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Carter, HH, Gong, P, Kirk, RW, Es'haghian, S, Atkinson, CL, Sampson, DD, Green, DJ and McLaughlin, RA (2016) Optical coherence tomography in the assessment of acute changes in cutaneous vascular diameter induced by heat stress. Journal of Applied Physiology (1985). ISSN 8750-7587

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Optical coherence tomography in the assessment of acute changes in cutaneous vascular diameter induced by heat stress

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Running Head:

Skin vasculature and optical coherence tomography

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Abstract

There are limited imaging technologies available that can accurately assess or provide surrogate markers of the in vivo cutaneous microvessel network in humans. In this study, we establish the use of optical coherence tomography (OCT) as a novel imaging technique to assess acute changes in cutaneous microvessel area density and diameter in humans. OCT speckle decorrelation images of the skin on the ventral side of the forearm up to a depth of 500 μm were obtained prior to and following 20-25 mins of lower limb heating in eight healthy males (30.3 ± 7.6 yrs). Skin red blood cell flux was also collected using laser Doppler flowmetry probes immediately adjacent to the OCT skin sites, along with skin temperature. OCT speckle decorrelation images were obtained at both baseline and heating time points. Forearm skin flux increased significantly (0.20 ± 0.15 to 1.75 ± 0.38 CVC, $P < 0.01$), along with forearm skin temperature (32.0 ± 1.2 to $34.3 \pm 1.0^\circ\text{C}$, $P < 0.01$). Quantitative differences in the automated calculation of vascular area densities (26 ± 9 to $49 \pm 19\%$, $P < 0.01$) and individual microvessel diameters (68 ± 17 to 105 ± 25 μm , $P < 0.01$) were evident following the heating session. This is the first in vivo within-subject assessment of acute changes in the cutaneous microvasculature in response to heating in humans and highlights the use of OCT as an exciting new imaging approach for skin physiology and clinical research.

New & Noteworthy

This manuscript examines for the first time the utility of optical coherence tomography (OCT) as a novel imaging technique to visualize and quantify changes in the cutaneous microvasculature in response to acute passive heating. We hypothesized that lower body heating would induce detectable microvascular changes in the skin of the forearm. Our findings demonstrate the capacity of OCT to image vascular structures over multiple time points, and to quantify these changes using automated techniques.

Keywords: Skin microvessels, optical coherence tomography, laser Doppler

Introduction

The skin contains dense networks of microvessels that are integral to the homeostatic regulation of core body temperature and blood pressure (35) in humans. Impairment in cutaneous microvascular function, observed in ageing (3) and diseases such as chronic heart failure (10, 22) and diabetes (36), has implications for integrated cardiovascular function. Non-invasive methods capable of quantifying change in the microvessels have the potential to provide insight into physiological responses and adaptations to stressors, such as body heating and exercise training, as well as the progression of microvascular dysfunction and disease.

The shallow depth of the cutaneous microvasculature has enabled the use of non-invasive in vivo optical techniques for blood flow assessment, with the most widely adopted techniques utilizing the Doppler effect on back-scattered light (9). Laser Doppler flowmetry is commonly used to provide a surrogate measure of cutaneous blood flow by measuring red blood cell movement, or flux. This technique provides reproducible dynamic measures of change in red blood cell flux (12), whilst higher spatial resolution variants on this approach include laser Doppler perfusion imaging (37, 39) and laser speckle perfusion imaging (5), both of which provide a two dimensional map of blood perfusion, often color-coded to provide a graphical indication of areas of flow. These techniques are all limited in that i) they do not provide direct visualization of the microvascular diameter and outputs therefore reflect, but are not direct indices of, blood flow, and ii) their depth of measurement is unknown.

Alternative optical techniques provide imaging capabilities at a much finer spatial scale, although typically restricted to far smaller fields of view. Video capillaroscopy (11, 18) is

able to distinguish individual capillaries that lie within $200 \mu\text{m}$ of the skin surface. Orthogonal Polarization Spectral imaging (23) and Sidestream Darkfield imaging (19) provide high-resolution images of superficial vessels, typically over a field of view of up to 1mm^2 , but provide no indication of vessel depth. Confocal microscopy has been used to provide cellular level resolution images of vessels over a field of view and depth of a few hundred microns (1). However, structural and functional changes in the cutaneous vasculature often manifest at a resolution between these two different scales. For example, the response to a heating challenge is multifaceted, involving dilation of individual vessels at a scale of tens of microns, the recruitment of previously non-perfused vessels and increased flow across the local vascular network, apparent over a field of view of several millimetres (32, 40). It is also important to note that glabrous (hairless) and non-glabrous (hairy) skin are viewed as distinct areas in terms of vascular structure and physiological control. Glabrous skin is characterized by numerous arteriovenous anastomoses while there are very few present, if any, in non-glabrous skin (2, 8). Additionally, both are innervated by vasoconstrictor adrenergic sympathetic fibers, whilst non-glabrous skin is also innervated by vasodilator cholinergic sympathetic fibers (24).

Optical coherence tomography (OCT) (14, 16, 30) is an optical imaging modality that enables non-invasive imaging to an imaging resolution of $1\text{-}20 \mu\text{m}$ (20, 28, 29), and provides the ability to directly visualize skin microarchitecture. Based on reflectance measurements using low-power non-ionizing near-infrared light, OCT has been used to visualize microvasculature in a range of dermatological applications, such as burns scar assessment (25), psoriasis (33) and basal cell carcinoma (4). Extensions of OCT allow visualization of blood vessels, typically using either analysis of the Doppler signal or speckle decorrelation analysis of the OCT intensity signal (28). It is important to note that as these techniques rely on blood cell

movement, the diameters reported therefore refer to the internal diameter of the vessels. In this study, we examine for the first time the utility of OCT as a novel technique to visualize and quantify changes in the cutaneous microvasculature in response to acute passive heating. We hypothesized that lower body heating would induce detectible microvascular changes in the skin of the forearm. Our findings demonstrate the capacity of OCT to image vascular structures over multiple time points, and to quantify these changes using automated techniques. We present the first published images showing changes in the vascular network in response to a thermoregulatory challenge, and report these findings alongside standard laser Doppler flowmetry.

Materials and Methods

Subject Characteristics

Eight healthy recreationally active males were recruited to undertake this study (Age; 30.3 ± 7.6 yrs, Height; 1.79 ± 0.08 m, Weight; 82 ± 9 kg and BMI; 26 ± 4 kg/m²). Participants who had a history of cardiovascular, musculoskeletal or metabolic disease; were smokers (or <6 months cessation); or were taking medication of any kind, were excluded. The study was approved by the University of Western Australia's Human Research Ethics Committee and conformed to the standards outlined in the Declaration of Helsinki. Participants were informed of all experimental procedures and any potential associated risks. Written informed consent was obtained from all participants prior to commencement of the study.

Study Design

Following familiarization with the study protocol, subjects underwent 30 mins of lower limb heating (40°C) in a custom-designed recovery bath (IC-iBody; iCoolSport, Queensland, Australia). The water bath temperature was maintained and continuously circulated via a

heating pump (IC-Heat; iCoolsport, Queensland, Australia). Subjects were submerged to the level of the waist and a thick plastic sheet was then placed over the top of the bath such that the subject's upper limbs were not heated during the sessions and remained under ambient conditions. Core temperature was measured using wireless temperature pills throughout the immersion (CorTemp Sensor, HQInc, Florida, USA). Participants ingested the pill 6-8 hrs prior to the heating session and all pills were successfully read throughout the protocol. Finally, raw beat-to-beat blood pressure and mean arterial pressure was recorded using a Finometer Pro (Finapres Medical Systems, Amsterdam, the Netherlands). To assess forearm cutaneous microvascular responses to this intervention, skin blood cell flux (laser Doppler) and structure (via optical coherence tomography) was measured prior to the heating bout and again after 20-25 mins of leg heating. All studies were performed in a quiet, temperature-controlled laboratory (room temperature at baseline v 30 mins; 25.5 ± 1.3 vs $25.7 \pm 1.5^\circ\text{C}$). Subjects arrived having fasted for a minimum of 8 hours and abstained from alcohol, caffeine and vigorous exercise for at least 24 hours.

Experimental Measures

Laser Doppler flowmetry

Forearm skin red blood cell flux, a surrogate for skin blood flow, was measured using 7 Doppler array laser probes (Model 413; Periflux 5000 System, Perimed AB, Sweden). The Doppler probe was positioned on the ventral side of the forearm using double-sided adhesive rings and reinforced with Fixomull tape. The skin site for the laser Doppler probe was shaved at least 24 hr prior to the assessment. Skin temperature sensors (MLT409, ADInstruments, Australia) were placed immediately adjacent to the Doppler probe. Cutaneous vascular conductance (CVC) was calculated by dividing raw skin flux in perfusion units (PU), by mean arterial pressure.

Optical Coherence Tomography

The OCT imaging scanner (Telesto II, ThorLabs, NJ, USA) comprises a spectral-domain OCT system with center wavelength of 1310 nm and specified lateral and axial imaging resolutions of, respectively, 13 μ m and 3.8 μ m in tissue, assuming a refractive index of 1.43 for the skin (17). In OCT imaging, the axial dimension is defined as the direction of the light beam, and the lateral dimension as perpendicular to this. In keeping with terminology taken from ultrasound, each 1-D depth scan (z) is referred to as an A-scan; a 2-D ($z \times x$) OCT image is referred to as a B-scan; and a sequence of these images may be acquired to form a 3-D C-scan. Scanning was performed using a handheld imaging probe (LSM03, ThorLabs, NJ, USA; Figure 1) with a working distance (distance from probe to scan lens focal plane) of 25.1 mm and depth of focus (distance over which beam width is $\leq \sqrt{2}$ of the minimum beam width) of 0.27 mm. The system was operated at an axial scan rate of 48 kHz. A metal spacer was affixed to the probe to ensure a constant distance between the skin and the focusing optics. The probe was affixed to a 5-degrees-of-freedom articulating arm to minimize movement artifact during scanning.

Image acquisition

As with the laser Doppler skin site, all hair on and surrounding the OCT site was trimmed and shaved 24 hr prior to the testing session to eliminate the potential for shadowing artifacts. A small metal fiducial marker was positioned securely to the skin on the ventral side of the forearm immediately adjacent to the laser Doppler site using double-sided adhesive tape. The 1-cm square brass shim (thickness: 170 μ m) fiducial marker had a 5-mm diameter hole in its center through which the skin was scanned. By marking the outline of the square marker on the skin with ink, we were able to ensure that subsequent scans were co-located to

sub-millimeter accuracy. A thin layer of ultrasound gel was applied between the marker and the imaging window of the spacer to reduce refractive index mismatch at the skin surface, and improve coupling of the light beam into and out of the skin (26). Each 3-D OCT scan was $6 \times 4.5 \times 3.6$ mm ($x \times y \times z$) in size and consisted of $1024 \times 600 \times 1024$ pixels, where x and y represent the lateral dimensions, and z the axial depth dimension. The OCT beam focus was set below the skin surface for optimal subsurface imaging. The scan acquisition time was ~30 sec.

Image analysis

Following the experimental sessions, the raw 3-D OCT data image files were transferred to a desktop computer and analyzed using bespoke analysis software developed in the C++ language (20, 21, 25). Delineation of the cutaneous vasculature was achieved using a speckle decorrelation algorithm, as described in Gong et al. (20). In brief, OCT images exhibit speckle at the scale of the imaging resolution, similar to ultrasound. For stationary tissue, the speckle pattern is temporally invariant, that is, it is unchanging over time. In regions of blood flow, the speckle pattern varies with movement of the red blood cells, which is also the origin of the flux measured in laser Doppler flowmetry and laser speckle perfusion imaging. However, unlike these techniques, OCT has the ability to acquire distinct measurements at each depth in the tissue, allowing it to acquire a 3-D speckle data volume at the imaging resolution of the system, in this case, $13 \times 13 \times 4$ μm ($x \times y \times z$). Speckle decorrelation at each (x,y,z) pixel is automatically quantified by calculating the Pearson's correlation coefficient between corresponding intensity values in a sequence of co-located pairs of B-scans within a small spatial window centered on the pixel. As speckle decorrelation is more subject to noise in areas of low signal-to-noise, these areas are masked to remove them from the calculations. The threshold mask was set to 1.5 times the noise floor of the OCT measurements. The same threshold mask value was used across all data sets, although we

note that the threshold may need to be adapted for different scanners with different imaging sensitivity. In our implementation, we restricted calculation of speckle decorrelation to pixels within $500 \mu\text{m}$ of the tissue surface to exclude regions of low signal-to-noise.

For visualization and quantification purposes, 2-D maximum intensity projection (MIP) images of speckle decorrelation were generated in the $x \times y$ plane (tangential to the skin surface), taken to a depth of $500 \mu\text{m}$, and blood vessels were segmented by thresholding the correlation coefficient values. The same empirically selected threshold value was used across all scans in the study. The vessel area density was calculated as the ratio of area of blood flow in the MIP to the total scanned tissue area. An estimate of total recruited vessel length was calculated by extracting the centerline of each vessel using a skeletonization algorithm (31) and summing the length of all centerlines within the field of view. Changes in total vessel length are reported as a percentage change between pre- and post-heating, normalized by the pre-heating total length. Finally, in each subject's pre- and post-heating OCT image, four corresponding pairs of microvessels were identified and, using edge detection software, microvessel inner diameter was measured. The same threshold value was used across all data sets to establish the edges of the vessel. These vessels were not selected at random, rather, they were prioritized based on their suitability to be accurately assessed at both time points. To provide an indication of reproducibility of the quantification approach, a parallel study (21) longitudinally monitored an area of unheated skin vasculature over a period of 17 days, and observed a maximum change in vessel area density of 2.3%, and a maximum change in mean vessel diameter of $6.9 \mu\text{m}$.

Statistical Methods

Statistical analysis was performed using SPSS 21.0 (SPSS, Chicago, IL) software. Paired

samples t-tests and Pearson's correlation analysis were performed on OCT skin vessel area and individual vessel diameter values between pre- and post-limb heating. Repeat-measure ANOVAs were performed on laser Doppler CVC, core temperature, mean arterial pressure and skin temperature data pre- and post-lower limb heating. Post hoc analysis t-tests were used where significant values were found. Statistical significance was assumed at $P < 0.05$. All data are reported as means \pm SD unless stated otherwise.

Results

Effect of passive heating on OCT derived forearm cutaneous microvessel densities, length and diameter

The acquisition of accurate and clear OCT images of the cutaneous vasculature was successful at baseline and again during the heating bout. Figure 2 shows representative pre- (Figures 2A, C and E) and post-heating (Figures 2B, D and F) MIP images from three subjects. Localized estimates of OCT speckle decorrelation are color-coded, with high decorrelation indicating vasculature. The same color scale was used for all images, allowing a graphical representation of the increase in vasculature flow and area. Baseline cutaneous vascular area densities were $26 \pm 9\%$ (Table 1). A significant increase in vessel area density was evident following 20-25 mins of lower limb heating (26 ± 9 to $49 \pm 19\%$, $P < 0.01$). Average total vessel length over the field of view increased from 149.2 ± 59.2 to $237.4 \pm 60.3 \text{mm}^2$ ($P < 0.01$, Table 1), while the average individual change in total vessel length was $+79 \pm 66\%$. Individual microvessels identified in both pre- and post-heating scans were subsequently measured for diameter. Figure 3 shows magnified views of the changes observed in individual vessels between baseline (Figures 3A, C and E) and heating scans (Figures 3B, D and F). Skin microvessel diameters were $68 \pm 17 \mu\text{m}$ at baseline and increased significantly to $105 \pm 25 \mu\text{m}$ following 20-25 mins of heating ($P < 0.01$, Figure 4). Finally, the OCT

outcome measures were directionally similar, as absolute change in microvessel density and diameter between pre- and post-heating were significantly correlated ($r=0.78$, $P<0.05$).

Effect of passive heating on forearm laser Doppler skin blood flow, temperature, core body temperature and hemodynamics

A repeat measures ANOVA revealed a significant increase in forearm PU across the 30-min passive heating bout (baseline v 30 mins; 19 ± 14 to 151 ± 23 PU, $P<0.01$). Similarly, CVC and skin temperature increased significantly throughout the 30-min passive heating bout (both $P<0.01$, Figures 5A and B). As expected, core temperature also increased significantly during the heating bout ($P<0.01$, Figure 5C) whilst mean arterial pressure decreased significantly ($P<0.05$, Figure 5D).

Discussion

The aim of the present proof of principle study was to test the utility of optical coherence tomography (OCT) as a novel technique to visualize changes in cutaneous microvasculature in humans *in vivo*. OCT images were acquired from a segment of forearm skin, before and during passive heating of the lower limbs in resting participants, along with the traditional approach of skin blood flow assessment using laser Doppler flowmetry. Our principle finding is that quantitative differences in OCT-derived skin morphology were evident following passive heating; specifically, a significant increase in total vessel area density, as well as increases in individual microvessel diameters. This is the first *in vivo* within-subject assessment of acute changes in the microarchitecture of the cutaneous vasculature in response to a physiologically relevant thermoregulatory challenge in humans.

In the present study, lower limb heating in 40°C water was successful at elevating core temperature and stimulating systemic thermoregulatory reflexes, resulting in increases in forearm skin blood flow. OCT images of the ventral surface of the forearm skin were obtained to a depth of 500 μm prior to and during heating. The use of a fiducial marker was successful at ensuring the same microvascular area was imaged at both study time points, which can be confirmed by visual inspection of the matched images, noting the presence of common structural vascular patterns, such as those highlighted in Figure 3. Two analysis approaches were used to quantify changes in cutaneous microvasculature, total cutaneous vessel area density and microvessel diameter change. The average resting diameters of the vessels was 68 μm . This increased by 54% to 105 μm following heating. To our knowledge, this is the first study to directly visualize and report changes in cutaneous diameter in the same microvessels pre and post an acute intervention in humans. The dilation response observed in the current study highlights the magnitude of vasomotor change that can occur in the skin and reinforces its active role in thermoregulation and blood flow distribution and blood pressure regulation (35). Braverman has previously characterized microvascular ultrastructure and reported skin capillary diameters in the range of 10-12 μm , and larger terminal arterioles and venules of the superficial papillary plexus are approximately 25-35 μm (6). The diameter of the vessels measured in the present study (~68 μm) in the region of the superficial papillary plexus most likely represent larger arterioles and collecting venules. Although these larger microvessels were chosen for comparison between pre- and post-heating in the current study, smaller vessels, such as the terminal arterioles and venules, were distinguishable in the speckle decorrelation OCT images (30-40 μm) and were included in quantification of vessel area density and total vessel length. Vessels below 30 μm were excluded due to partial volume effects.

Laser Doppler flux was also measured in the present study immediately adjacent to the OCT skin sites. Laser Doppler flowmetry has been used extensively and has provided valuable insights into basic skin vascular physiology and control mechanisms, as well as indications of chronic changes in skin microvessel function in response to aging, exercise and pathological conditions (3, 22). The outcome parameter of laser Doppler flux is in arbitrary units, defined as perfusion units, and is based on measurement of the Doppler shift created by moving red blood cells (34). However, current commercially available laser Doppler systems cannot assess microvascular structure or absolute blood flow, as the measured flux value is a combined function of both average flow speed and red blood cell concentration, being linear for flow and non-linear for concentration. In addition, the angle of the emitting laser beams relative to the moving red blood cells is unknown. In keeping with previous literature and consistent with the OCT data, we observed significant increases in skin flux in response to lower limb heating (7). The laser Doppler system used in the present study has 7 laser array Doppler probes with a source-detector separation of 0.25 mm and a laser wavelength of 780 nm. Monte Carlo simulations by Fredriksson et al., using an ideal skin model, calculated that the majority of the measured signal came from depths below 530 μ m in forearm skin (15). Because of the non-linear relationship between measured flux and red blood cell concentration, their work found that this depth increases to 660 μ m in forearm skin undergoing a thermoregulatory challenge. This suggests that one confounding factor in laser Doppler flux measurements may be changes in the depth of the skin interrogated under varying physiological conditions. In contrast, the OCT signal was depth-limited to 500 μ m, providing insight to the more superficial vasculature. The depth range of the measured OCT signal is controlled and set through OCT's use of low-coherence interferometry of the near infrared light (14), hence remaining consistent between both pre- and post-heating measurements. Indeed, it is likely that differences in the depth of measurement may be

responsible for the lack of correlation between the two techniques in the current study. Regardless, these two assessment techniques provide complementary information regarding the microvasculature at different depths for a more complete assessment of acute changes.

We note that some imaging artifacts are visible in the OCT images. Specifically, these present as parallel lines in the MIP speckle decorrelation images visible in Figures 2 and 3. These artifacts are due to movements in the tissue occurring between the acquisition of adjacent B-scans. Analysis of the individual B-scans showed the bulk movement to be localized within the B-scan, not global across the entire B-scan. As movement of the scanning probe would result in global movement, this suggests that it reflects physiological movement of the tissue, such as the pulsatile movement of tissue mechanically coupled to larger blood vessels. Supporting this, we note that the periodicity of the artifact is comparable to the cardiac rate. The effect of these artifacts on the calculation of vessel area density was minimized with a simple post-processing algorithm that detected and removed these lines from calculations, using their characteristic orientation parallel to the B-scan plane, and omitting B-scans with total speckle decorrelation greater than an empirically selected threshold. We note that pulsatile variation in the diameter of the measured superficial vessels was not evident in our scans. Whilst such changes may be expected, we believe that such variations may lie below the imaging resolution of the OCT system. We also note that limiting calculations to a fixed depth ($500\ \mu\text{m}$ in these experiments) may lead to omitting vessels as limb diameter increases with increased blood flow. However, we were unable to find evidence of this effect in the pre- and post-heating MIP images generated as part of this study.

Future Developments

We note that the OCT speckle decorrelation measurements presented provide quantification of microvessel geometry, however as the rate of speckle decorrelation is not directly related to blood flow velocity, it does not demonstrate relationships such as Poiseuille's law. Future development of the OCT technique could provide accurate measures of skin blood flow velocity and recent work has proposed quantitative speckle analysis techniques that are robust over a wide range of signal-to-noise ratios (38). Combined with the development of automated user-independent blood vessel diameter measurement software, which would allow for the comparison of all microvessel dilation/constriction changes, true blood flow quantification is conceivable, with the potential to be validated against skin-like phantoms with embedded vascular structures (13). In addition, a recent study has successfully tracked and compared the same cutaneous microvasculature within 1-2mm accuracy across a time period of up to 5 months (20). The utility of OCT in a longitudinal study setting is very promising and this development would represent a significant step forward in the field of skin physiology and clinical research, as functional changes have been documented in response to a number of interventions, such as exercise, passive heating and ageing, yet very little is known about structural adaptations that occur in the skin. In this proof of principle study, we chose to compare four microvessels between the pre and post heating scans, however as can be seen in Figures 2 and 3, after identifying similar vascular patterns between scans, the potential for the identification and comparison of a larger cohort of vessels is possible. Finally, at present, OCT can image blood vessels with diameters in the range of 25-150 μm , therefore, capillary networks ($\sim 10\text{-}12\ \mu\text{m}$) are not yet distinguishable. Recent developments, improving the imaging resolution of OCT to 1-2 μm , have the potential to enable delineation of the capillary beds in future (27).

In summary, this study confirms that OCT can provide clear and accurate non-invasive morphological depictions of the cutaneous vasculature. This is the first study to directly visualize and report changes in cutaneous microvascular diameter following a passive heating intervention in humans. Two quantification methods were used to assess changes in cutaneous microvascular morphology in response to passive heating, which revealed significant increases in blood vessel numbers and size. OCT speckle decorrelation imaging is a very exciting new technique for the assessment of changes in cutaneous microvascular structure in response to acute provocations, as well as longer term chronic interventions in humans (20).

Grants

P. Gong, S. Es'haghian and R. A. McLaughlin are supported by grants from the National Health and Medical Research Council, Australia. R. W. Kirk is supported by a grant from Chevron Australia and the Fiona Wood Foundation. H. Carter is supported by a grant from the National Heart Foundation. D. J. Green is a National Health and Medical Research Council Principal Research Fellow (APP1080914) and this research is supported by a grant from the Australian Research Council (ARC 130103793).

Disclosures

None of the authors have conflicts to disclose.

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Figure Legends

Figure 1. A demonstration photo showing the handheld OCT imaging probe and scanner set up, with the probe covering the metal fiducial marker on the skin surface.

Figure 2. Examples of acquired OCT speckle decorrelation MIP images of the forearm cutaneous vasculature for three subjects at baseline (A, C and E) and again during heating (B, D and F). Color-scale indicates localized measurements of OCT speckle decorrelation. The same color-scale was used for all images.

Figure 3. Pre- and post-heating OCT images of a fourth subject (A and B). Insets show magnified images of corresponding microvessels (C, E and D, F), providing a graphical indication of acute changes in blood vessel diameter in response to a thermoregulatory challenge.

Figure 4. Mean and individual microvessel diameter change for all participants at baseline and during heating.

Figure 5. Change in laser Doppler forearm cutaneous vascular conductance (CVC) (A), skin temperature (B), core temperature (C) and mean arterial pressure (D) throughout the 30 minute heating protocol. #significantly different from baseline at $P=0.05$. *significantly different from baseline at $P<0.05$.

Table 1. Cutaneous blood vessel area (out of 27 mm²) and vascular density area values (%) pre and during passive heating.

Participant	Baseline			Heating		
	Blood vessel area (mm ²)	Density (%)	Total length (mm ²)	Blood vessel area (mm ²)	Density (%)	Total length (mm ²)
1	2.3	9	40.1	6.1	23	129.8
2	6.0	22	126.4	9.7	36	206.4
3	6.4	24	134.9	10.3	38	215.5
4	11.0	41	255.9	19.9	74	271.7
5	7.2	27	152.7	16.8	62	302.2
6	6.6	25	148.1	19.9	74	317.6
7	7.7	29	169.5	10.5	39	213.6
8	8.2	30	165.6	12.4	46	242.6
Average	6.9 ± 2.4	26 ± 9	149.2±59.2	13.2 ± 5.1*	49 ± 19*	237.4±60.3*

*Significantly different from Baseline at P<0.01.