to a standardized local heating protocol. The primary finding of these studies is that there are at least two independent mechanisms contributing to the rise in SkBF when heat is directly applied to the skin at a temperature below the threshold for pain sensation. The initial rise in SkBF in response to local heating appears to be predominantly mediated by an axon reflex mechanism that remains robust when NOS is inhibited. In agreement with the study by Kellogg et al. (10), we found that the secondary, sustained rise in SkBF to a plateau appears to be primarily mediated by NO (Fig. 5). Additionally, full expression of this secondary rise in SkBF is not dependent on the initial peak response.

Blocking the nerves involved in the axon reflexes greatly reduced the initial rise in SkBF but did not alter the secondary rise to plateau. Simultaneous NOS inhibition did not reduce the initial peak response to a greater extent than axon block alone (31 ± 3 vs. 32 ± 2%, respectively), suggesting that the neurotransmitter(s) released during axon reflex activation stimulate some NO release but that the neurotransmitters themselves are not dependent on NO for release. In the present study, we can only speculate on the neurotransmitters involved in the initial response to our local heating protocol because they were not measured. Recent evidence using microdialysis suggest that CGRP is the most likely neurotransmitter involved in the axon reflex (19). However, substance P cannot be ruled out because of the difficulty in measuring this peptide. CGRP is one of the most abundant neuropeptides in the skin and is found alone or colocalized with either substance P or somatostatin (24). Interestingly, intradermal injection of CGRP together with substance P converts the prolonged vasodilator response of CGRP to a transient increase in SkBF (300–400% increase over baseline) that remains only slightly elevated over baseline levels (4). The pattern of SkBF response to intradermal injection of these neuropeptides is similar to the results in the present study and may suggest that both substance P and CGRP are involved in the initial peak response.
released at the initiation of heating. When local heating is painful, much higher concentrations of CGRP may be released from these nerves or adjacent nerves, resulting in a greater and more prolonged SkBF response. The fact that the increase in SkBF with CGRP is largely independent of NO (3) is in agreement with this hypothesis.

We are not able to determine why blocking the axon reflexes did not completely abolish the initial peak SkBF response to local heating. It is possible that we were unable to completely block the nerves mediating the axon reflex, although this seems unlikely. We were able to completely eliminate the flare response to a thermode heated to 45°C placed adjacent to the treated area for a few seconds. In the untreated area, the hot thermode was perceived as being very painful and a significant flare response was observed (visually and by laser-Doppler flowmetry) extending throughout the untreated area but not within the treated area. When the thermode was placed within the treated area, no flare response was observed. Combined, these results suggest that the nerves in the treated area involved in the axon reflex were sufficiently blocked. It is possible that the locally applied heat stimulated Ca2+ channels at the nerve terminal, causing a transient release of vasodilator substances.

In contrast to the finding that blockade of the cutaneous nerves proximal to the site of local heating did not alter the SkBF response to local heating, blocking the antebrachial cutaneous nerve proximal to the site of local heating did not alter the initial or sustained SkBF response to local heating or the contribution of NO to these responses (Fig. 3, A and B). As expected, we did observe a higher baseline when these nerves were blocked (16) but not when NO production was simultaneously inhibited. This result was not surprising in light of the fact that both adrenergic vasoconstrictor fibers and NO have been shown to contribute to resting cutaneous vascular tone (10, 16).

L-NAME infusion before local heating changed the initial SkBF response compared with the control trials. The initial peak was reduced from 73% without NOS inhibition to 56%, suggesting that NO contributes only modestly to this initial response to local heating. After the initial peak, SkBF declined to near baseline levels and then gradually increased to ~40% of maximal dilation. This observation provides further evidence that NO primarily mediates the secondary rise in SkBF with local heating. However, the secondary rise in SkBF with L-NAME infusion before heating was significantly higher than SkBF with NOS inhibition during heating (40 ± 5 vs. 26 ± 3%; P < 0.05). In a few of the subjects (n = 4), we increased the concentration of L-NAME infused during this secondary phase to 50 mM, with no further reduction in SkBF.

We designed our local heating protocol on the basis of preliminary studies in our laboratory and the published reports that a high rate of skin temperature rise or a sustained skin temperature >41°C may activate specific nociceptive neurons and a sensation of pain (14). We found that even a very brief sensation of mild pain (<5–10 s) on the initiation of heating eliminated the bimodal increase in SkBF and rendered the vasodilation insensitive to NOS inhibition, similar to the observations of Kellogg et al. (10). Furthermore, some subjects in our preliminary studies reported a mild burning sensation when skin temperature was sustained above 41.0°C for a prolonged period. In these pilot subjects, NOS inhibition did not reduce SkBF in the heated area, providing further evidence that a mild sensation of pain elicits the release of neuropeptides that are independent of NO. Magerl and Treede (14) found that release of vasoactive neuropeptides by nociceptive primary afferents contributes to local hea-
evoked vasodilation at temperatures above 40°C even in the absence of a conscious perception of pain. These authors also reported that the rate of local heater temperature rise lowers the temperature at which pain is perceived. Thus we chose a rate of temperature rise (0.1°C/s in our study vs. 4.0°C/s by Magerl and Treede) and a peak temperature that did not result in a sensation of pain in any of our subjects. Although our heating elements attained a temperature of 42.0°C, this resulted in a skin temperature of 39.5–40.0°C.

Our local heating protocol allowed us to reliably evaluate the bimodal SkBF response to local heating in a large number of subjects. Interestingly, SkBF started to gradually decline after 50–70 min of continued heating in approximately one-third of the subjects. This response was first observed by Barcroft and Edholm in 1943 (2), during prolonged heating of the forearm in a warm water bath. These authors termed this phenomenon “die away” and speculated that the vasodilator substance may be washed out because of the high blood flows. It is possible that the precursor for NO, L-arginine, may be reduced because of the prolonged heating, resulting in less production of NO. Along these lines, the die-away phenomenon was not observed in our study when NOS was inhibited throughout the heating period. However, the heating period in these experiments was usually <60 min. It is also possible that prolonged heat causes tachyphylaxis of the vessel. However, Barcroft and Edholm reported that the die-away phenomenon was not observed when the forearm was heated in 45°C water (2), suggesting that tachyphylaxis of the vessel does not occur. Interestingly, these data provide additional evidence that a vasodilator mechanism other than NO may be activated at very high skin temperatures. At the termination of the heating protocol, we observed a paradoxical rise in SkBF in most subjects when the local heater temperature was lowered back to baseline (33°C). At this time, we are not able to speculate on the mechanism that may underlie this response.

In summary, nonpainful local heating of the skin results in a consistent bimodal rise in SkBF that is mediated by at least two independent mechanisms: a fast-responding vasodilator system mediated by the axon reflexes and a more slowly responding vasodilator system that relies on local production of NO. Furthermore, our results (and our heating protocol) may be useful in evaluating the nature and mechanisms of cutaneous microvascular dysfunction in aging (8, 12, 15) and in patients with Type 1 (13, 17) and Type 2 diabetes (1, 18, 20, 21, 23), with hypertension (5, 20), and after irradiation therapy (7). Finally, additional studies that explore which substances are involved in the initial cutaneous vasodilation during local heating and the mechanisms of NO generation during the secondary rise in SkBF seem warranted at this time.

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