Insight in Human Skin Microcirculation Using In Vivo Reflectance-Mode Confocal Laser Scanning Microscopy

Mehmet Ali Altintas,¹ A. A. Altintas,² M. Guggenheim,³ A. E. Steiert,¹ M. C. Aust,¹ A. D. Niederbichler,¹ C. Herold,¹ and P. M. Vogt¹

Reflectance-mode confocal laser scanning microscopy allows in vivo imaging of the human skin. We hypothesized that this high-resolution technique enables observation of dynamic changes of the cutaneous microcirculation. Twenty-two volunteers were randomly divided in two groups. Group 1 was exposed to local heating and group 2 to local cold stress. Confocal microscopy was performed prior t_0 (control), directly t_1 and 5 min t_2 after local temperature changes to evaluate quantitative blood cell flow, capillary loop diameter, and density of dermal capillaries. In group 1, blood flow increased at t_1 ($75.82 \pm 2.86/\text{min}$) and further at t_2 ($84.09 \pm 3.39/\text{min}$) compared to the control ($61.09 \pm 3.21/\text{min}$). The control capillary size was $9.59 \pm 0.25 \mu\text{m}$, increased to $11.16 \pm 0.21 \mu\text{m}$ (t_1) and $11.57 \pm 0.24 \mu\text{m}$ (t_2). The dermal capillary density increased in t_1 ($7.26 \pm 0.76/\text{mm}^2$) and t_2 ($8.16 \pm 0.52/\text{mm}^2$), compared to the control ($7.04 \pm 0.62/\text{mm}^2$). In group 2, blood flow decreased at t_1 ($41.73 \pm 2.61/\text{min}$) and increased at t_2 ($83.27 \pm 3.29/\text{min}$) compared to the control ($60.73 \pm 2.90/\text{min}$). The control capillary size was $9.55 \pm 0.25 \mu\text{m}$, decreased at t_1 ($7.78 \pm 0.26 \mu\text{m}$) and increased at t_2 ($11.38 \pm 0.26 \mu\text{m}$). Capillary density decreased at t_1 ($5.01 \pm 0.49/\text{mm}^2$) and increased at t_2 ($7.28 \pm 0.53/\text{mm}^2$) compared to the control ($7.01 \pm 0.52/\text{mm}^2$). Confocal microscopy is a sensitive and noninvasive imaging tool for characterizing and quantifying dynamic changes of cutaneous microcirculation on a histomorphological level.

KEY WORDS: Diagnostic imaging, clinical application, vasodilatation, vasoconstriction, microcirculation

INTRODUCTION

Adequate microcirculation is a prerequisite for tissue nutrition and oxygen supply. In the past decades, various techniques have been used to assess the cutaneous microcirculation, such as laser Doppler flowmetry,¹⁻⁴ hydrogen clearance,⁵ near-infrared spectroscopy,⁶ hemoglobin oxygena-

tion,⁷ pulsoxymetry,⁸ nuclear magnetic resonance imaging,⁹ and dorsal skinfold chamber models.¹⁰ Whereas some techniques were solely used for basic research, the most frequently utilized noninvasive bedside method to analyse blood circulation was laser Doppler flowmetry. However, observations using laser Doppler were based mainly on dynamic flow velocity changes of relatively large vessels. Moreover, the flow velocity is a calculated value based on spectral analysis of backscattering laser light. Hence, laser Doppler provides no information on the smallest cutaneous vessels or the microvascular morphology. Thus, an adequate imaging technique to visualize the cutaneous capillaries easily and directly even on a histomorphological level was missing so far. In vivo reflectance-mode confocal laser scanning microscopy is a noninvasive high-resolution imaging technique that allows real-time visualization of the human skin up to a depth of 350 μm on cellular

¹From the Department of Plastic, Hand and Reconstructive Surgery, Medical School Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany.

²From the Department of Plastic and Reconstructive Surgery, Cologne-Merheim University of Witten-Herdecke, Cologne, Germany.

³From the Division of Plastic and Reconstructive Surgery, Burn Centre, Department of Surgery, University Hospital Zurich, Zurich, Switzerland.

Correspondence to: Mehmet Ali Altintas, Department of Plastic, Hand and Reconstructive Surgery, Medical School Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany; tel: +49-511-5328865; fax: +49-511-5328869; e-mail: altintas.mehmet@mh-hannover.de

Copyright © 2009 by Society for Imaging Informatics in Medicine

Online publication 10 June 2009

doi: 10.1007/s10278-009-9219-3

and subcellular levels. By optical sectioning, it is possible to acquire data repetitively without biopsy collection and thus causing no damage to the areas under investigation. In several studies, structures imaged by confocal microscopy were compared to conventional histological sectioning^{11–13} and a high sensitivity (88.15%) and specificity (97.60%) was determined for diagnosis of malignant skin tumors.¹⁴ Accordingly, the widespread biological application of confocal microscopy is publicized in both dermatology^{15–18} and the burn care arena.^{19,20} The purpose of the present preliminary study was to evaluate reflectance-mode confocal laser scanning microscopy for *in vivo* imaging of temperature-dependant changes in cutaneous microcirculation on a histomorphological level.

PATIENTS AND METHODS

Patients

Twenty-two healthy volunteers were randomly divided in two groups and subjected to the following procedures:

1. Group 1 (aged 22.4 ± 3.1 years, five females, six males)—local heating by immersion in a water bath at $42 \pm 3^\circ\text{C}$ for a period of 120 s
2. Group 2 (aged 24.1 ± 5.3 years, six females, five males)—local cold stress by immersion in a water bath at $3 \pm 1^\circ\text{C}$ for a period of 120 s

Measurements were performed prior (t_0 , control), directly (t_1), and 5 min (t_2) after local temperature variation using confocal laser scanning microscopy. The previously marked area of interest on the middle volar forearm was covered with an adhesive waterproof impermeable thin sheet (OpSite foil, Smith and Nephew Germany, Hamburg, Germany) prior to immersion in the water bath. All observations were performed in a standardized manner at $22 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ relative humidity. Subjects who were on medication and had a history or evidence of diabetes, hypertension, and venous or arterial disease were excluded. Ethics approval was sought from and subsequently granted by the Local Ethics Committee of the hospital, and written informed consent was obtained from all subjects.

Instrument

Reflectance-mode confocal laser scanning microscopy was performed with the Vivascope1500 (Lucid Inc, Rochester, NY, USA; cf. Fig. 1). This commercial device was designed for basic and clinical research and contrary to other confocal microscopes, it uses a gallium/arsenide laser at 830 nm wavelength. Thus, it is in the “optical window” of the skin and enables investigation of the epidermis and papillary dermis, including the upper dermal plexus and capillary loops and even the reticular dermis up to a depth of 350 μm . With a lateral resolution of 0.4 μm and a vertical resolution of 1.9 μm , this device generates cellular and subcellular images by optical sectioning. The contrast in confocal images is due to different naturally occurring refractive indexes of tissue structures, such as melanin, keratin, cytoplasm, etc. Due to the laser’s low power of 30 mW at the skin surface, no tissue damage occurs. The single frame field of view is $500 \times 500 \mu\text{m}$. The standard

Fig 1. Reflectance-mode confocal laser scanning microscopy: Vivascope1500 (Lucid Inc, Rochester, NY, USA).

interfaces are a high-speed Universal Serial Bus (USB 2.0), at a rate of 480 Mbit/s, maximum 500 mA; a FireWire S400 rate 400 Mbit/s, maximum 1.5 A; a 1000BASE-T, 1 Gbit/s, Cat 5e cable, 25 m, 82 ft; and a SATA 3 Gbit/s (internal). The resolution of a single scanned image is $1,000 \times 1,000$ pixel, 8 Bit. Digital images are saved in BMP format and videos are saved as AVI. No compression of images is used. The Vivascope uses a patented magnetic tissue ring with glass window. These features provide excellent tissue stabilization and allow tissue imaging without applying pressure. Thus, the investigated area in the center of the tissue ring is free of pressure in order to avoid the transmission into deeper layers and possibly on feeding and draining vessels.

Parameters

In confocal images, the epidermal–dermal junction reflects strongly due to its melanin content, as bright circles of basal layer with the dark focus corresponding to the dermis. The circles are surrounded by the spinous layer. In the focus of the dermal papillae, the lumina of capillary loops are visible as black holes (cf. Fig. 2). Blood flow can be clearly observed in real-time imaging as brightly reflecting erythrocytes flowing through the capillary loops. The following parameters were assessed using confocal microscopy in the epidermal–dermal junction:

- Quantitative blood cell flow per minute was established by counting in the capillary loops of the same two papillae in each volunteer, at each time point, by offline analysis of four fields of view, which were digitally recorded for 30 s. Digitally recorded video sequences were exported and stored in uncompressed AVI format directly on the computer for offline analysis. The storage size required for one movie second is about 20 MB, so a 30-s movie will require about 600 MB of disk space.
- The capillary loop diameter was evaluated in digital images of the epidermal–dermal junction using the image analysis program Image Tool (cf. “Data and Statistical Analysis” section). In four fields of view, the same two papillae were measured at each time point.
- The density of dermal capillaries per area was counted in the dermal papillae of the epider-

Fig 2. In confocal images, the epidermal–dermal junction appears as a bright circular basal layer with the dark focus corresponding to the dermal papillae. The circles are surrounded by the spinous layer. Focusing on the circles, the lumina of capillary loops are apparent as black holes. The bright structures in the focus of the black holes correspond to blood cells. After local cold stress (b), the size of the capillary loops appeared to have a smaller aspect, whereas 5 min after heating (c), larger size of capillaries were evident, compared with the controls (a).

mal–dermal junction in each volunteer, at each time point, in real-time images of eight fields of view.

To stabilize and isolate the imaging skin site, a magnetic tissue ring was applied to the skin surface. To avoid pressure, the corresponding magnetic lens-head was positioned over the ring

to capture the tissue ring. Moreover, the investigated area in the center of the tissue ring was anytime free of pressure.

Data and Statistical Analysis

The evaluation of the capillary size was performed using the image analysis program Image Tool (version 3.0, UTHSCA, San Antonio, TX, USA); download and detailed description of this noncommercial software is available at <http://ddsdx.uthscsa.edu/dig/itdesc.html>. For the evaluation of the capillary size, the first step is to import confocal images into the image analysis program. Subsequently, a bar scale was constructed from a digital image of a slide micrometer taken at the same magnification and added to all images. Each image measurement was calibrated using the tool "settings—calibrate spatial measurements". Based on the digitized images, capillary size measurements between two reference points were done by tracing a horizontal line between clearly distinguishable inner borders of the reference points of $\times 50$ magnified images. The software calculated the intracapillary distance in micrometers according to the previous calibration. The results were transferred to statistical software. Two independent examiners performed the measurements for each image. The evaluation of the confocal microscopy parameters/images was performed in a blinded manner by a statistician who was unaware of the status of the measured/recorded area. Intra- and interobserver reliabilities were estimated by using intraclass correlation coefficient (ICC) tests under a two-way mixed effects model with measures of absolute agreement. Intra-observer ICCs were calculated for each examiner considering their eight measurement values for each time point. Interobserver ICC was estimated by using the average measurement value of each time point (mean of the eight measurements) per observer. Statistical analyses were performed with SPSS 13.0 for Windows. Analysis of distribution was carried out by Kolmogorov–Smirnov test. Time values were averaged and a standard deviation calculated. Two-tailed Student's *t* test was used for comparisons of paired samples (within the time values) and for comparison between the time values, independent sample *t* test was used, since the data fit a normal distribution. *P* values < 0.05 were considered significant.

RESULTS

Group 1

Quantitative blood cell flow in group 1 at baseline was 61.09 ± 3.21 /min, increased to 75.82 ± 2.86 /min at t_1 and further to 84.09 ± 3.39 /min 5 min after local heat stress (t_2). The baseline diameter of capillary loops was 9.59 ± 0.25 μm (t_0), increased to 11.16 ± 0.21 μm at t_1 , and further to 11.57 ± 0.24 μm at t_2 . The density of the dermal capillaries increased in group 1 to $7.26 \pm 0.76/\text{mm}^2$ at t_1 and to $8.16 \pm 0.52/\text{mm}^2$ at t_2 , respectively, compared to the baseline (t_0 , $7.04 \pm 0.62/\text{mm}^2$). In group 1, the statistical analysis of both blood flow and capillary size measurements within each time point differed not significantly ($P > 0.05$; two-tailed Student's *t* test). However, comparison of the averaged time point values both for t_1 and t_2 to the baseline demonstrated significant differences (cf. Figs. 2 and 3). Statistical comparison of capillary density measurements both for each time point and for the averaged time point values for each time point to the baseline yielded no significant differences ($P > 0.05$; two-tailed Student's *t* test).

Group 2

In group 2, baseline blood cell flow was 60.73 ± 2.90 /min and decreased to 41.73 ± 2.61 /min at t_1 . By contrast, blood flow increased to 83.27 ± 3.29 /min 5 min after local cold stress (t_2). The baseline for capillary size was 9.55 ± 0.25 μm (t_0) and decreased to 7.78 ± 0.26 μm at t_1 . However, it re-increased to 11.38 ± 0.26 μm at t_2 . The statistical comparison of both blood flow and capillary size measurements within each time point was insignificant ($P > 0.05$; two-tailed Student's *t* test). However, comparison of the averaged time point values to the baseline demonstrated significant differences (cf. Figs. 3 and 4). Capillary density baseline value (t_0) was $7.01 \pm 0.52/\text{mm}^2$ and decreased significantly ($P < 0.05$) at t_1 to $5.01 \pm 0.49/\text{mm}^2$. Compared with the baseline (t_0), capillary density decreased significantly ($P < 0.05$) at t_1 (cf. Fig. 4); however, the re-increase toward baseline values at t_2 to $7.28 \pm 0.53/\text{mm}^2$ was insignificant ($P < 0.05$) when compared to the baseline value. The increase from t_1 to t_2 was significant ($P < 0.05$). Intra-observer ICCs

Fig 3. Both blood cell flow and capillary size increased significantly directly (t_1) and 5 min (t_2) after local heating, compared to the baseline (t_0). However, the capillary density per area increased insignificantly. * $P < 0.05$; # $P > 0.05$ analyzed by Student's t test for independent samples and in comparison to the baseline (t_0), - $P > 0.05$ analyzed by Student's t test for independent samples and in comparison to t_1 .

were 0.979 and 0.989 for examiners 1 and 2, respectively. Interobserver ICC was 0.718.

DISCUSSION

Our findings obtained by reflectance-mode confocal laser scanning microscopy confirm previous observations about thermoregulatory processes in cutaneous microcirculation.^{11-14,21}

Currently, this technique is used in dermatology to evaluate skin lesions suspected of malignancy.^{13,14,18,21} Accordingly, using earlier-type confocal microscopy, Sauermann et al. investigated micro-morphologic features in basal cell carcinoma.²¹ In patients with histological diagnosis of basal cell carcinoma, they observed typical alterations in the tumor parenchyma and stroma. Although confocal

Fig 4. Compared to the baseline (t_0), blood flow and capillary size decreased significantly directly (t_1) after local cooling. By contrast, both parameters increased 5 min (t_2) after local cold stress. The capillary density decreased significantly and re-increased insignificantly compared to the baseline (t_0). However, the increase from t_1 to t_2 was significant. * $P < 0.05$; # $P > 0.05$ analyzed by Student's t test for independent samples and in comparison to the baseline (t_0), + $P < 0.05$ analyzed by Student's t test for independent samples and in comparison to t_1 .

microscopy was evaluated for observing detailed morphological features of tumor parenchyma, an investigation of dynamic alterations of microcirculation using this high-resolution imaging technique has been missing so far. In the present study, an enhanced confocal laser scanning microscopy was used to investigate the cutaneous microcirculation influenced by local temperature changes on a histomorphological level. The influence of local heat stress^{1,23} or local cooling²⁴ on cutaneous microcirculation was reported in a number of papers. Based on laser Doppler flowmetry measurements, Christen et al. reported heating-induced local cutaneous vasodilatation.³ The underlying mechanism was explained as two independent effects: an initial rapid peak followed by a slower vasodilator response. Local sensory nerves were described as responsible for the vasodilatation, causing the initial rapid peak, whereas the second phase was attributed to a substantial NO component. Using confocal laser scanning microscopy, we observed a significant cutaneous vasodilatation of over 16% following local heating in all volunteers, probably due to a similar effect of local sensory nerves. Recently, Johnson et al., utilizing laser Doppler, found that hyperemia induced by local heat stress of 41°C was significantly greater than that observed with the other temperatures measured.²⁵ Although the aim of our study was not to compare different temperatures, we similarly observed vasodilatation with a significantly increased blood cell flow of over 37% following heating to 42°C. Based on the heat-induced in vivo observations, we were further able to demonstrate significant increases in blood flow and capillary size (vasodilatation) using confocal microscopy. Local cooling-induced cutaneous vasoconstriction was previously reported as a part of local control, arising from sympathetic, sensory, and autonomic nerves.²⁶ Confocal laser scanning microscopy observations confirm and quantify this fact on a histomorphological level, as we observed cooling-dependent significant vasoconstriction of over 18%. This was accompanied by a concomitant decrease in blood flow of the dermal capillaries of over 31%. Five minutes after local cooling, we observed, in accordance with previous studies, a significant reactive hyperemia of up to 37%. The density of perfused dermal capillaries, as an estimation of the absolute density of tissue perfusion, showed no significant temperature-de-

pendant variation. However, it decreased significantly immediately after cutaneous cooling, as an indication of the reduced tissue perfusion. Compared to this initial decrease, it re-increased as a sign of reactive hyperemia, as demonstrated by the observed vasodilatation and increased blood flow 5 min after cooling. Based on local temperature changes, we could thus demonstrate significant alterations on cutaneous microvascular circulation using confocal microscopy. Various diseases are associated with functional changes of microvessels and/or structural defects. Reflectance-mode confocal laser scanning microscopy provides a relatively easy approach to noninvasive investigation of structural or dynamic changes in the cutaneous microcirculation of the human skin, much like a "virtual biopsy". By optical sectioning, microcirculatory modification resulting from topical cosmetic products or chemical agents could thus be observed in real time. In pathology, several conditions could possibly be examined in an improved manner using this technique. Thus, confocal imaging offers a variety of fields of application, such as direct observation of the vascularization of biomaterials, the developing of capillary sprouts, the effects of angiogenetic drugs, long-term observation of the microcirculation, the pathophysiology of wound healing as well as the healing course of chronic or diabetic wounds. We believe that given the potential of confocal microscopy, it will probably extend its application to the assessment of the influence of arterial and venous diseases on the skin microcirculation and thus makes it also a promising tool for basic microcirculation research. Furthermore, this device may provide new diagnostic and/or therapy control options in the future. It is, however, not without limitations, as this technique allows the acquisition of high resolution images up to a depth of 350 μm only. Beyond this depth, image resolution is limited. In spite of this, in contrast to other noninvasive methods, confocal laser scanning microscopy is currently the only method that allows observation of the cutaneous micromorphology, including the microcirculation in vivo and in real time on cellular and subcellular levels. In conclusion, in vivo reflectance-mode confocal laser scanning microscopy is a sensitive and noninvasive imaging tool for characterizing and quantifying dynamic changes of the microcirculation in the human skin.

ACKNOWLEDGMENT

There are no funding sources for this study.

Conflict of interest All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence this work.

REFERENCES

1. Alvarez GE, Zhao K, Kosiba WA, Johnson JM: Relative roles of local and reflex components in cutaneous vasoconstriction during skin cooling in humans. *J Appl Physiol* 100(6):2083–2088, 2006
2. Forst T, Caduff A, Talary M, Weder M, Brandle M, Kann P, Flacke F, Friedrich C, Pfutzner A: Impact of environmental temperature on skin thickness and microvascular blood flow in subjects with and without diabetes. *Diabetes Technol Ther* 8(1):94–101, 2006
3. Christen S, Delachaux A, Dischl B, Golay S, Liaudet L, Feihl F, Waeber B: Dose-dependent vasodilatory effects of acetylcholine and local warming on skin microcirculation. *J Cardiovasc Pharmacol* 44(6):659–664, 2004
4. Arildsson M, Nilsson GE, Stromberg T: Effects on skin blood flow by provocation during local analgesia. *Microvasc Res* 59(1):122–130, 2000
5. Thomson JG, Kerrigan CL: Hydrogen clearance: assessment of technique for measurement of skin-flap blood flow in pigs. *Plast Reconstr Surg* 88(4):657–663, 1991
6. Irwin MS, Thorniley MS, Dore CJ, Green CJ: Near infrared spectroscopy: a non-invasive monitor of perfusion and oxygenation within the microcirculation of limbs and flaps. *Br J Plast Surg* 48(1):14–22, 1995
7. Wolff KD, Kolberg A, Mansmann U: Cutaneous hemoglobin oxygenation of different free flap donor sites. *Plast Reconstr Surg* 102(5):1537–1543, 1998
8. Strauss JM, Neukam FW, Krohn S, Schmelzeisen R, Borchard F: Postoperative monitoring of microvascular flap repair with pulse oximetry—initial experience. *Handchir Mik-rochir Plast Chir* 26(2):80–83, 1994
9. Franiel T, Schmidt S, Klingebiel R: First-degree burns on MRI due to nonferrous tattoos. *AJR Am J Roentgenol* 187(5):W556, 2006
10. Langer S, Sinitsina I, Biberthaler P, Krombach F, Messmer K: Revascularization of transplanted adipose tissue: a study in the dorsal skinfold chamber of hamsters. *Ann Plast Surg* 48(1):53–59, 2002
11. Gonzalez S, Gonzalez E, White WM, Rajadhyaksha M, Anderson RR: Allergic contact dermatitis: correlation of in vivo confocal imaging to routine histology. *J Am Acad Dermatol* 40(5 Pt 1):708–713, 1999
12. Rajadhyaksha M, Gonzalez S, Zavislan JM, Anderson RR, Webb RH: In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. *J Invest Dermatol* 113(3):293–303, 1999
13. Pellacani G, Cesinaro AM, Seidenari S: In vivo confocal reflectance microscopy for the characterization of melanocytic nests and correlation with dermoscopy and histology. *Br J Dermatol* 152(2):384–386, 2005
14. Gerger SKA, Kern T, Massone C, Steiger K, Richtig E, Kerl H, Smolle J: Konfokale in vivo-Laserscanmikroskopie in der Melanomdiagnostik. *Akt Dermatol*; 30 (Jahrestagung der Arbeitsgemeinschaft Dermatologische Forschung, München, 21.–23. 10. 2004), 2004
15. Agero AL, Busam KJ, Benvenuto-Andrade C, Scope A, Gill M, Marghoob AA, Gonzalez S, Halpern AC: Reflectance confocal microscopy of pigmented basal cell carcinoma. *J Am Acad Dermatol* 54(4):638–643, 2006
16. Gambichler T, Sauermaun K, Altintas MA, Altmeyer P, Hoffmann K: Acute effect of solar-simulated radiation on epidermal thickness assessed by confocal laser scanning microscopy in vivo. *J Eur Acad Dermatol Venereol* 18(5):638–639, 2004
17. Gambichler T, Sauermaun K, Altintas MA, Paech V, Kreuter A, Altmeyer P, Hoffmann K: Effects of repeated sunbed exposures on the human skin. In vivo measurements with confocal microscopy. *Photodermatol Photoimmunol Photomed* 20(1):27–32, 2004
18. Gonzalez S, Tannous Z: Real-time, in vivo confocal reflectance microscopy of basal cell carcinoma. *J Am Acad Dermatol* 47(6):869–874, 2002
19. Altintas MA, Altintas AA, Guggenheim M: In vivo evaluation of histomorphological alterations in first degree burn injuries by means of confocal-laser-scanning microscopy—more than “virtual histology”. *J Burn Care Res* 30:315–320, 2009
20. Altintas MA, Altintas AA, Guggenheim M: Differentiation of superficial-partial vs. deep-partial thickness burn injuries in vivo by confocal-laser-scanning microscopy. *Burns* 35:80–86, 2009
21. Sauermaun K, Gambichler T, Wilmert M, Rotterdam S, Stucker M, Altmeyer P, Hoffmann K: Investigation of basal cell carcinoma [correction of carcionoma] by confocal laser scanning microscopy in vivo. *Skin Res Technol* 8(3):141–147, 2002
22. Yue K, Zhang X, Zuo YY: Noninvasive method for simultaneously measuring the thermophysical properties and blood perfusion in cylindrically shaped living tissues. *Cell Biochem Biophys* 50(1):41–51, 2008
23. Thompson-Torgerson CS, Holowatz LA, Flavahan NA, Kenney WL: Cold-induced cutaneous vasoconstriction is mediated by Rho kinase in vivo in human skin. *Am J Physiol Heart Circ Physiol* 292(4):H1700–H1705, 2007
24. Kellogg Jr., DL, Liu Y, Kosiba IF, O'Donnell D: Role of nitric oxide in the vascular effects of local warming of the skin in humans. *J Appl Physiol* 86(4):1185–1190, 1999
25. Johnson JM, Yen TC, Zhao K, Kosiba WA: Sympathetic, sensory, and nonneuronal contributions to the cutaneous vasoconstrictor response to local cooling. *Am J Physiol Heart Circ Physiol* 288(4):H1573–H1579, 2005
26. Weiss T, Windthorst C, Weiss C, Kreuzer J, Bommer J, Kubler W: Acute effects of haemodialysis on cutaneous microcirculation in patients with peripheral arterial occlusive disease. *Nephrol Dial Transplant* 13(9):2317–2321, 1998