Microvascular Research 148 (2023) 104540

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Microcirculatory response to cold stress test in the healthy hand

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ARTICLE INFO

Keywords:
Cold intolerance
Cold stress test
Microcirculation
Perfusion

ABSTRACT

Objective: Cold sensitivity of the fingers is common in several conditions. It has been linked to digital vasospasm, microvascular dysfunction, and neural mechanisms. This study aimed to investigate the normal digital microvascular response to a cold stress test in healthy individuals using Laser Speckle Contrast Imaging (LSCI).
Methods: Twenty-six healthy individuals, mean age 31 (SD 9) years were included. Skin perfusion of digits II-V was measured using Laser Speckle Contrast Imaging before and after a standardized cold stress test. Changes in skin perfusion from baseline were analyzed between hands, digits, and sexes.
Results: Skin perfusion was significantly ($p < 0.0001$) affected by cold provocation in both the cold exposed and the contralateral hands in all participants of the study. This effect was significantly different between the radial (digit II and III) and the ulnar (digit V) side of the hands ($p < 0.001$). There was a trend towards a larger decrease in perfusion in men (ns), and a faster recovery to baseline values in women (ns). A larger inter subject variability was seen in perfusion values in women.
Conclusions: The normal microvascular response to cold provocation may involve both centrally and regionally mediated processes. When exposing one hand to a cold stress test, the contralateral hand responds with simultaneous but smaller decreases in perfusion.

1. Introduction

Hands and feet are important thermoregulators of the body (Taylor et al., 2014). The normal physiological response to cold exposure of the hands includes a decrease in finger temperature and blanching of fingers, indicating that microvascular blood flow decreases because of the exposure (Cheung, 2015). In pathological conditions, such as in autoimmune connective tissue diseases, Raynaud's phenomenon, or after an injury to the hand, these responses are more pronounced and the return to normal blood flow slower (Rosato et al., 2011; Della Rossa et al., 2013). Such responses are often referred to as cold sensitivity or cold intolerance (Campbell and Kay, 1998), and are associated with experiences of pain, sensory changes, weakness and stiffness, with or without discoloration (Irwin et al., 1997). Cold sensitivity has further been associated with high levels of disability and poor health-related quality of life (Carlsson et al., 2010; Novak and Mackinnon, 2016). Cold sensitivity often remains a debilitating symptom that is often persistent (Collins et al., 1996).

To better understand the mechanisms involved in cold sensitivity, it is important to thoroughly understand the normal physiologic response to cold exposure. Considering that the sensory function of the hand is controlled by different nerve trunks, and assuming that neural mechanisms may be involved (Cheung, 2015), the question arises whether these physiological responses differ between radial fingers innervated by the median nerve or ulnar fingers innervated by the ulnar nerve.

Earlier studies have been performed to investigate the reaction of the fingers to a cold stress test. These studies have, however, been limited to measurements on isolated fingers and single point measurements on very small measurement areas in single fingers, preferentially using laser doppler flowmetry (LDF) techniques (Rosato et al., 2011; Kanetaka et al., 2004) and thermography techniques (Ruijs et al., 2009). With Laser Speckle Contrast Imaging (LSCI), which is a camera-based method,

Abbreviations: CST, cold stress test; NRS, Numerical Rating Scale; LSCI, Laser Speckle Contrast Imaging; LDF, laser doppler flowmetry; CIVD, cold induced vasodilatation; ROI, regions of interest; Dig, digit; HAVS, Hand-arm-vibration syndrome; CL, contralateral; PU, perfusion units.

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https://doi.org/10.1016/j.mvr.2023.104540
Received 22 February 2023; Received in revised form 17 April 2023; Accepted 17 April 2023
Available online 20 April 2023

changes in skin blood flow in the hands can be measured with a much higher spatial and temporal resolution as it allows for video recordings of all fingers and both hands simultaneously (Mirdell et al., 2019a; Heeman et al., 2019; Guven et al., 2023). LCSI is an optical method which uses a divergent laser to illuminate the area being measured. When an area is illuminated the laser light is backscattered after interaction with a biological tissue and then forms an interference pattern (speckle pattern) which is captured by the detector. The variance in the observed speckle pattern is quantified through analysis of pixel fields. At the wavelength in question the variance in the speckle patterns arises mainly due to motion of hemoglobin molecules and correlates strongly to the perfusion of the tissue (Heeman et al., 2019; Senarathna et al., 2013). The advantage of the LSCI, to the LDF is that it offers the possibility of examining larger areas simultaneously over an extended period of time (Roustit et al., 2010), and hence performing for example analyses of vasomotion in large areas, for in depth analysis of autonomic functions of the microcirculation. This may, in the future, be used for clinical follow up of patients with for example Raynaud's phenomenon, systemic sclerosis and cold sensitivity after injuries to the hand (Della Rossa et al., 2013). It can also provide an assessment tool for treatment results in patients suffering from cold sensitivity due to an injury to the hand or other diagnoses with disturbed microcirculation. In the clinical setting, the possibility also arises of investigating the healthy hand and the injured hand of the same patient simultaneously.

A recent study by Stjernbrandt et al. used LSCI to investigate the microcirculatory pattern in individuals who experienced cold sensitivity but without underlying traumatic injuries to the hand. Their findings included disturbances in the microvascular regulation and it was stated that LSCI could be a useful tool in future studies on cold sensitivity (Stjernbrandt et al., 2020), and that LSCI's large spatial resolution had the ability to test if cold stimulation has central microcirculatory effects on the contralateral hand. It has not been clearly elucidated if there is a difference in the reaction to cold exposure between men and women, and if sex hormones have an impact on the microcirculation after cold exposure (Bartelink et al., 1993; Jay and Havenith, 2004). Recent studies indicate that there is no such difference in the response to a cold stress test under normoxic conditions (Hohenauer et al., 2022), however this has not been studied with the benefits of the LSCI system.

The overall aim of this study was therefore to investigate the physiological digital microvascular response to a standardized cold stress test over time in healthy individuals using Laser Speckle Contrast Imaging (LSCI). We also wanted to investigate the response in the contralateral hand when the other hand was exposed to cold, and whether there was a difference in normal response to cold exposure in between the sexes. It was hypothesized that there is a difference in reaction pattern between the fingers innervated by the median nerve (radial fingers) and the fingers innervated by the ulnar nerve (ulnar fingers). A slower return to baseline was suspected, due to cold sensitivity being more common in patients with ulnar nerve injuries than in median nerve injuries (Lenoble et al., 1990).

2. Materials and methods

2.1. Participants

Twenty-six healthy individuals were included in the study (Table 1). They were recruited by advertisement on flyers placed around Linköping University hospital and the Medical Faculty of Linköping University. The inclusion criteria were age between 18 and 60 years, non-smokers, healthy without any ongoing medication (excluding oral contraceptives and hormonal replacement therapy) and without pathological cold sensitivity. The exclusion criteria were previous nerve injury of the hand, pharmacological treatment (except for contraceptives), smoking or nicotine use, previous surgery to the hand, neuropathies of the hand, severe cold sensitivity and not being able to understand Swedish in oral or written language. The study participants were requested to abstain from caffeine intake at the days of the study and to abstain from strenuous physical exercise two hours prior to the experiments. The study was done at Linköping University Hospital between March 2019 and March 2020. Before the inclusion the participants gave their oral informed consent. The study was approved by the Linköping University Ethics Board (2018/187-31).

Table 1
Demographics of the participants.

	Mean (SD)
Number of participants	26
Age (years)	31 (9)
Sex (male/female)	13/13
BMI (kg/m^2)	24 (4)
Dexterity (dx/sin)	24/2
Blood pressure (mm Hg)	119/76 (16/10)
Saturation (%)	99 (1)
Pulse/min	68 (10)

BMI = Body Mass Index, SD = standard deviation, Dx = Dexter, Sin = Sinister.

2.2. Equipment

Changes in microvascular perfusion in the digits were measured using Laser Speckle Contrast Imaging (PeriCam PSI System, Perimed AB, Järfälla, Sweden). The LSCI uses a near infrared laser, operating at a wavelength of 785 nm and with a resolution of up to 33 μm/pixel. The measurement distance was set to 30-35 cm and at the given distance, the resolution was around 1.3 measurement points/mm^2. The images in the study were acquired with 5,3 images/s. The LSCI technique has been considered a reliable technique compared to other commonly used techniques for monitoring the blood flow and has also shown excellent reproducibility (Roustit et al., 2010). This has been investigated in studies of reconstructive surgery to assess tissue viability (Berggren et al., 2021a; Zötterman et al., 2019; Zötterman et al., 2020; Berggren et al., 2021b) and in the assessment of burn wound healing (Mirdell et al., 2019b; Mirdell et al., 2018), as well as in the dermatological (Margouta et al., 2022) and ophthalmological field (Heeman et al., 2019). The main source of error to the method is considered to be the susceptibility to motion artifacts (Mahé et al., 2011).

The LSCI system was calibrated according to the manufacturer's guidelines (PeriCam psi System, Perimed AB Järfälla Sweden).

2.3. Cold stress test

Cold stress tests were performed according to the method described in ISO 14835-1:2016, which is designed for evaluation of peripheral vascular function in several conditions such as Hand-arm-vibration syndrome (HAVS) (Standardization IOf, 2016).

Study participants were seated with their feet stable on the floor and arms resting on the armrests to minimize disturbances. The hands were placed on a desk with the palmar side down, the thumbs and the index fingers pointed towards each other and with a small space between each fingertip. The hands were placed on a green non reflective surgical sheet under the LSCI camera perpendicular at a distance of 30–35 cm (Fig. 1a). Each participant was then acclimatized to room temperature for 25 min while measuring the perfusion (Fig. 1b). The mean room temperature was 21,9 °C (SD 0,5 °C). After the initial baseline recording, the registration was paused shortly to equip the right hand with a water-proof glove to avoid subsequent evaporation-effects upon emerging (Suizu and Harada, 2005). The right hand was then completely immersed, to 5 cm distal to the radiocarpal joint, into a water bath with a temperature of 11.9 ± 0.2 °C for 5 min. At minute 1 and 5 the participants were asked about current experience of pain or discomfort according to a Numerical Rating Scale (NRS scale). During the cold provocation, the perfusion in the other, non-exposed hand was continuously registered with a

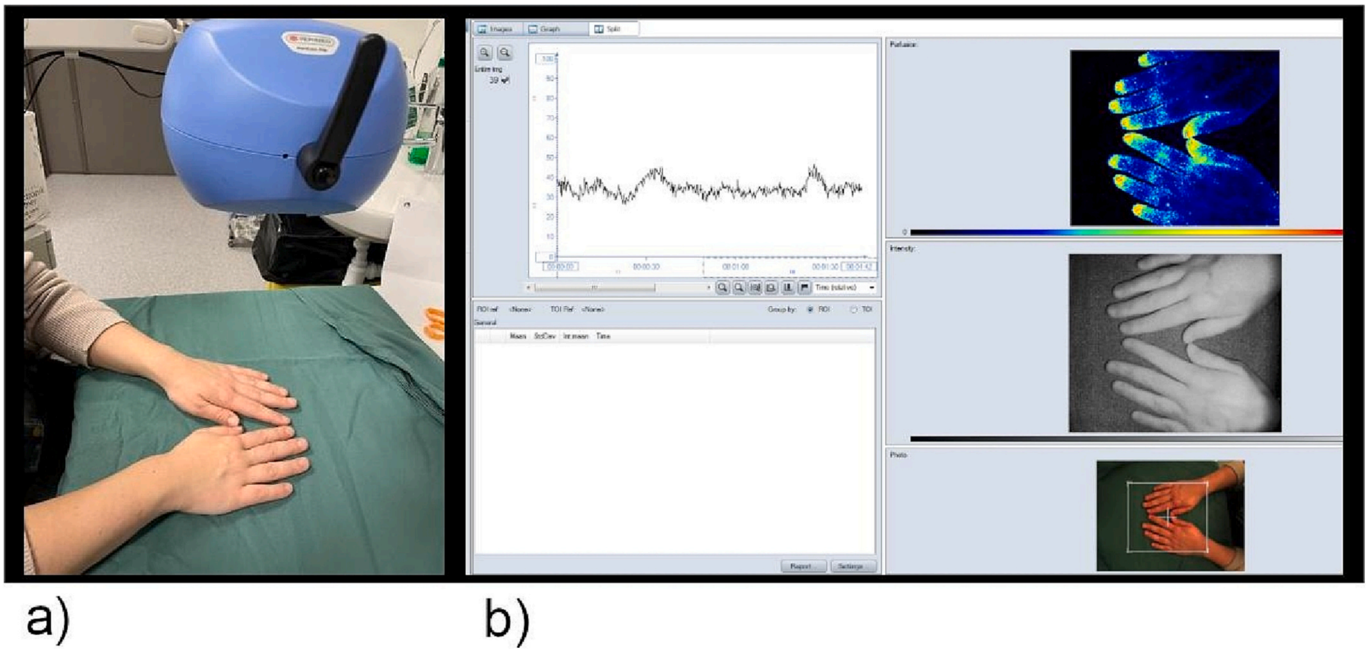

Fig. 1. Placement of the hands for measurement of skin blood flow before and after cold stress test (left). Laser Speckle Contrast Imaging software showing trend in average perfusion, perfusion map, intensity image and live image (right).

temporal resolution of 1 image per second. When 5 min had passed, the recording was paused again while the cold-exposed hand was taken out of the water and the glove removed. The hand was then again placed under the LSCI camera to continue the perfusion measurement for another 25 min. During this time, the participant was repeatedly asked about experiences of pain or discomfort at 1, 5, 6, 10 and 15 min after the initiation of the cold provocation (Fig. 2). After a recovery time of 25 min the registration was completed. The total duration of the registration was 55 min. After the recording the saturation, pulse and blood pressure were measured with a pulse oximeter and an automatic blood pressure cuff on the left index finger and left arm respectively (Table 1).

2.4. Data analysis

The images were analyzed using the LSCI system's software (PimSoft 1.5, Perimed AB, Järfälla, Sweden). Circular regions of interest (ROI) were placed on the nailbed of digits II to V of both hands. The ROI were marked manually in the first images and then adjusted to correct for movement of the hands in subsequent images. In each ROI and for each image, the mean perfusion was calculated by the software and presented in perfusion units (PU) as earlier described (Heeman et al., 2019; Senarathna et al., 2013). Mean perfusion values were then exported for further statistical analysis. The mean perfusion values in each ROI were analyzed at specific time points: at 5 min before CST, and at 1, 5 and 20 min after the CST. Measurements at 1, 5 and 20 min after the CST are presented as change from baseline (i.e., perfusion value 5 min before the CST). We used the D'Agostino and Pearson normality test to check for normality of the data. The results indicated that the data were normally distributed, with a few exceptions as a result of outliers, mainly during cold provocation.

Differences in perfusion between cold-exposed and contralateral hands, between the radial digits (II, III) and the ulnar digits (IV and V) and between women and men were analyzed using two-way analysis of variance. Sidak's post-tests were used to correct for multiple comparisons. In the analyses of differences between digits, digit IV was excluded because of its double innervation by both the median and ulnar nerves.

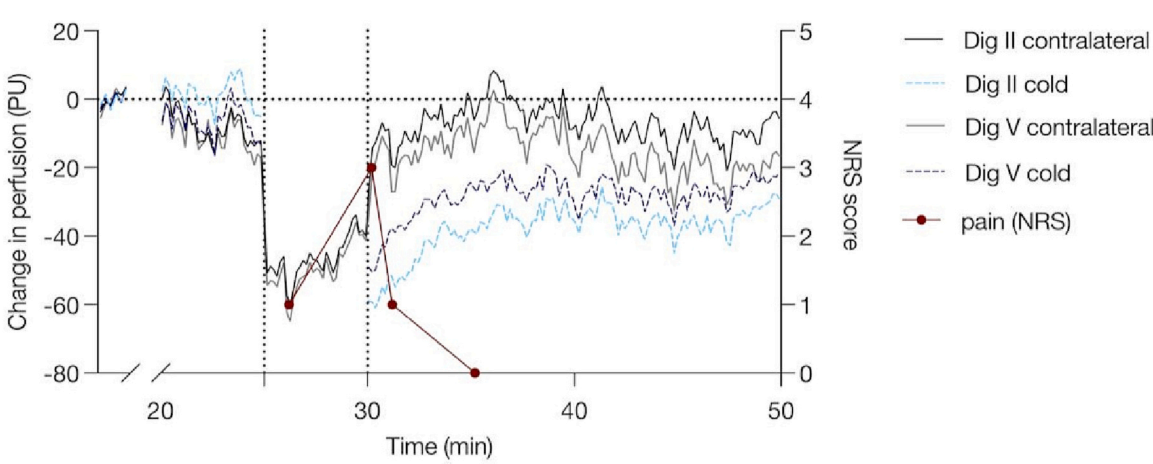

Fig. 2. Change in microvascular perfusion in the fingertips of digits II and V of the hands before (0–25 min) during (25–30 min) and after (30–50 min) cold stress test of the right hand. Error bars are left out for clarity. NRS: Numerical Rating Scale. PU: perfusion units.

Effect sizes were calculated using Cohen's *d*.

All statistical analyses were done using GraphPad Prism (version 9.0.0, GraphPad Software, San Diego, CA). A p-value of <0.05 was considered statistically significant.

3. Results

All study participants tolerated the experiments. An overview of the physiological parameters of the participants is given in Table 1.

Mean (SD) values of absolute perfusion, relative (%) change from baseline, and effect sizes in digits II-V at baseline, 1 min after the start of CST and 5 and 20 min after the CST are shown in Table 2 for women, men, and all participants.

The typical change in perfusion in the fingertips before (0–25 min), during (25–30 min) and after (30–50 min) the CST is shown in Fig. 2. In the cold exposed hand, the perfusion was significantly reduced for at least 20 min after the exposure. During the CST, a pronounced decrease in the perfusion was seen in all fingers of the contralateral hand, which recovered within 5 min after the initiation of the CST.

Baseline perfusion values are further presented in Fig. 3. At baseline, there was no significant difference in perfusion between digits. For all time points after the CST (1, 5, 20 min) (Fig. 4), there was a significant difference in perfusion between the radial fingers (dig II-III) and the ulnar finger (dig V) that was subject to the CST ($p < 0.001$). No significant difference between the radial and ulnar fingers was seen for the contralateral hand ($p > 0.08$) (Fig. 2). The effects described above are seen in both men and women, and there was no difference in perfusion in radial or ulnar fingers between men and women (Fig. 3). Although the decrease in perfusion after CST appeared larger in men and the recovery to baseline appeared slower than in women, the difference in perfusion at 1, 5 or 20 min after CST between men and women was not statistically significant. In general, a larger interindividual variability was seen in perfusion values in women compared to men throughout the test protocol (Fig. 3).

The degree of discomfort at all time points is included in Fig. 2. There was no significant difference in how the participants rated their cold tolerance in normal life between men and women ($p = 0.39$, data not shown), measured by NRS. At the end of the CST at 5 min there was no difference in discomfort between men and women ($p = 0.65$). The highest degree of discomfort was found at 5 min during the CST. The degree of discomfort rapidly returned to normal after the CST was discontinued and returned to 0 at 10 min.

4. Discussion

The aim of this study was to investigate the possibility of using LSCI for continuous measurement of the skin perfusion of both hands when exposing one hand to cold. It was also hypothesized that a difference in perfusion would be seen between the radial and the ulnar side fingers. Skin perfusion was significantly affected by cold provocation in both the cold exposed and the contralateral hands in all participants of the study. This effect was significantly different between the radial (digit II and III) and the ulnar (digit V) side of the hands. There was a trend towards a larger decrease in perfusion in men (ns), and a faster recovery to baseline values in women (ns). A larger inter subject variability was also seen in perfusion values in women.

When exposed to cold, the blood flow to the hand is rapidly decreased, likely due to a vasoconstriction mediated by the sympathetic nervous system. In case of a prolonged period of cold exposure, a compensatory temporary vasodilatation, cold-induced vasodilatation (CIVD) is commonly observed. CIVD has been suggested as a protective mechanism to avoid damages to the extremities (Ruijs et al., 2011).

In this study one hand was exposed to CST and the other one served as control. We were not able to study perfusion changes in the exposed hand during the actual cold exposure, as this hand was immersed into the water bath. However, we found an initial, rapid decrease in perfusion in the contralateral control hand as soon as the exposed hand was placed in the cold water. This was followed by an increase in perfusion after about 1 min. One might hypothesize that the same pattern would be seen also in the cold exposed hand, as a sign of the CIVD, however this is hard to test for with LSCI. The vasoconstriction in the non-exposed hand was found to be much more rapidly reversed, compared to in the

Table 2
Absolute perfusion values and perfusion changes from baseline.

		Baseline	Cold provocation			5 min			20 min		
		Abs.	Abs.	% change	Eff. size	Abs.	% change	Eff. size	Abs.	% change	Eff. size
Cold hand											
Digit II	Women	148 (57)	91 (48)	−36 (22)	1,1	105 (49)	−27 (19)	0,8	115 (67)	−24 (31)	0,5
	Men	144 (36)	85 (28)	−33 (47)	1,8	90 (31)	−29 (48)	1,6	107 (41)	−22 (31)	1,0
	All	146 (47)	88 (39)	−35 (36)	1,3	98 (41)	−28 (36)	1,1	111 (54)	−23 (30)	0,7
Digit III	Women	143 (57)	93 (57)	−32 (27)	0,9	108 (57)	−23 (23)	0,6	117 (69)	−18 (37)	0,4
	Men	141 (27)	80 (29)	−39 (38)	2,2	84 (28)	−36 (37)	2,1	97 (37)	−29 (29)	1,4
	All	142 (44)	86 (45)	−36 (33)	1,3	96 (45)	−29 (31)	1,0	107 (55)	−24 (33)	0,7
Digit IV	Women	146 (63)	90 (49)	−35 (22)	1,0	105 (52)	−26 (19)	0,7	119 (72)	−21 (25)	0,4
	Men	136 (30)	80 (28)	−34 (47)	1,9	87 (27)	−29 (46)	1,7	108 (38)	−17 (35)	0,8
	All	141 (48)	85 (39)	−35 (36)	1,3	96 (41)	−28 (34)	1,0	114 (57)	−19 (30)	0,5
Digit V	Women	124 (57)	90 (51)	−22 (31)	0,6	105 (50)	−11 (25)	0,4	111 (71)	−11 (35)	0,2
	Men	132 (35)	86 (27)	−24 (56)	1,5	93 (22)	−17 (58)	1,4	106 (36)	−12 (44)	0,7
	All	128 (47)	88 (40)	−23 (44)	0,9	99 (39)	−14 (44)	0,7	109 (55)	−12 (39)	0,4
Contralateral hand											
Digit II	Women	130 (48)	73 (35)	−43 (12)	1,4	115 (58)	−15 (23)	0,3	122 (64)	−10 (27)	0,1
	Men	134 (44)	97 (32)	−30 (22)	1,0	147 (35)	6 (25)	−0,3	134 (20)	−3 (16)	0,0
	All	132 (45)	86 (35)	−36 (18)	1,2	131 (50)	−4 (26)	0,0	128 (47)	−7 (22)	0,1
Digit III	Women	133 (57)	74 (45)	−44 (15)	1,2	120 (66)	−11 (27)	0,2	135 (66)	1 (28)	0,0
	Men	146 (27)	97 (29)	−32 (20)	1,8	149 (29)	4 (25)	−0,1	131 (24)	−10 (14)	0,6
	All	140 (44)	86 (39)	−38 (18)	1,3	134 (52)	−4 (27)	0,1	133 (49)	−4 (22)	0,2
Digit IV	Women	140 (57)	78 (51)	−46 (13)	1,1	128 (75)	−12 (29)	0,2	136 (69)	−5 (28)	0,1
	Men	140 (24)	94 (29)	−32 (21)	1,7	142 (26)	4 (27)	−0,1	127 (22)	−9 (15)	0,6
	All	140 (43)	86 (41)	−39 (19)	1,3	135 (55)	−4 (29)	0,1	131 (51)	−7 (22)	0,2
Digit V	Women	130 (60)	76 (45)	−38 (17)	1,0	116 (70)	−14 (25)	0,2	119 (64)	−10 (24)	0,2
	Men	140 (24)	96 (30)	−30 (21)	1,6	144 (27)	5 (21)	−0,2	124 (23)	−11 (11)	0,7
	All	135 (45)	86 (39)	−34 (19)	1,2	130 (54)	−4 (25)	0,1	122 (47)	−10 (18)	0,3

Abs.: absolute perfusion value, % = % change from baseline, eff. size = effect size.

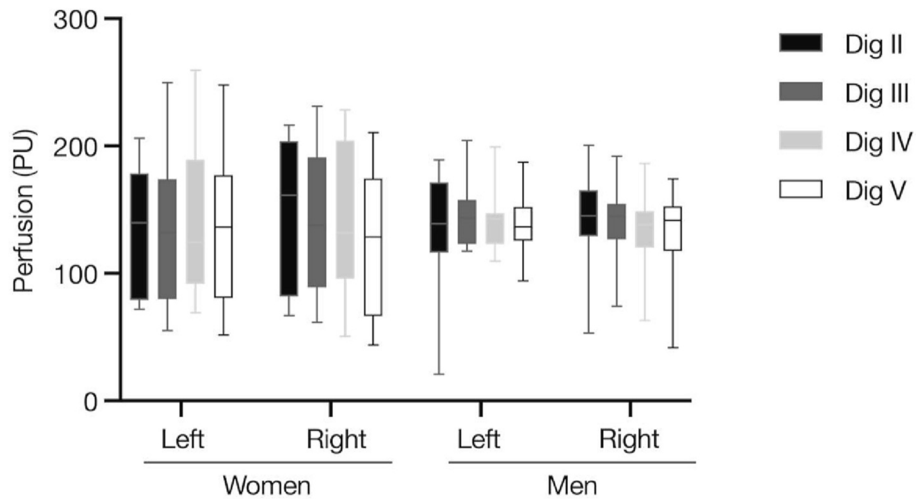

Fig. 3. Baseline perfusion in digits II-V of the left and right hands of women (n = 13) and men (n = 13).

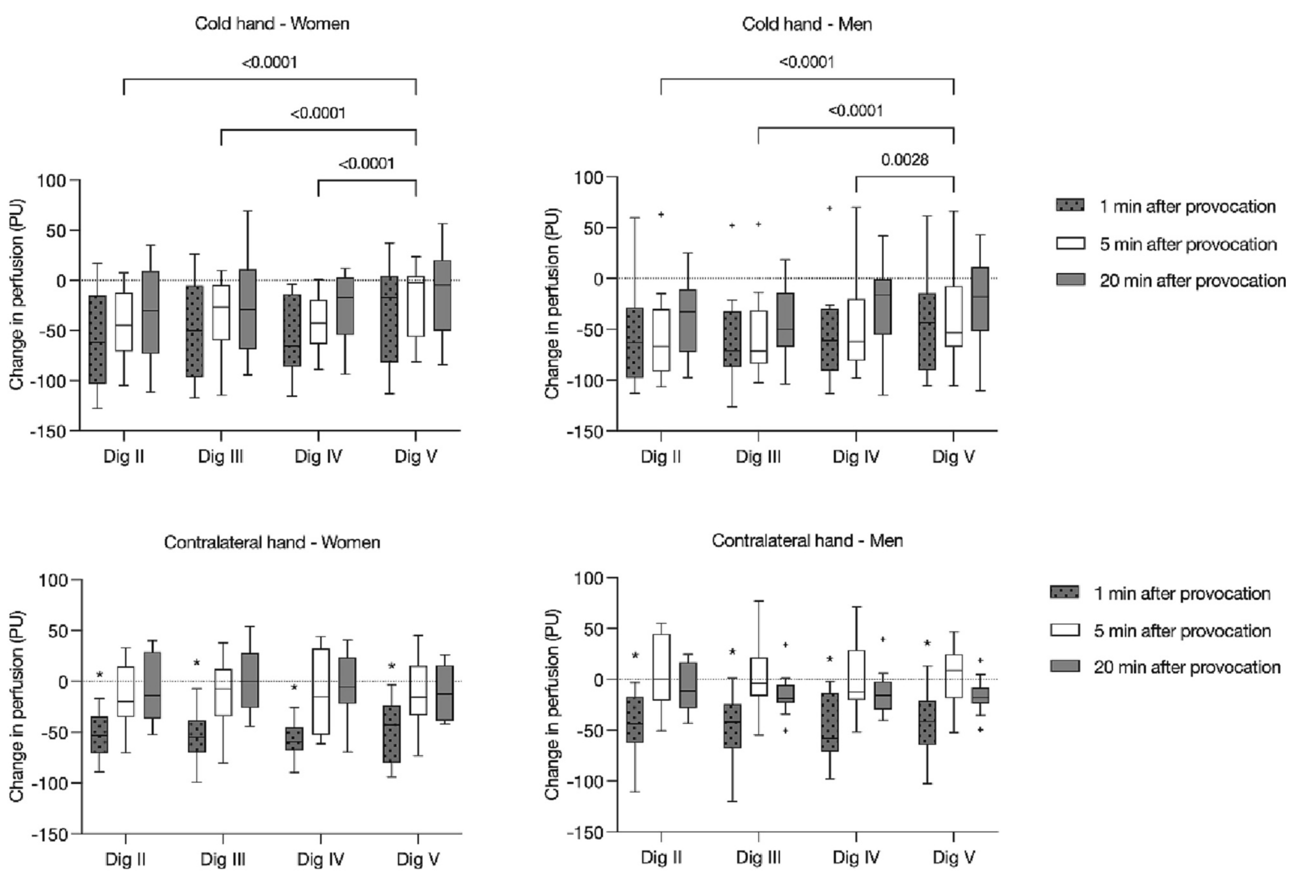

Fig. 4. Box plots showing the change in perfusion from baseline during the first, fifth and 20th minute after cold stress test in the cold-exposed and the non-exposed hand in women (n = 13) and men (n = 13). In the cold-exposed hands, perfusion in all digits was significantly reduced at all three time points after cold stress test ($p < 0.0001$). In the non-exposed hands, perfusion was decreased in all digits only during the first minute after cold stress test, as indicated by asterisks ($p < 0.001$).

hand that was exposed to the CST. The finding that, during CST, the perfusion is affected in the hand that is not exposed to cold, confirms previous data suggesting that central mechanisms (Cheung, 2015) are involved in perfusion changes in the digits in reaction to a CST in healthy individuals.

The mechanisms involved in cold sensitivity are still unclear, but it has been suggested that vascular mechanisms, as well as central and peripheral neural mechanisms are involved in altering the microvascular blood flow after exposure to low temperatures. In a retrospective paper by Klocker et al. (2012), patients who had undergone arterial repair after upper extremity injuries presented with cold intolerance, without having a concomitant nerve injury. This may indicate that cold intolerance is not only mediated through the sympathetic nervous system, but also by local circulatory regulation. Similar conclusions have been drawn from studies on patients with recurrent digital ischemia, where periarterial sympathectomy has been used to reduce symptoms (Rudolph et al., 2021). Furthermore, a discrepancy has been demonstrated between patients that were improved by a pre-operative sympathetic block compared to those who improved by periarterial sympathectomy, indicating that release of local constriction rather than sympathetic block alone may explain clinical improvement (Rudolph et al., 2021).

The present study shows a difference in the perfusion of fingers innervated by the median nerve and by the ulnar nerve when being exposed to cold. This difference was not seen in the contralateral hand. Such an effect may have implications on the outcome after nerve injuries to either of to the two nerve trunks. Whether there are differences in cold sensitivity in median nerve injuries compared to ulnar nerve injuries is still unclear, as the literature is inconsistent. In a study by Ruijs et al. (2007) no significant difference in cold sensitivity between median and ulnar nerve injury was shown. In contrast to what was shown by Lenoble et al. (1990) where 50 % of patients suffered from cold sensitivity after a median nerve lesion, compared to 75 % of the patients with an injury to the ulnar nerve. Cold intolerance has also been seen to be more common in patients with combined median and ulnar nerve injuries than in the groups separately (Ruijs et al., 2007). Cold intolerance is further on seen in compression neuropathies and has been well explored in carpal tunnel syndrome. Studies have shown that cold sensitivity improved considerably after open carpal tunnel release (Zimmerman et al., 2020). Wendt et al. (2018) also concluded in their material that 52 % of their patients with nerve compression neuropathies reported cold sensitivity. The use of LSCI is unique in the possibility of in the future being able to investigate the response of the healthy hand, meanwhile as the hand with an injury or pathology to the other hand.

In research on motor function and sensory recovery after nerve injuries, studies have shown poorer function in patients with ulnar nerve injuries and combined median and ulnar nerve injuries, than in singular median nerve injuries. Recent data also show that cold sensitivity is associated with worse sensory recovery (Magistroni et al., 2020), and that restoration of certain thermophysiological responses could indicate nerve recovery (Cesim and Oksuz, 2020).

As seen in Ruijs et al. (2009) the rewarming pattern after nerve injuries was altered in the fingers innervated by the affected nerve with a loss of the active rewarming phase which was seen in healthy individuals. The rewarming returned to more normal patterns correlated to the degree of cold intolerance and sensory recovery in the innervated area. Contrary to this, the same group showed that the presence or absence of abnormal subjective symptoms of cold sensitivity after nerve injury, as measured by the CISS questionnaire (Cold Intolerance Severity Score), does not seem to correlate with the quality of the thermophysiological response to a cold stimulus. Thus, even with a normal cold induced vasodilation reaction in both injured and uninjured digits, patients still might experience symptoms of cold sensitivity (Ruijs et al., 2011). In our findings there was a trend towards a difference in the perfusion response to CST between men and women. Men had a larger decrease in perfusion during CST. It was also seen that there was a tendency towards a faster recovery to baseline values in women than in men. In various studies there have also been reports on differences between men and women, especially concerning cold sensitivity after nerve injuries. Women score significantly higher on CISS after nerve injuries, even when the degree of sensory recovery is at a similar level (Ruijs et al., 2007). This difference in reported cold sensitivity between men and women has not been seen in a healthy normative study population (Ruijs et al., 2006). It is somewhat surprising that our findings indicate that perfusion in women, and in ulnar nerve innervated fingers, seem to return faster to baseline after a CST. It raises the question whether this can predict the severity of cold sensitivity after nerve injuries in these groups. This needs to be further investigated in future studies.

A similar study to ours, using the LSCI technique, was performed (Wilkinson et al., 2018) on patients with Raynaud's phenomenon related to systemic sclerosis. Their findings included the presence of the so-called "edge effect" where lower perfusion values were seen at the edges of the LSCI images than in the center. The same phenomenon was observed in the present study with a 4.3 % reduction in measured perfusion of calibration fluid in the periphery of the measurement area. To prevent this from affecting the measurements, we normalized all perfusion values by subtracting the baseline perfusion, i.e., the measured perfusion values in each ROI at the start of the measurement. The data is therefore presented in change from baseline. We also aimed to follow previously described methodological guidelines for the LSCI, and placement of the laser beam relative to the subject (Zotterman et al., 2017). It was stated that a significant decrease in the perfusion values was only seen in measurements with the laser beam meeting the measured surface at angles larger than 45^0. The same study also concluded that there were no significant differences in the perfusion values when obtained within distances between 15 and 40 cm. In the current study, the maximum measurement distance was never >35 cm, and the camera head was always placed at a 0^0 angle above the hands. The incident angle of the laser beam may however have been larger in the more lateral i.e. ulnar digits and this may have contributed to the above-mentioned edge effects. One other limitation of the LSCI method is the uncertainty of ROI that are marked in the perfusion images. These were located on the dorsum of the distal phalanx of the finger, on the fingernails with a limited measurement area. The small size of the ROIs may increase the uncertainty of the measurements.

A limitation of our study was the inclusion of women with oral contraceptives. In our 13 female participants, 4 were on a continuous treatment with oral contraceptives and 2 participants had a hormonal intrauterine device. Despite the inclusion criteria, no participants were on hormonal replacement therapy. The possibility of the impact of oral contraceptives on the differences in microcirculation and cold sensitivity between the sexes, cannot be completely excluded (Bartelink et al., 1993), especially since studies have shown that estrogen may potentiate cold-induced vasoconstriction (Fardoun et al., 2020), and the fact that standard deviation of measurement was larger in the female group compared to the male group. However, the proportion of participants with oral hormone treatment was still a minority of the participants. The limitations also include the absence of vasomotion analysis in the study, which may have given clearer insight into possible effects of cold exposure on the microvascular response. Such analysis may in the future be used to further explore the effects of autonomic nervous system on vascular tone as an explanation of the mechanisms behind altered cold sensitivity after nerve injury. The advantage of our study is the continuous simultaneous registration of the reactions in both hands to an ISO standardized CST of one hand. Previous studies have mainly done CST on both hands simultaneously and focused on the differences between healthy individuals and patients with microvascular disorders.

5. Conclusion

When exposing one hand to cold the contralateral hand reacts simultaneously with a decrease in perfusion, which recovers after a few minutes. A trend towards a larger decrease in perfusion in men (ns), and a faster recovery to baseline values in women (ns) was seen. There was also a larger inter subject variability in perfusion values in women. In the cooled hand, there was a significant difference between the radial and the ulnar fingers, with the ulnar fingers showing lower perfusion values.

The LSCI technique offers a possibility to compare the microcirculatory perfusion of both hands over time which may be of interest in the investigation of microcirculatory patterns in conditions with cold sensitivity, such as in patients with nerve injuries or autoimmune connective tissue diseases.

CRediT authorship contribution statement

Hedvig Detert: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, **Astrid Karlernäs:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Carin Rubensson:** Conceptualization, methodology, Writing – Review and Editing. **Erika Nyman:** Conceptualization, methodology, Writing – Review and Editing. **Erik Tesselaar:** Conceptualization, methodology, Software, Formal Analysis, Writing – Review and Editing, Visualization. **Simon Farnebo:** Conceptualization, methodology, Formal analysis, Writing – Review and Editing, Supervision.

Funding statement

This work was supported by ALF Grants (register number RÖ-900731 and LIO-823361), Region Östergötland, Sweden.

Ethics approval statement

The study was approved by the Linköping University Ethics Board (2018/187-31).

Patient consent statement

All participants gave their oral consent before inclusion.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Data availability statement

The original data presented in the study are included in the article. Inquiries can be addressed to the corresponding author.

Acknowledgements

The authors would like to thank all the study participants for taking their time to participate in the study.

References

Bartelink, M.L., De Wit, A., Wollersheim, H., Theeuwes, A., Thien, T., 1993. Skin vascular reactivity in healthy subjects: influence of hormonal status. J. Appl. Physiol. (1985) 74 (2), 727–732.
Berggren, J., Castelo, N., Tenland, K., Dahlstrand, U., Engelsberg, K., Lindstedt, S., et al., 2021. Reperfusion of free full-thickness skin grafts in periocular reconstructive surgery monitored using laser speckle contrast imaging. Ophthalmic Plast. Reconstr. Surg. 37 (4), 324–328.
Berggren, J.V., Sheikh, R., Hult, J., Engelsberg, K., Malmsjö, M., 2021. Laser speckle contrast imaging of a rotational full-thickness lower eyelid flap shows satisfactory blood perfusion. Ophthalmic Plast. Reconstr. Surg. 37 (4), e139–e141.
Campbell, D.A., Kay, S.P., 1998. What is cold intolerance? J. Hand Surg. Br. 23 (1), 3–5.
Carlsson, I.K., Edberg, A.K., Wann-Hansson, C., 2010. Hand-injured patients' experiences of cold sensitivity and the consequences and adaptation for daily life: a qualitative study. J. Hand Ther. 23 (1), 53–62.
Cesim, O.B., Oksuz, C., 2020. More severe cold intolerance is associated with worse sensory function after peripheral nerve repair or decompression. J. Hand. Surg. Eur. 45 (3), 231–236.
Cheung, S.S., 2015. Responses of the hands and feet to cold exposure. Temperature (Austin) 2 (1), 105–120.
Collins, E.D., Novak, C.B., Mackinnon, S.E., Weisenborn, S.A., 1996. Long-term follow-up evaluation of cold sensitivity following nerve injury. J Hand Surg Am. 21 (6), 1078–1085.
Della Rossa, A., Cazzato, M., d'Ascanio, A., Tavoni, A., Bencivelli, W., Pepe, P., et al., 2013. Alteration of microcirculation is a hallmark of very early systemic sclerosis patients: a laser speckle contrast analysis. Clin. Exp. Rheumatol. 31 (2 Suppl 76), 109–114.
Fardoun, M.M., Issa, K., Maaliki, D., Nasser, S.A., Baydoun, E., Eid, A.H., 2020. Estrogen increases expression of vascular alpha 2C adrenoceptor through the cAMP/Epac/JNK/AP-1 pathway and potentiates cold-induced vasoconstriction. Vasc. Pharmacol. 131, 106690.
Guven, G., Dijkstra, A., Kuijper, T.M., Trommel, N., van Baar, M.E., Topeli, A., et al., 2023. Comparison of laser speckle contrast imaging with laser doppler perfusion imaging for tissue perfusion measurement. Microcirculation 30 (1), e12795.
Heeman, W., Steenbergen, W., van Dam, G., Boerma, E.C., 2019. Clinical applications of laser speckle contrast imaging: a review. J. Biomed. Opt. 24 (8), 1–11.
Hohenauer, E., Taube, W., Freitag, L., Clijsen, R., 2022. Sex differences during a cold-stress test in normobaric and hypobaric hypoxia: a randomized controlled crossover study. Front. Physiol. 13, 998665.
Irwin, M.S., Gilbert, S.E., Terenghi, G., Smith, R.W., Green, C.J., 1997. Cold intolerance following peripheral nerve injury. Natural history and factors predicting severity of symptoms. J. Hand. Surg. Br. 22 (3), 308–316.
Jay, O., Havenith, G., 2004. Finger skin cooling on contact with cold materials: a comparison between male and female responses during short-term exposures. Eur. J. Appl. Physiol. 91 (4), 373–381.
Kanetaka, T., Komiyama, T., Onozuka, A., Miyata, T., Shigematsu, H., 2004. Laser doppler skin perfusion pressure in the assessment of Raynaud's phenomenon. Eur. J. Vasc. Endovasc. Surg. 27 (4), 414–416.
Klocker, J., Peter, T., Pellegrini, L., Mattesich, M., Loescher, W., Sieb, M., et al., 2012. Incidence and predisposing factors of cold intolerance after arterial repair in upper extremity injuries. J. Vasc. Surg. 56 (2), 410–414.
Lenoble, E., Dumontier, C., Mériaux, J.L., Mitz, V., Sokolow, C., Lemerle, J.P., 1990. Cold sensitivity after median or ulnar nerve injury based on a series of 82 cases. Ann. Chir. Main. Memb. Super. 9 (1), 9–14.
Magistroni, E., Parodi, G., Fop, F., Battiston, B., Dahlin, L.B., 2020. Cold intolerance and neuropathic pain after peripheral nerve injury in upper extremity. J. Peripher. Nerv. Syst. 25 (2), 184–190.
Mahé, G., Rousseau, P., Durand, S., Bricq, S., Leftheriotis, G., Abraham, P., 2011. Laser speckle contrast imaging accurately measures blood flow over moving skin surfaces. Microvasc. Res. 81 (2), 183–188.
Margouta, A., Anyfanti, P., Lazaridis, A., Nikolaidou, B., Mastrogiannis, K., Malliora, A., et al., 2022. Blunted microvascular reactivity in psoriasis patients in the absence of cardiovascular disease, as assessed by laser speckle contrast imaging. Life (Basel) 12 (11).
Mirdell, R., Farnebo, S., Sjöberg, F., Tesselaar, E., 2018. Accuracy of laser speckle contrast imaging in the assessment of pediatric scald wounds. Burns 44 (1), 90–98.
Mirdell, R., Lemstra-Idsardi, A.N., Farnebo, S., Tesselaar, E., 2019. The presence of synchronized perfusion dips in the microcirculation of the resting nail bed. Microvasc. Res. 121, 71–81.
Mirdell, R., Farnebo, S., Sjöberg, F., Tesselaar, E., 2019. Interobserver reliability of laser speckle contrast imaging in the assessment of burns. Burns 45 (6), 1325–1335.
Novak, C.B., Mackinnon, S.E., 2016. Evaluation of cold sensitivity, pain, and quality of life after upper extremity nerve injury. Hand (N Y) 11 (2), 173–176.
Rosato, E., Rossi, C., Molinaro, I., Giovannetti, A., Pisarri, S., Salsano, F., 2011. Laser doppler perfusion imaging in systemic sclerosis impaired response to cold stimulation involves digits and hand dorsum. Rheumatology (Oxford) 50 (9), 1654–1658.
Roustit, M., Millet, C., Blaise, S., Dufournet, B., Cracowski, J.L., 2010. Excellent reproducibility of laser speckle contrast imaging to assess skin microvascular reactivity. Microvasc. Res. 80 (3), 505–511.
Rudolph, M., Butler, K., Prabhu, S., Browne, D., Koman, L.A., Li, Z., 2021. Revision periarterial sympathectomy for recurrent digital ischaemia: a report with 11 patients. J. Hand. Surg. Eur. 46 (8), 883–890.
Ruijs, A.C., Jaquet, J.B., Daanen, H.A., Hovius, S.E., 2006. Cold intolerance of the hand measured by the CISS questionnaire in a normative study population. J. Hand. Surg. Br. 31 (5), 533–536.
Ruijs, A.C., Jaquet, J.B., van Riel, W.G., Daanen, H.A., Hovius, S.E., 2007. Cold intolerance following median and ulnar nerve injuries: prognosis and predictors. J. Hand. Surg. Eur. 32 (4), 434–439.
Ruijs, A.C., Niehof, S.P., Selles, R.W., Jaquet, J.B., Daanen, H.A., Hovius, S.E., 2009. Digital rewarming patterns after median and ulnar nerve injury. J Hand Surg Am. 34 (1), 54–64.
Ruijs, A.C., Niehof, S.P., Hovius, S.E., Selles, R.W., 2011. Cold-induced vasodilatation following traumatic median or ulnar nerve injury. J. Hand. Surg. Am. 36 (6), 986–993.
Senarathna, J., Rege, A., Li, N., Thakor, N.V., 2013. Laser speckle contrast imaging: theory, instrumentation and applications. IEEE Rev. Biomed. Eng. 6, 99–110.
Standardization IOf, 2016. International Organization for Standardization (ISO). 14835-1:2016 – Mechanical Vibration and Shock – Cold Provocation Tests for the Assessment of Peripheral Vascular Function – Part 1: Measurement and Evaluation of Finger Skin Temperature. Geneva.
Stjernbrandt, A., Bjor, B., Pettersson, H., Lundstrom, R., Liljelind, I., Nilsson, T., et al., 2020. Manifestations of cold sensitivity - a case series. Int. J. Circumpolar Health 79 (1), 1749001.
Suizu, K., Harada, N., 2005. Effects of waterproof covering on hand immersion tests using water at 10 degrees C, 12 degrees C and 15 degrees C for diagnosis of hand-arm vibration syndrome. Int. Arch. Occup. Environ. Health 78 (4), 311–318.
Taylor, N.A., Machado-Moreira, C.A., van den Heuvel, A.M., Caldwell, J.N., 2014. Hands and feet: physiological insulators, radiators and evaporators. Eur. J. Appl. Physiol. 114 (10), 2037–2060.
Wendt, M., Novak, C.B., Anastakis, D.J., 2018. Prevalence of cold sensitivity in upper extremity nerve compression syndromes. J. Hand. Surg. Eur. 43 (3), 282–285.
Wilkinson, J.D., Leggett, S.A., Marjanovic, E.J., Moore, T.L., Allen, J., Anderson, M.E., et al., 2018. A multicenter study of the validity and reliability of responses to hand cold challenge as measured by laser speckle contrast imaging and thermography: outcome measures for systemic sclerosis-related Raynaud's phenomenon. Arthritis Rheumatol. 70 (6), 903–911.
Zimmerman, M., Nyman, E., Dahlin, L.B., 2020. Occurrence of cold sensitivity in carpal tunnel syndrome and its effects on surgical outcome following open carpal tunnel release. Sci. Rep. 10 (1), 13472.

Zotterman, J., Mirdell, R., Horsten, S., Farnebo, S., Tesselaar, E., 2017. Methodological concerns with laser speckle contrast imaging in clinical evaluation of microcirculation. PLoS One 12 (3), e0174703.
Zötterman, J., Tesselaar, E., Farnebo, S., 2019. The use of laser speckle contrast imaging to predict flap necrosis: an experimental study in a porcine flap model. J. Plast. Reconstr. Aesthet. Surg. 72 (5), 771–777.
Zötterman, J., Opsomer, D., Farnebo, S., Blondeel, P., Monstrey, S., Tesselaar, E., 2020. Intraoperative laser speckle contrast imaging in DIEP breast reconstruction: a prospective case series study. Plast. Reconstr. Surg. Glob. Open 8 (1), e2529.