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# Real-Time Investigation of Skin Blood Flow Changes Induced by Topical Capsaicin

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**ABSTRACT** Capsaicin induces a localized inflammatory process known as neurogenic inflammation upon its topical administration on the skin, due to the release of various neuropeptides from the cutaneous sensory nerve endings. In this study, we investigated real-time skin blood flow changes that occur in neurogenic inflammation induced by topical capsaicin by means of *in vivo* reflectance confocal microscopy. 27 healthy subjects (15 women and 12 men, mean age ± Standard Deviation: 22.62±4.47) were administered topical capsaicin solution (Capsaicin group) or immersion oil (Control group) on the dorsal side of their non-dominant hand. At different time intervals during administration (0, 10, 25, and 40 minutes), cutaneous blood flow was evaluated using reflectance confocal microscopy and compared between the two groups. Blood flow values were higher during topical capsaicin, with significant increase after 25 ($P$=0.0160, Dunn's multiple comparisons test) and 40 minutes ($P$=0.0132, Dunn's multiple comparisons test) after its administration when compared with the initial 0 min value. Furthermore, the differences in the blood flow changes between the two groups were significant at 25 min ($P$=0.0182, Dunn's multiple comparisons test) and 40 min ($P$=0.0296, Dunn's multiple comparisons test) after capsaicin administration. Reflectance confocal microscopy allows *in vivo*, real-time evaluation of cutaneous blood flow changes within the capsaicin-induced inflammation, and this method might serve as a research model to test neurovascular reactivity.

**KEY WORDS:** skin, blood flow, *in vivo* reflectance confocal microscopy, capsaicin

## INTRODUCTION

Capsaicin, the pungent ingredient of chili pepper, mediates its effects by activating the transient receptor potential vanilloid type 1 (TRPV1) channel, expressed in the skin mainly in unmyelinated type C nerve fibers (1) but also in the thin myelinated A-delta fibers (2). Local administration of capsaicin induces a skin reaction known as neurogenic inflammation, which highlights the complex pathways linking the

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nervous system and skin, involving nerve fibers, dermal microvasculature, epidermal keratinocytes, mast cells, and other immune cells (3,4). Applied on the skin, capsaicin initially induces a burning pain associated with a transient local inflammation (5), followed by allodynia and hyperesthesia to mechanical and heat stimulation (6). Subsequent applications of capsaicin produce a progressive reduction of the initially local effects, explaining the potential use of topical capsaicin in the treatment of neuropathic conditions (7) and chronic inflammatory skin diseases (8). Moreover, the initial local inflammatory response triggered by the release of neuropeptides from the cutaneous sensory nerve endings might serve as a research model to investigate the neurovascular reactivity and also as a diagnostic tool in various functional alterations of cutaneous nerve fibers (9). In a previous paper (10), we showed that *in vivo* reflectance confocal microscopy (RCM) can be used for the evaluation of cutaneous neurogenic inflammatory reaction induced by topical capsaicin by the assessment of structural parameters of dermal blood vessels. Herein, using the same technique, we investigate the functional changes in skin capillaries by assessing the real-time blood flow variations upon topical application of capsaicin.

## PATIENTS AND METHODS

**Ethics**. The study protocol was in accordance with the ethical standards of the institutional committee on human experimentation. Before enrollment, written informed consent was obtained from all participants after receiving complete information about the study.

**Subjects**. Twenty-seven healthy, Caucasian volunteers (Female patients (F) = 15, Male patients (M) = 12) between 18 and 35 years old (mean age ± Standard Deviation (SD): 22.62±4.47) were recruited. None of the participants had a past or present history of systemic or skin diseases, infections, or allergies to capsaicin or its derivatives and they were not on any treatment. Pregnant and breastfeeding women were excluded from the study. Consumption of psychoactive substances, including alcohol and caffeine, as well as smoking and intense physical effort were prohibited 12 hours prior to the study. The subjects included in the study were randomly divided in two groups, namely the Capsaicin group with 15 subjects (F=7, M=8) and the Control group with 12 subjects (F=8, M=4).

**Capsaicin solution** was obtained by dissolving capsaicin powder (M-2020; Sigma Chemical Co, St Lois, MO, USA) at a concentration of 1% in the immersion oil (Crodamol STS oil; Croda Inc., Edison, NJ) used for RCM image acquisition.

**Study protocol**. The study was performed in a quiet room with a constant temperature of 22±1 ºC and a humidity of 50±5%, while participants were seated in comfortable chairs. After a period of accommodation with the new location, the area to be investigated was delimited on the dorsal side of the non-dominant hand. Cutaneous blood flow was evaluated using RCM before and during topical administration of capsaicin for subjects assigned in the Capsaicin group or immersion oil without capsaicin for subjects assigned in the Control group. Initial recordings allowed the assessment of baseline cutaneous blood flow in the area of interest. Then, a volume of 7.5 µL of solution (capsaicin 1%/immersion oil) was administered with a pipette (Biohit Proline Single-Channel Pipettor, 0.5-10 µL) on a plastic adhesive disc that was applied on the investigated skin area and attached to the metallic ring that was connected to the reflectance confocal microscope objective. Subsequent cutaneous blood flow changes in the investigated area were recorded at 0, 10, 25, and 40 minutes during administration.

**Measurement of blood flow using *in vivo* RCM videos**. Blood flow was analyzed using a reflectance confocal laser scanning microscope (Vivascope 1500, Lucid Inc., Rochester, NY, USA). This system uses a near infrared, less than 30 mW power laser to provide horizontal, grey-scale images or videos of the skin at different levels, down to a depth of 200-250 µm. A single field of view, usually of 500 µm × 500 µm, has a lateral resolution of approximately 1 µm and an axial resolution of 3-5 µm. At the level of the dermoepidermal junction, dermal capillaries appear in transversal section as black holes inside dermal papillae appearing as dark roundish areas surrounded by bright circles

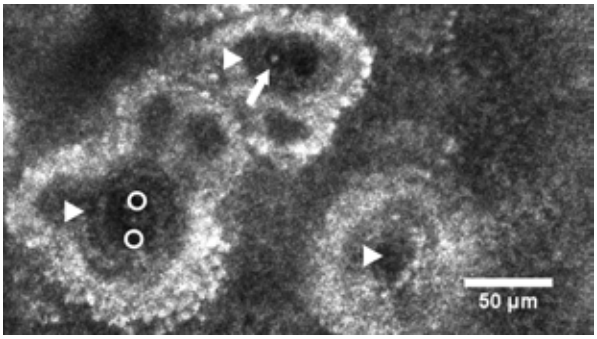


**Figure 1.** *In vivo* RCM image at the level of the dermoepidermal junction. Dermal papillae (arrow heads) appear as dark areas surrounded by bright circles corresponding to epidermal basal cells. Dermal capillaries (white circles) appear in transversal section as black holes inside dermal papillae. Blood cells (white arrow) can be observed as bright elements inside the lumina of dermal capillaries.

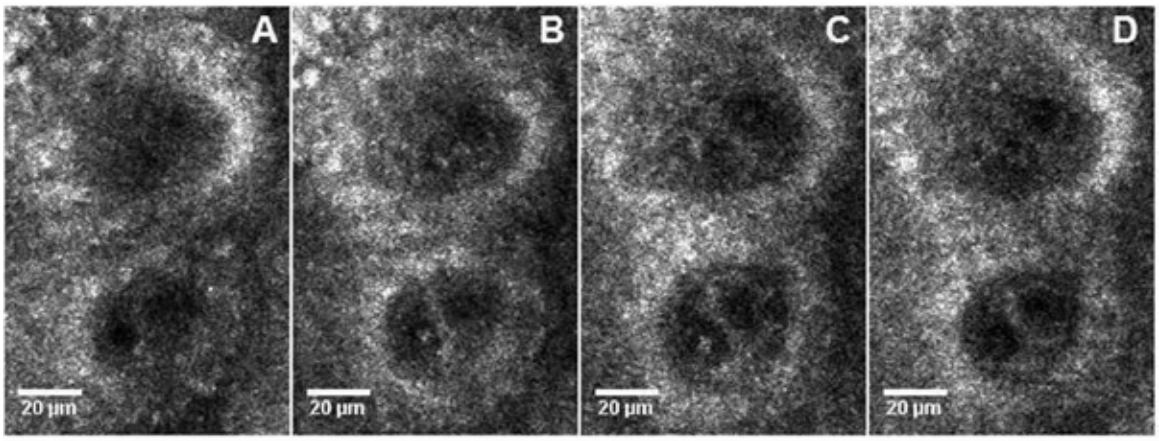


**Figure 2.** (A) *In vivo* RCM sequential images at 0 min, 10 min, 25 min, and 40 min after the administration of capsaicin, showing the blood cell flow inside the lumina of dermal capillaries. Blood cell flow becomes obvious after 10 min, with stronger effects after 25 and 40 min of topical capsaicin. (B) Mean blood flow obtained at each examination point within the Capsaicin and Control groups (**$P$<0.01, Dunn's multiple comparisons test). (C) Comparative analysis of mean blood flow at each investigation point between the two groups (*$P$<0.05, Dunn's multiple comparisons test). Mean blood flow was normalized to baseline and expressed in percent change. Error bars represent Standard Deviation (SD).

that correspond to the epidermal basal layer (Figure 1). During real-time examination, blood cells can be observed as moving bright elements in the lumina of dermal capillaries. Video sequences of 10 second duration were recorded before and at 0, 10, 25, and 40 minutes during capsaicin administration using RCM to monitor the dermal microvascular changes (Figure 2, A). At least 6 dermal papillae were analyzed in a blinded manner for each subject and at each step of the experiment. Blood cell flow was assessed in a semi-quantitative manner using a four-point grading scale corresponding to negative (0 points), weak (1 point), moderate (2 points), and intense (3 points).

**Statistical analysis.** After the assessment of blood flow for each subject after every investigation interval, the values were normalized to baseline and were expressed as percentage of basal measurements. For statistical analysis, we used SPSS 15.0 (SPSS, Chicago, IL, USA) and GraphPad Prism (Graphpad Software, Inc., San Diego, CA, USA). The differences of baseline blood flow between sexes and between the two groups were analyzed using the Mann-Whitney U test. The analysis of blood flow changes within each group was performed using the Kruskal-Wallis test, followed by Dunn's multiple comparisons test. The comparative analysis between the two groups for every investigation interval was performed using

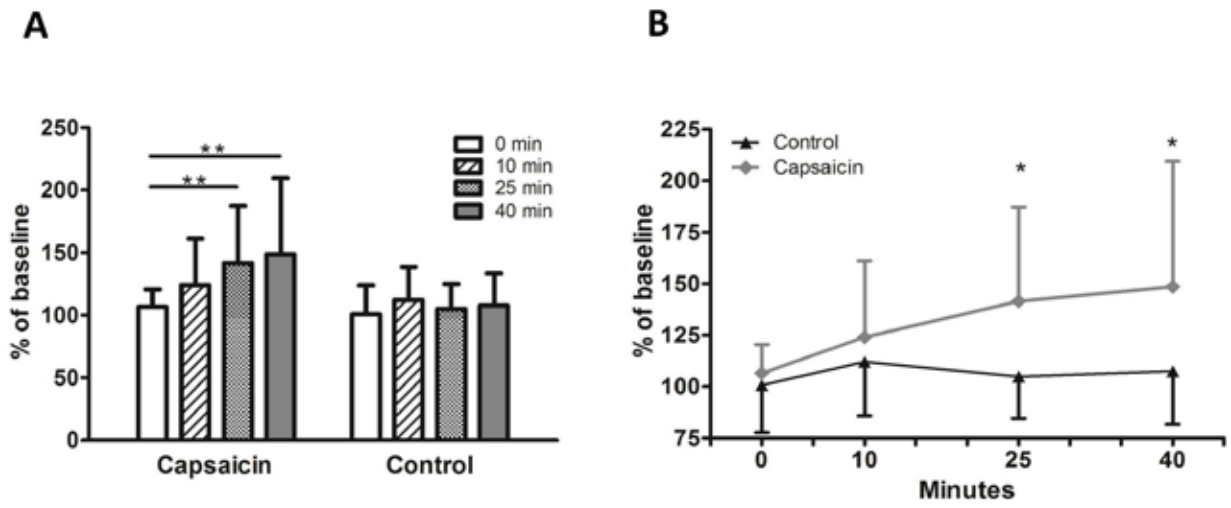


**Figure 3.**

Dunn's multiple comparisons test. The results were presented as mean ± SD. Statistical significance was accepted at a level of $P<0.05$.

## RESULTS

**Mean baseline values.** Baseline capillary blood flow measurements showed no statistically significant differences between sexes (F=1.55±0.52; M=1.59±0.33; $P>0.05$). Likewise, mean baseline blood flow values between the two groups (Capsaicin = 1.42±0.40; Control = 1.69±0.44) were analyzed and no significant differences ($P>0.05$) were revealed.

**Post-application of capsaicin or immersion oil.** The mean blood flow values normalized to baseline and expressed in percent change were further analyzed within each group at 0, 10, 25, and 45 minutes during topical administration of capsaicin or immersion oil (Figure 2, B). Overall, the blood flow values were higher during topical capsaicin in the Capsaicin group, but the increase was not statistically significant after 10 min when compared to the 0 min value. After 25 min, the blood flow increase was statistically significant as compared with 0 min ($P=0.0160$, Dunn's multiple comparisons test), but not to the 10 min value. At the 40 min investigation point, the blood flow was slightly increased compared with the previous 25 min and 10 min values, but the differences were not statistically significant. However, 40 min after topical capsaicin, the blood flow increase remained statistically significant in comparison with the initial 0 min value ($P=0.0132$, Dunn's multiple comparisons test). Conversely, the mean blood flow did not change in the Control group significantly during immersion oil topical application, with relatively constant values at the 0, 10, 25, and 40 minutes investigation points.

Finally, we performed a comparative analysis between the two groups of the semi-quantitative blood flow changes obtained at each investigation point (Figure 3, B). No statistically significant differences were observed at 0 min ($P>0.05$, Dunn's multiple comparisons test) or 10 min after capsaicin administration ($P>0.05$, Dunn's multiple comparisons test). However, the differences between the two groups were statistically significant at 25 min ($P=0.0182$, Dunn's multiple comparisons test) and 40 min ($P=0.0296$, Dunn's multiple comparisons test) after capsaicin administration.

## DISCUSSION

In the present study, we show that cutaneous blood flow changes within local neurogenic inflammation induced by topical capsaicin can be investigated *in vivo* and in real-time using RCM. Capsaicin is well-known for its local vasodilatory response in neurogenic inflammation due to the release of neuropeptides, mainly SP and CGRP from unmyelinated C nerve fibers (11). Therefore, capsaicin-induced inflammation represents an ideal research model to investigate cutaneous neurovascular reactivity (9). In order to exclude known ethnic differences in the cutaneous response to topical capsaicin (12) and to minimize the effect of age on the microvascular reactivity (13), only Caucasians and young volunteers were included in our study. For the first time, RCM was used to investigate capsaicin effects on cutaneous microcirculation, emphasizing the advantages of this novel method (10). In contrast to previously used techniques, RCM allows a noninvasive, *in vivo*, morphological, and dynamic, real-time evaluation of the cutaneous capillaries within capsaicin-induced neurogenic inflammation, with a high, quasi-microscopic resolution. As it allows the examination of the epidermis and superficial dermis, this method is considered to be ideal for the evaluation of the capillary ansae located at the dermoepidermal junction, also allowing the observation of blood cell flow within dermal capillaries (14). We examined blood cell flow within the same dermal capillaries at different time intervals. Topical capsaicin induced a significant increase of the cutaneous blood flow at 25 minutes after its administration, followed by a minor amplification at 40 minutes. These changes in dermal circulation can be easily monitored at different time intervals with RCM as it has the capacity of performing serial assessment of the same cutaneous area and can also record video sequences.

## CONCLUSION

*In vivo* RCM allows the dynamic, noninvasive, and real-time investigation of dermal blood flow within cutaneous neurogenic inflammation induced by topical administration of capsaicin. This novel method might provide useful functional information regarding cutaneous microvascularization and thus might have various applications in the assessment of peripheral neuropathies and microangiopathies.

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## References:

1. Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, *et al*. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. J Comp Neurol 2005;493:596-606.
2. Lumpkin EA, Caterina MJ. Mechanisms of sensory transduction in the skin. Nature 2007;445:858-65.
3. Roosterman D, Goerge T, Schneider SW, Bunnett NW, Steinhoff M. Neuronal control of skin function: the skin as a neuroimmunoendocrine organ. Physiol Rev 2006;86:1309-79.
4. Căruntu C, Boda D, Musat S, Căruntu A, Mandache E. Stress-induced mast cell activation in glabrous and hairy skin. Mediators Inflamm 2014;2014:105950.
5. Căruntu C, Negrei C, Ghiță MA, Căruntu A, Bădărău AI, Buraga I, *et al*. Capsaicin, a hot topic in skin pharmacology and physiology. FARMACIA 2015;63:487-91.
6. Geppetti P, Nassini R, Materazzi S, Benemei S. The concept of neurogenic inflammation. BJU Int 2008;101(Suppl 3):2-6.
7. Peppin JF, Pappagallo M. Capsaicinoids in the treatment of neuropathic pain: a review. Ther Adv Neurol Disord 2014;7:22-32.
8. Ständer S, Moormann C, Schumacher M, Buddenkotte J, Artuc M, Shpacovitch V, *et al*. Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures. Exp Dermatol 2004;13:129-39.
9. Căruntu C, Negrei C, Boda D, Constantin C, Căruntu A, Neagu M. Biotechnological advances for diagnosis of peripheral diabetic neuropathy. Rom Biotehnol Letters 2014;19:9846-58.
10. Căruntu C, Boda D. Evaluation through in vivo reflectance confocal microscopy of the cutaneous neurogenic inflammatory reaction induced by capsaicin in human subjects. J Biomed Opt 2012;17:085003.
11. Holzer P. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 1991;43:143-201.
12. Gazerani P, Arendt-Nielsen L. The impact of ethnic differences in response to capsaicin-induced trigeminal sensitization. Pain 2005;117:223-9.
13. Lee YM, Kang SM, Chung JH. The role of TRPV1 channel in aged human skin. J Dermatol Sci 2012;65:81-5.
14. Altintas MA, Altintas AA, Guggenheim M, Steiert AE, Aust MC, Niederbichler AD, *et al*. Insight in human skin microcirculation using in vivo reflectance-mode confocal laser scanning microscopy. J Digit Imaging 2010;23:475-81.