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Original Article

The effect of topical capsaicin-induced sensitization on heat-evoked cutaneous vasomotor responses

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Abstract: Brief, localized, cutaneous, non-painful thermal stimuli can evoke a transient vasomotor response, causing increased cutaneous blood flow and elevated skin temperature. The aims of this study were to investigate 1) if cutaneous sensitization by topical application of capsaicin (TRPV1 receptor agonist) can facilitate the size, duration and spatial extent of this vasomotor response and 2) if males and females respond differently. Thermal pulses (43°C for 60 seconds) were applied on left/right volar forearms of 15 age-matched males and females. Skin temperature and cutaneous blood flow were measured 1, 5, 10, 15, and 30 minutes after heat application before and after topical capsaicin (1%, 30 min application) with contralateral arm serving as the control. Recordings were made from the region of interest at distances of 2, 4, 6, 8, and 10 cm from the capsaicin application site. Sensitization significantly enhanced skin temperature for up to 30 min and compared with non-sensitized skin at 10 min. Females showed the strongest response after sensitization, but the response lasted longer and spread more widely in males. The blood flow responses were significantly longer after capsaicin (from 5 to 30 minutes after thermal application). This increased blood flow extended outside the treated area up to 10 min after stimulation. After sensitization, the area under the blood flow response curves showed significantly stronger responses in females, spreading 4 cm outside the stimulation site. Cutaneous sensitizing caused prolonged and spatially expanded vasomotor responses to standardized thermal stimulation with sex specific differences.

Keywords: Neurogenic inflammation, vasomotor response, thermal stimulation, sensitization, capsaicin, cutaneous

Introduction

A brief, transient controlled heat stimulus (43°C for 60 seconds) applied to the skin has recently been shown to provoke a profound localized vasomotor reflex response (elevated blood flow and temperature) without provoking pain [1]. This novel mechanism may have a number of diagnostic and pharmaco-technological applications.

In recent years, transdermal drug delivery has gained more focus, with a significant increase in FDA-approved transdermal drugs [2]. It would be of interest if transiently changing the status of the superficial vascular plexus could result in a higher systemic absorption of a transdermally delivered intracutaneous drug. This vasomotor provocation technology could also be envisioned to enhance absorption of intradermally placed macromolecules. Such an "on-demand" option, consisting of a passive delivery process in combination with accelerated controllable absorption could offer new opportunities for pain control, for example.

Drug penetration and absorption depends on many factors such as lipophilicity, molecule size, diffusion rates, skin barrier, capillary density, vascular permeability, and the rate of vascular perfusion [3, 4]. For small diffusible molecules, clearance from the dermis is highly dependent on local blood flow [5]. Transdermal absorption of several drugs (e.g., nicotine and nitroglycerine) is enhanced by heat [6-11]. However, most techniques to deliver heat have relied on longer periods or generalized heating, whereas few have explored the effect of local-

ized, brief, standardized heating and the associated local vasomotor responses. Studies using increased ambient temperature as a method to enhance transdermal delivery have found increased absorption of nicotine [12] or nitroglycerine from patches [13] and insulin from a subcutaneous depot [14].

Recently, two studies have tried to utilize the local heat-evoked vasomotor response for enhancing the absorption of a drug (nicotine) from a patch [15] and an intradermally injected macromolecule (insulin) [16]. For nicotine, a significantly positive correlation was found between absorption and vasomotor response, whereas for insulin, no correlation between heating and absorption was found.

The optimal stimulus temperature and duration to provoke the maximal local cutaneous vasomotor response has also been investigated [1]; 43°C for 60 seconds induced the most stable response. This study, however, did not investigate the spatial extent of the response away from the site of thermal stimulation, nor did it investigate possible sex differences. Furthermore, from both a basic and a pharmacological point of view, it would be of interest to know if the local vasomotor reflex response could be enhanced by sensitization of the skin using topical capsaicin and thereby opening new opportunities for developing technologies to provoke enhanced dermal absorption.

Capsaicin is the transient receptor potential vanilloid subtype 1 (TRPV1) receptor agonist, activating cutaneous mechano-heat sensitive afferents and facilitating the response to controlled heat stimulation [17]. Topical application of capsaicin is a technique widely used for pain treatment [18] and in medical research for inducing cutaneous thermal hyperalgesia [19, 20] and for generating a neurogenic inflammatory response [21]. Capsaicin-provoked sensitization is known to be stronger in females compared with males [22].

The aims of this human experimental study were to assess 1) the size of the vasomotor response to locally controlled heat in normal and capsaicin-sensitized skin, 2) the temporal response profile, 3) the spatial response profile, and 4) possible sex differences of those responses.

Material and methods

Subjects and study design

Thirty young, healthy, non-smoking Caucasian age- and sex-matched volunteers (15 males and 15 females) were recruited from students at Aalborg University, Denmark.

Initial screening involved recording of demographic information and a review of medical history. The use of alcohol and caffeine was prohibited 24 hrs prior to the experiments. No subject had a past or present history of current systemic or skin diseases, known allergies or hypersensitivity (except seasonal allergic rhinitis), infections, and none were taking any medication. In the case of females, they were not pregnant and had no irregularities in their menstrual cycles. Experiments were performed on females when they were in the beginning of their menstrual phase because it has been shown that hormonal changes influence the vasomotor responses to capsaicin [23]. Caucasians were included in the study due to ethnic differences in the response to capsaicin [24] and only young volunteers were included to minimize the effect of age on the response to capsaicin [25].

Application of topical creams, lotions or cosmetics on the test sites was not allowed. Written informed consent was obtained from all participants prior to the study. The study protocol was approved by the local ethics committee (No. N-20120010). The study was conducted in accordance with the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP), and was performed at the experimental and clinical pharmacology laboratory, Aalborg University, Denmark.

The subject sat at a table with both forearms facing upwards. The measurement techniques for recording vasomotor changes were objective (thermography and speckle contrast imaging). All experiments were performed in a quiet room with a stable temperature (23-24°C).

The magnitude, temporal and spatial pattern, and sex differences of the vasomotor responses to controlled mild heat (43°C for 60 seconds) before and after topical capsaicin (cream, 1%) were assessed. The non-treated contralateral area (homologous to the stimulation site

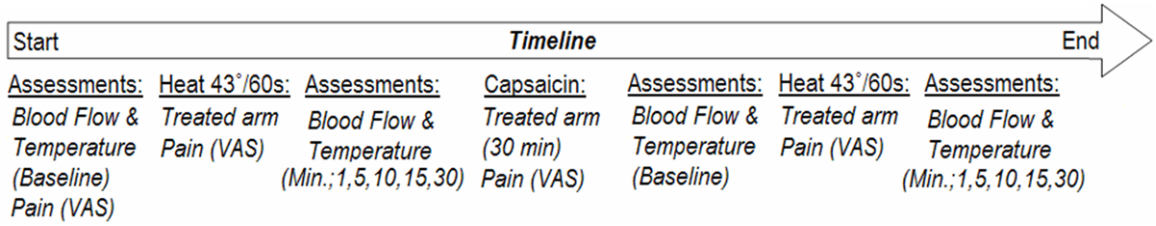

Figure 1. The study overview. Forearms were exposed to heat in a randomized order. One forearm was treated with heat and capsaicin and the other forearm served as a control, with no capsaicin application. Min: minute; VAS: visual analogue scale.

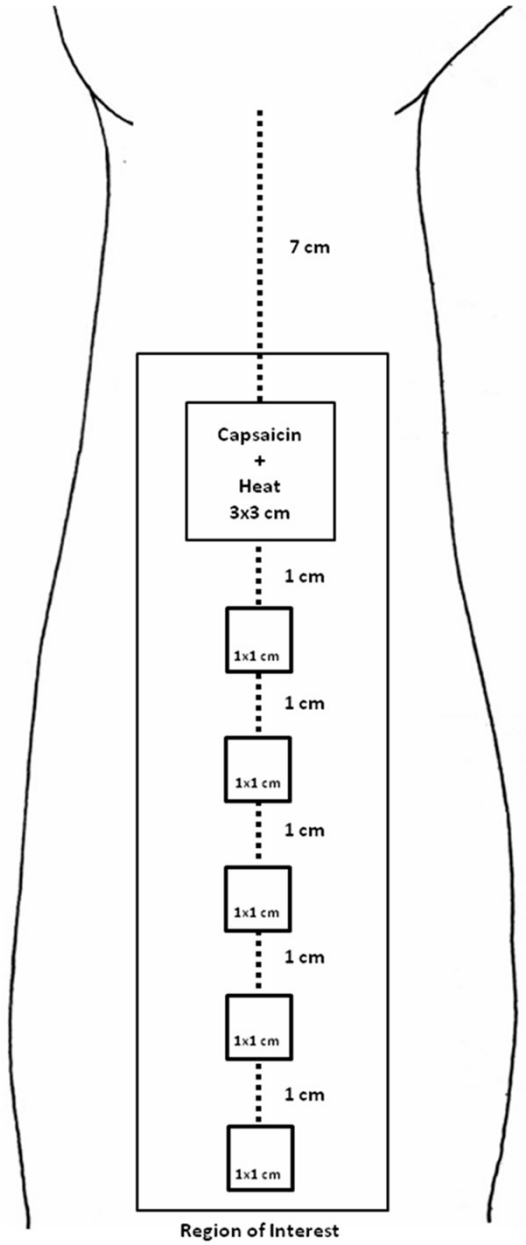

Figure 2. The regions of interest (ROI). The large 3 x 3 cm region (ROI 1) is where the thermal stimulus was applied (and later where the capsaicin was applied on the treatment arm). Regions of interest (ROI 1-6) for measurements of blood flow and temperature are also shown.

(ROI 1)) was used for control recordings to see if there was a systemic vasomotor response to the thermal stimulus. The study consisted of one session lasting approximately 2.5 hours. Recordings were made at baseline, during and after the treatment of skin (**Figure 1**).

Baseline

The regions of interest (ROI 1-6) were marked on the volar forearms (**Figure 2**) as 1 x 1 cm squares, 5 regions, 1 cm apart (border to border distance) from the 3 x 3 cm stimulation/application area (ROI 1). Baseline recordings were performed before any stimulation. The responses to thermal stimulation were then provoked and measured simultaneously from both arms at baseline and at 1, 5, 10, 15, and 30 minutes after thermal stimulation.

Treatment

Capsaicin was applied for 30 min. Vasomotor recordings were performed on both arms simultaneously, immediately (within 5 min) after the cream was removed (no heat provocation).

Post-treatment

Vasomotor responses were provoked in the treated area and vasomotor recordings were made simultaneously from both arms 1, 5, 10, 15, and 30 minutes after thermal stimulation of the treated arm.

Experimental methods

Stimulation methods

Thermal stimulation: A controlled thermal stimulus was delivered by a thermal stimulator (PATHWAY Pain & Sensory Evaluation System, Medoc Ltd. Advanced Medical Systems, Ramat Yishai, Israel) with a rectangular thermode (3 x 3 cm). The stimulus intensity was adjusted to 43°C for 60 seconds on the treated area (ROI 1).

Capsaicin provocation: Approximately 0.5 mL of capsaicin cream 1% (Aalborg University Hospital Pharmacy) was applied under occlusion to a 3 x 3 cm area on the volar aspect of one forearm to sensitize the skin (**Figure 2**). The cream was left on the skin for 30 minutes and then gently wiped off. Assessments of skin blood flow and temperature were taken before and immediately after the removal of the cream. Unknown allergic reactions to capsaicin are very rare, but precautions for anaphylactic shock were taken with an Epipen (adrenalin/epinephrine, 0.3 mg) being available throughout the experiments.

Assessment methods

Assessment of pain: Subjects were asked to rate their pain intensity to thermal stimulus on an electronic continuous 10 cm VAS scale (Visual Analogue Scale) from 0 (no pain) to 10 (maximum imaginable pain). This measurement was performed after each thermal stimulus before and after the capsaicin. The thermal stimulation before capsaicin was considered to be mild and not painful, but in the sensitized area, the stimulus may cause mild pricking pain due to the sensitization [20].

Assessment of skin temperature: Measurements of skin temperature were taken using a thermography camera (FLIR ThermoVision A40 M, Sweden). The temperature resolution of the device is 0.09°C. The thermographic images were stored on a computer for off-line analysis (ThermaCAM Researcher Professional 2.10) of the profile and local changes in temperature of the region of interest (**Figure 2**). Recordings were made from both arms simultaneously at a distance of 45 cm. Assessments were made at baseline and 1, 5, 10, 15, and 30 min after thermal stimulation.

Assessment of blood flow by speckle contrast imager: A speckle contrast imager (FLPI, Moor Instruments, Devon, UK), using a full field laser technique, was used to provide real time images of dynamic changes in blood flow. The technique illuminates an area of tissue with laser light and produces a high contrast random interference effect (speckle pattern). Blood cells flowing through the region of interest cause the speckle pattern to change and appear blurred, leading to a reduction in local contrast. High flow rates show up as areas of low contrast and conversely, low flow rates are regions of high contrast.

Measurements were performed in a semi-dark room to eliminate artifacts from ambient room lighting. The laser wavelength was 785 nm. The laser head was placed at a distance of 45 cm from the skin. The scanning images were stored on a computer for off-line analysis (Moor FLPI Measurement v. 3.0) of the profile and local changes in blood flow in the region of interest (**Figure 2**). Recordings were made from both arms simultaneously at baseline and 1, 5, 10, 15, and 30 min after thermal stimulation.

Assessment of blood flow by laser doppler imaging: As most published studies have measured blood flow using laser Doppler imaging, a side experiment was performed where speckle and laser Doppler imaging were used simultaneously following the above protocol in three female volunteers. This would allow comparing the results from the present study with the literature. The Laser Doppler Imager (LDI, version 2, Moor Instruments, Devon, United Kingdom) is based on the Doppler principle, which is a shift in frequency of waves caused by movement of the reflecting object relative to the observer [26]. This principle allows assessment of the movement of red blood cells in the most superficial skin layers (1-1.5 mm) [27]. The measurements, in arbitrary units, reflect the blood flow at a proportional rate, allowing relative comparisons. The scanner setup was adjusted using the following parameters: 0.25-15 kHz bandwidth, 116 x 70 pixel resolution, 3.0 x 3.0 cm scan area, 4 ms/pixel scan speed, and 30 cm scan distance. Laser scans were stored on the hard disc of a computer for off-line analysis (Moor LDI software v. 5.3).

To compare FLPI and LDI, blood flow before and 30 min after the application of capsaicin cream was assessed. The responses were measured before and 1, 5, 10, 15, and 30 min after removal of the capsaicin cream and the absolute responses in skin perfusion were compared. The ratio between LDI and FLIP responses was calculated for the individual time points.

Statistics

A linear mixed model analysis of variance with factors of treatment and time was performed. Dependent variables included temperature,

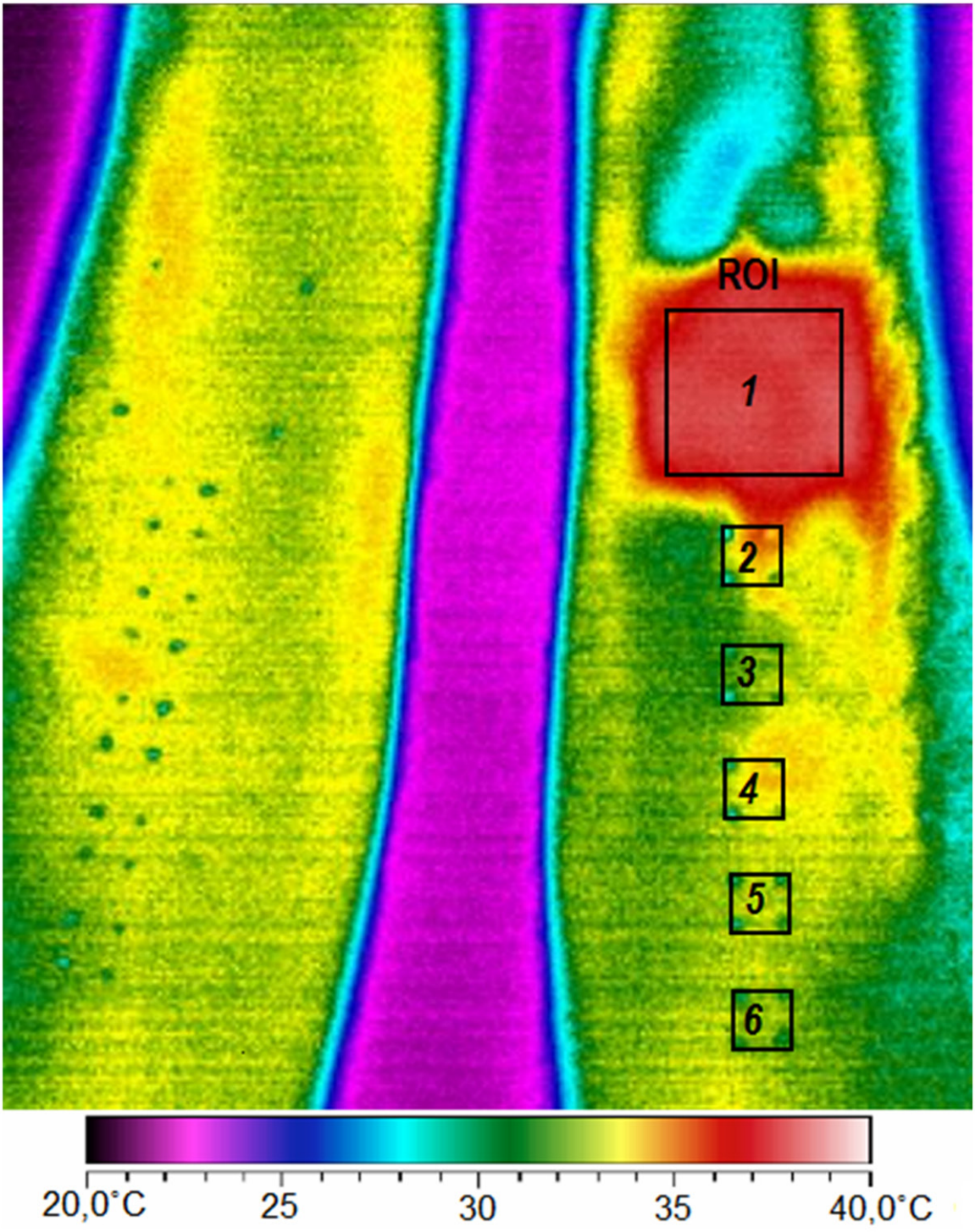

Figure 3. Typical thermographic image of the right and left forearms 1 min following the thermal stimulus applied to the region of interest (ROI 1). Regions of interest (ROI 1-6) are marked on the figure. Red is high temperature and blue is low temperature.

blood flow and VAS ratings. Bonferroni adjustment for multiple comparisons was used as a post hoc test. Shapiro-Wilk and Q-Q plot tests confirmed a normal distribution of the dataset. In all tests, $P \leq 0.05$ was considered statistically significant. The statistics were performed with

IBM SPSS Statistics version 19. The data are presented as the mean values ± standard deviations (SD) unless otherwise stated.

Results

All participants completed the tests and no safety issues were recorded. The mean (±SD) age of the subjects was 24 ± 3 years. The baseline temperature and blood flow measurements from the right and left arms showed no differences.

Pain ratings

The pain ratings to thermal stimulation after capsaicin were significantly (P<0.005) stronger (4.8 ± 2.3) compared with recordings before capsaicin (0.9 ± 1.5), with no sex differences.

Temperature

The averaged ROI 1 response (pooled data from males and females) 1 min after thermal stimulation was significantly (P<0.01) higher after capsaicin application (5.5°C ± 2.0°C) compared with baseline (3.7°C ± 1.5°C). A typical thermographic image 1 min after heat application is shown in **Figure 3** with the ROI marked.

The temperature remained significantly (P< 0.01) higher than baseline for 10 min before capsaicin application and for 30 min after application (P<0.01). Before capsaicin, the temperature responses were similar for males and females (**Table 1**).

After capsaicin, the females responded significantly (P<0.003) stronger than males, with a total increase in temperature of 6.5°C, compared with 4.5°C for males (stimulation site (ROI 1), 1 min, **Figure 4**). For all regions except the stimulation site (ROI 1) and the region 1 cm away from stimulation (ROI 2), the temperature remained significantly (P<0.003) higher for males throughout the 30 min (longer duration), compared with females.

No changes throughout the session were found in the contralateral arm.

Blood flow

Before capsaicin, the maximal ROI 1 response (males and females pooled, **Table 1**) occurred one minute after the thermal stimulation. After application of capsaicin, the maximal provoked response was observed 10 min after thermal stimulation.

At ROI 1, the average baseline blood flow increased by 135% after the capsaicin application. In **Table 1** and **Figure 5**, the relative baseline-normalized percent changes in blood flow are given. Typical speckle contrast images to a thermal stimulus are presented in **Figure 6**.

The provoked increases in baseline normalized blood flow at 5 to 30 minutes after stimulation were significantly (P<0.001) stronger after sensitization compared with the response before sensitization.

For the region 2 cm away from the stimulation area (ROI 2), the baseline-normalized increase was significantly (P<0.005) larger after capsaicin at the 1, 5 and 10-minute time points compared with baseline. None of the other regions showed a significant difference in blood flow response at any time point.

When adding the 135% capsaicin-provoked increase in baseline flow, the increases after thermal stimulation were 251%, 271%, 283%, 257%, and 289% (**Table 1**).

No sex differences between responses were found before the capsaicin application. The increase in background blood flow (before heat provocation) from before capsaicin application to immediately after removal of the capsaicin was similar for males and females.

When comparing the responses from males and females (baseline normalized) using the calculated area under the curve (summation of flow values over time) after capsaicin, females showed a significantly stronger response within ROI 1 (p<0.0001), ROI 2 (p<0.0001), and ROI 3 (p<0.015). The maximal relative increase in flow response of 261% (**Table 1**) was observed 10 minutes after stimulation in females after capsaicin sensitization. Adding the capsaicin-provoked increased flow of 135%, the maximal flow increase could, on average, be estimated as 396% over baseline flow, before any stimulation or sensitization.

No changes throughout the session were found in the contralateral arm.

Table 1. Changes in cutaneous temperature and blood flow (expressed in percent) in the area of thermal stimulation (ROI 1) before and after application of topical capsaicin (Mean ± SD). Data are given for pooled values of males and females and for each sex separately

Temperature increase (%) after thermal stimulation		*1 min*	*5 min*	*10 min*	*15 min*	*30 min*
Males + Females	Before capsaicin	12% ± 5%	2% ± 2%	1% ± 3%	2% ± 3%	1% ± 3%
	After capsaicin	17% ± 8%	8% ± 4%	9% ± 4%	9% ± 5%	9% ± 5%
Males	Before capsaicin	11% ± 4%	2% ± 3%	2% ± 3%	2% ± 3%	3% ± 3%
	After capsaicin	14% ± 6%	7% ± 3%	7% ± 3%	7% ± 3%	7% ± 3%
Females	Before capsaicin	12% ± 7%	2% ± 2%	1% ± 3%	1% ± 2%	0% ± 2%
	After capsaicin	22% ± 8%	11% ± 4%	11% ± 4%	11% ± 5%	11% ± 5%
Blood flow increase (%) after thermal stimulation		*1 min*	*5 min*	*10 min*	*15 min*	*30 min*
Males + Females	Before capsaicin	201% ± 58%	28% ± 31%	12% ± 21%	6% ± 28%	10% ± 47%
	After capsaicin	116% ± 118%	136% ± 175%	148% ± 178%	122% ± 123%	154% ± 180%
Males	Before capsaicin	195% ± 55%	34% ± 32%	8% ± 21%	5% ± 19%	20% ± 57%
	After capsaicin	63% ± 73%	46% ± 53%	57% ± 65%	62% ± 67%	71% ± 97%
Females	Before capsaicin	209% ± 64%	21% ± 29%	16% ± 21%	8% ± 37%	-1,6% ± 27%
	After capsaicin	183% ± 133%	248% ± 211%	261% ± 210%	196% ± 138%	258 % ± 208%

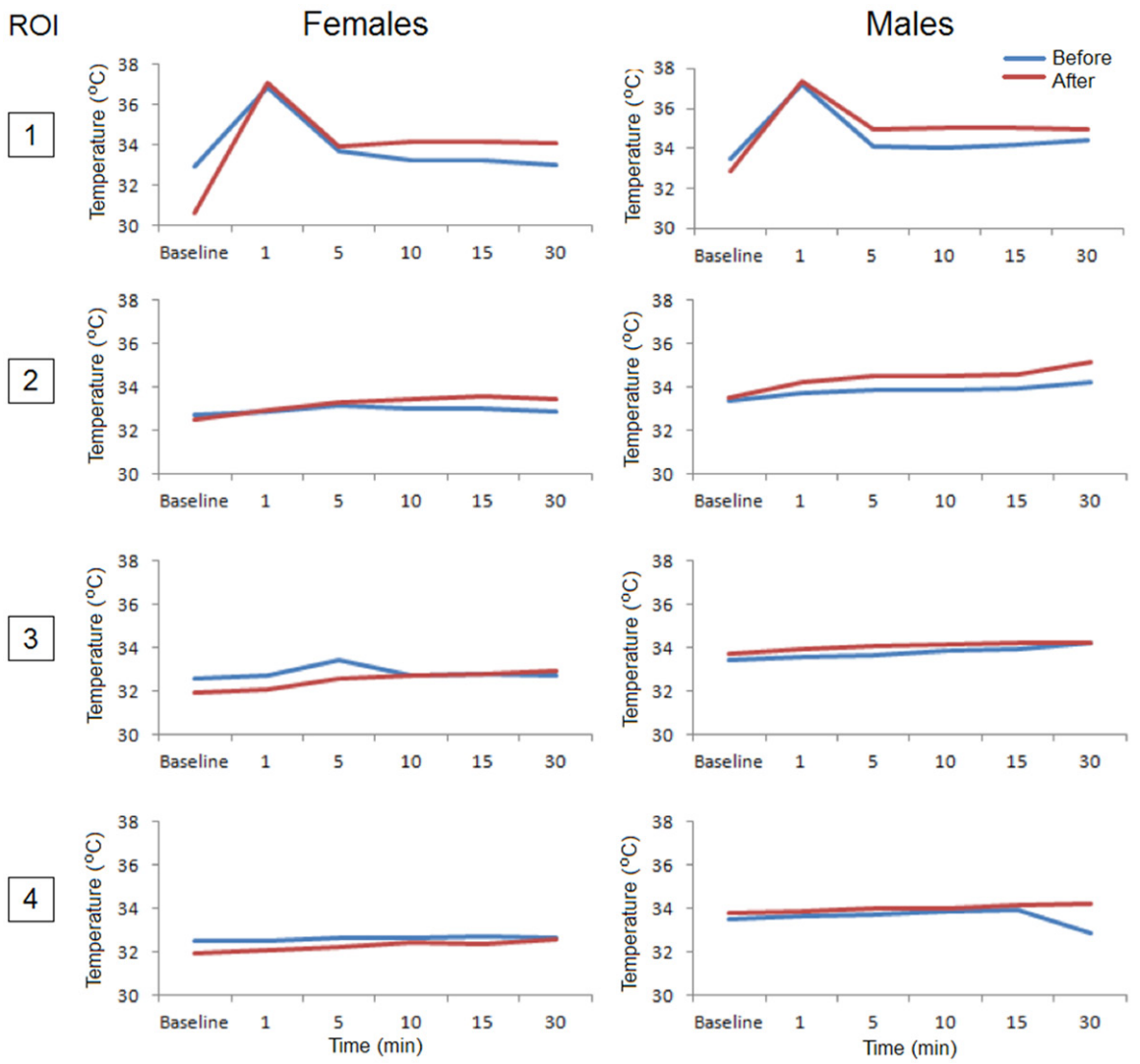

Figure 4. Absolute temperature changes (°C) following heat-evoked responses before and after capsaicin application in males and females. Temporal (baseline and 1, 5, 10, 15, 30 min after stimulation) and spatial distributions (ROI 2, ROI 3, ROI 4) proximal from the stimulation site (ROI 1) are illustrated. ROI 5 and ROI 6 are not shown due to the lack of detectable changes. For error bars see **Table 1**.

Comparing FLPI and LDI

For the baseline and provoked conditions before capsaicin application, the ratio between LDI and FLPI was 1.02, but for the high flow condition after capsaicin, the LDI showed a much stronger response and the ratio was 2.60, indicating a non-linear relationship between the two techniques.

Discussion

The present study investigated the fundamental properties of the cutaneous heat-provoked (43°C, 60 seconds) vasomotor response before and after sensitization with topical capsaicin. The heat-provoked temperature response was facilitated and lasted longer after sensitization of skin by capsaicin. Females showed the strongest temperature response but the response lasted longer and extended more widely in males. The provoked blood flow responses were facilitated and temporally/spatially extended by capsaicin. After sensitization, females showed stronger and more spatially extended blood flow responses than males.

Vasomotor responses

The predominant mechanism behind the heat-provoked vasomotor reflex is believed to result from an interaction between the cutaneous

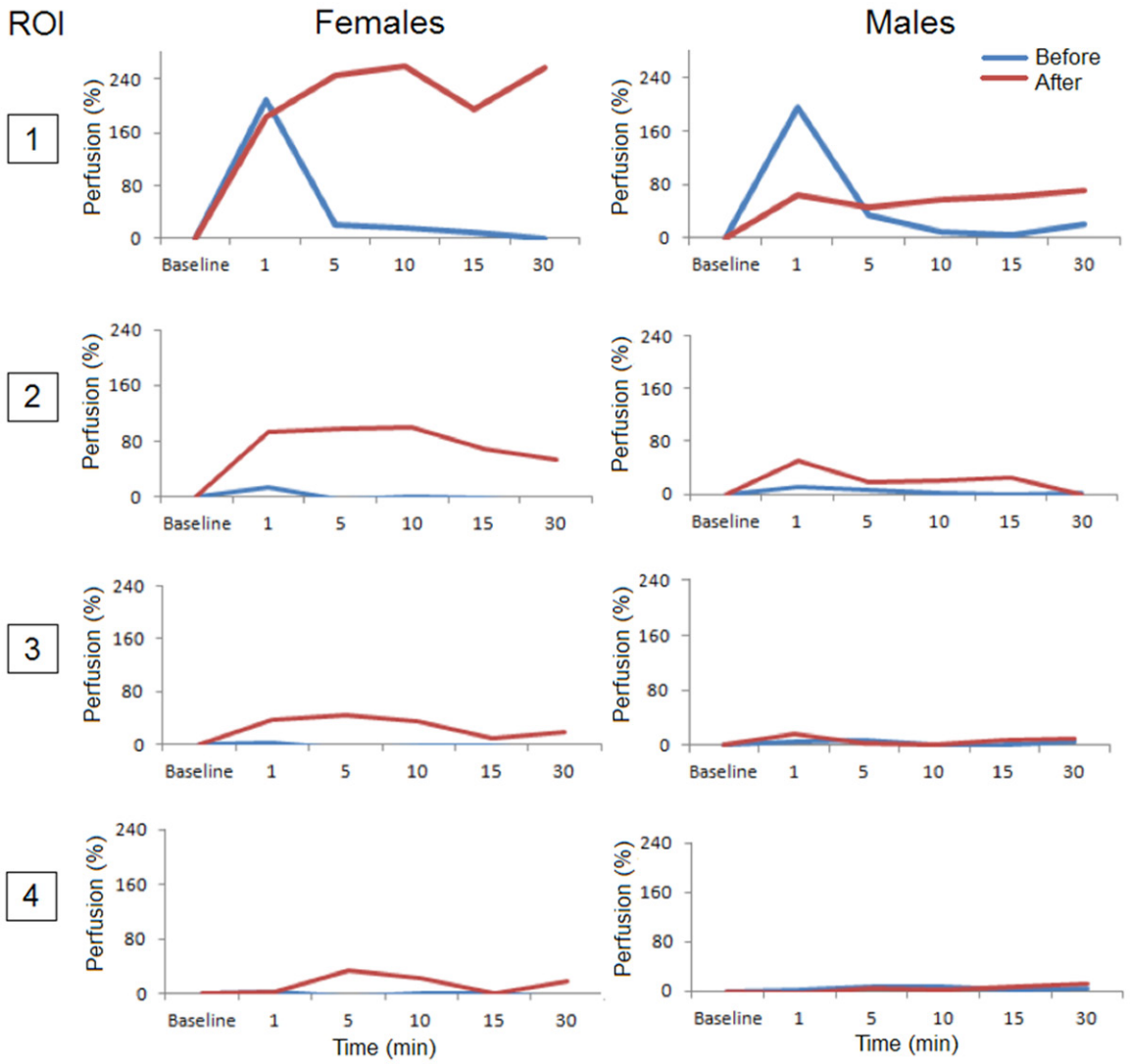

Figure 5. Blood flow (normalized to baseline, expressed in percent change) before and after capsaicin application in males and females. The temporal (baseline and 1, 5, 10, 15, 30 min after stimulation) and spatial distributions (ROI 2, ROI 3, ROI 4) proximal from the stimulation site (ROI 1) are illustrated. ROI 5 and ROI 6 are not shown due to the lack of detectable changes. For error bars see **Table 1**.

neuronal network and the superficial vascular plexus [1]. In addition to sensitizing the peripheral TRPV1-sensitive receptors (peripheral sensitization by topical capsaicin), central sensitization may also be involved, as secondary hyperalgesia may develop around a capsaicin-treated area (ROI 1 in the present study) [28]. How this contributes to the facilitated amplitude, prolonged duration, and extended spatial distribution of the vasomotor reflex is not known. Multiple animal studies have shown that dorsal root reflexes can be elicited by cutaneous capsaicin stimulation [29] and contribute to neurogenic inflammation. The peak in cutaneous blood flow after intradermal capsaicin provocation occurs after 30 min and lasts for approximately 2 hours in rats [30]. In the present study, the heat-evoked vasomotor response, when provoked in a capsaicin-treated area, peaked quickly and was restricted to the region close to the stimulation site. Therefore, this response is likely not generated by a dorsal root reflex, which would be expected to cause blood flow changes in a larger area. No changes on the contralateral side were observed, excluding spreading to the contralateral dermatome.

In the present study, an averaged 135% increase in speckle-assessed baseline cutane-

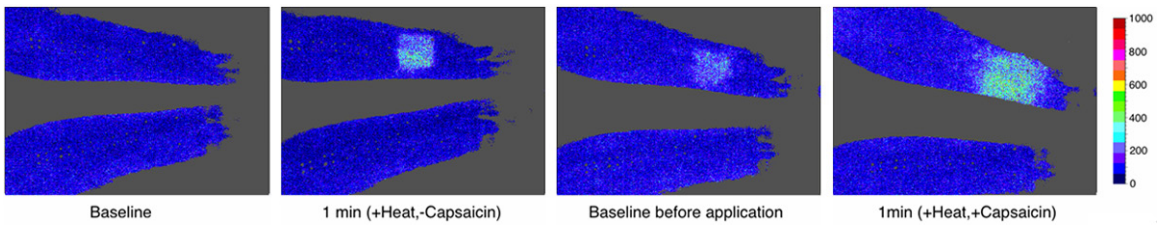

Figure 6. Typical images taken with a speckle contrast laser (FLPI). Recordings were from the two forearms at baseline, 1 min after thermal stimulation (before capsaicin), baseline before capsaicin application, and 1 min after thermal stimulation of the capsaicin treated area.

ous blood flow was found after 30 min of capsaicin application. When the thermally provoked averaged (males + females) response was added, a maximal increase between 250-290% could be found. This is expected to be around the maximum dynamic range of the peripheral vasomotor network responsible for superficial vasodilatation when assessed by the speckle technique. The maximum perfusion increase after thermal stimulation for males and females was 250% and 370%, respectively, when assessed by speckle imaging, which would most likely correspond to an increase of approximately 650-960% using LDI.

The hypothesis of the present study was that the heat-provoked response from a sensitized area would consist of a facilitated, prolonged and spatially extended vasomotor response, which may provide a new opportunity for controlled facilitated drug absorption from a cutaneous depot. The study revealed new information about the temporal and spatial distribution of the heat-evoked vasomotor response in human skin.

Sex differences

The topical capsaicin-provoked sensitization had different effects on heat-provoked vasomotor responses (size, duration and expansion) in males and females. There are numerous studies showing that females respond more strongly to an experimental pain stimulus (in particular heat, as utilized in the present study) compared with males [31] and that they experience more pronounced neurogenic inflammation and hyperalgesia after capsaicin provocation [23]. This is also supported by animal data [32]. In the present study, the heat-provoked vasomotor responses could not be considered painful (0.9 on the VAS) and were equally perceived in males and females. The sex differences, however, became very obvious after sensitization, indicating that the difference in capsaicin-induced sensitization is an important factor for the facilitated response amplitude in females. In addition, the spatial distribution as assessed by speckle contrast imaging was also larger in females (up to 6 cm (ROI 3) away from the stimulation site). The skin temperature in the capsaicin-treated area was lower (particularly in females) compared with the temperature before capsaicin, which was attributed to the immediate cooling effect of the cream when removed. In a previous experiment, topical capsaicin application was also shown to cause an initial reduction in cutaneous temperature [33].

Numerous review papers have addressed possible underlying reasons for sex differences in response to pain and sensitization [31], and most of those reasons could also play a role in explaining the sex differences in the provoked cutaneous vasomotor response after skin sensitization in this study.

Topical capsaicin

Topical capsaicin has been widely used in human experimental pain studies as a model of sensitization and neurogenic inflammation but has not been used before as a model to provoke facilitated local vasomotor responses. Capsaicin is a TRPV1 receptor agonist, which provokes activation and sensitization of peripheral mechano-heat sensitive afferents (and possibly other non-neuronal epidermal cells) in Caucasians [34] and causes local release of inflammatory mediators resulting in neurogenic inflammation [21]. Topical capsaicin releases vasoactive and inflammatory mediators such as neurokinins, prostaglandins, and acetylcho-

line [35]. In addition, capsaicin causes antidromic activation of the superficial neuronal network, and substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) release causes local vasodilation and extravasation known as neurogenic inflammation [36], which was shown in the present study as an average 135% increase in blood flow (assessed by speckle) in the area.

As repeated application of capsaicin is neurotoxic to sensory nerves and inhibits peripheral nociceptor responses and vasomotor responses (neurogenic inflammation) [37-39], many clinical applications for the treatment of pain have been suggested [40, 41]. In the present study, only one application was used and hence the neurotoxic effect was not predominant. However, if the present study had shown a massive sex-independent capsaicin facilitation of the vasomotor response amplitude, the neurotoxic effect of repeated capsaicin applications would hamper utilizing this technique on a daily basis. While no studies have investigated if repeated topical capsaicin application causes an inhibition of the heat-evoked vasomotor response, this would be expected based on the discussion above.

The 30 min capsaicin application in the present study caused primary thermal hyperalgesia resulting in increased ratings to the standardized 43°C, 60 seconds thermal stimulus (changing from almost no pain to moderate pain) and is in line with previous studies [19, 20]. In addition, the increased blood flow following application of capsaicin was confined to a few centimeters around the treated area, as previously shown [33]. A larger spatial extent of the evoked vasomotor response was specifically seen in females.

Skin temperature and blood flow for assessing vasomotor responses

In the present study, cutaneous blood flow was assessed by speckle contrast images. Speckle contrast images were more sensitive to detect sex differences in the spatial distribution after sensitization compared with thermography. Recently, the sensitivity of speckle contrast imaging and thermography was compared for assessing microvascular function [42]. The authors reported comparable reproducibility, but because the two techniques reflect different underlying mechanisms, they should be used in combination. As the different techniques have different sensitivity and resolution (temporal and spatial), it can be difficult to recommend one over the other.

The widely used LDI method estimates cutaneous vascular perfusion by scanning the tissue, which provides lower temporal resolution compared with laser speckle contrast imaging [43]. Another advantage of the speckle method is the option to measure from large areas [44]. Both of these advantages were utilized in the present study in which both arms were assessed simultaneously and sequentially.

In our previous study [1], we used LDI and found that a 43°C for 60 seconds thermal stimulus caused an increase of approximately 200%, comparable to the response in the present study using speckle imaging (for low flow rates, the conversion factor between the two techniques was 1.02).

Conclusion

The present study showed that heat-evoked cutaneous vasomotor responses are not massively facilitated by sensitization provoked by topical application of capsaicin, possibly due to a ceiling effect of the superficial vasomotor network provoked by capsaicin-induced neurogenic inflammation. Capsaicin-induced cutaneous sensitization causes sex-specific changes in the size, duration, and spatial distribution of vasomotor responses.

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

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