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Previous recreational cold exposure does not alter endothelial function or sensory thermal thresholds in the hands or feet

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Abstract

In this study, we investigated whether cold-sensitive (CS) individuals, who rewarm more slowly after a mild cold challenge, have impaired endothelial function and sensory thermal thresholds (STTs) and whether this is related to reported cold exposure. Twenty-seven participants with varying previous cold exposure undertook three tests: an STT test, i.e. determination of warm and cold STTs of the fingers and dorsal foot; an endothelial function test, i.e. measurement of cutaneous vascular conductance (CVC) during iontophoresis of ACh on the forearm, finger and foot; and a CS test, involving immersion of a foot for 2 min in water at 15°C followed by 10 min of rewarming in air at 30°C. Toe skin temperature (T_{sk}) measured during the CS test was used to form a CS group (<32°C before and 5 min after immersion) and an otherwise closely matched control group [T_{sk} >32°C; $n = 9$ (four women) for both groups]. A moderate relationship was found between cold exposure ranking and T_{sk} rewarming ($r = 0.408$, $P = 0.035$, $n = 27$) but not STT or endothelial function. The T_{sk} and blood flow were lower in CS compared with control subjects before and after foot immersion [T_{sk}, mean (SD): 30.3 (0.9) *versus* 34.8 (0.8) and 27.9 (0.8) *versus* 34.3 (0.8)°C, $P < 0.001$; and CVC: 1.08 (0.79) *versus* 3.82 (1.21) and 0.79 (0.52) *versus* 3.45 (1.07) flux $mmHg^{-1}$, $n = 9$, $P < 0.001$, respectively]. However, no physiologically significant differences were observed between groups for endothelial function or STT. A moderate correlation between previous cold exposure and toe T_{sk} rewarming after foot immersion was observed; however, CS was not associated with impaired endothelial function or reduced thermal detection.

KEYWORDS

cold sensitivity, extreme environments, non-freezing cold injury, vascular function

1 | INTRODUCTION

Non-freezing cold injury (NFCI) is caused by prolonged exposure to cold and often wet conditions (Kuht, Woods, & Hollis, 2019; Ungley & Blackwood, 1942). Historically, NFCI has mainly affected military personnel, and during World War II four stages of NFCI were identified (Ungley, Channell, & Richards, 1945). During cold exposure (stage 1) the tissue is ischaemic and numb, which then becomes mottled blue and painful on rewarming (stage 2). Stage 3 may last for up to 4 weeks and involves hyperaemia, where the tissue becomes swollen, red and hot, with pain that may be persistent and severe. Stage 4, the chronic state, is characterized by cold sensitivity (reduced skin blood flow in thermoneutral ambient temperatures and poor rewarming after a cold challenge; Eglin, Golden, & Tipton, 2013), numbness, hyperhidrosis and persistent pain (Ungley et al., 1945). These symptoms may last for many years and can therefore have life-changing consequences for the individual. The cold sensitivity alone may cause protracted peripheral vasoconstriction, leading to an increase in peripheral cooling and associated pain and numbness, thus increasing an individual's risk of subsequent cold injury (Golden, Francis, Gallimore, & Pethybridge, 2013).

Although NFCI is still present within the military (DeGroot, Castellani, Williams, & Amoroso, 2003; O'Donnell & Taubman, 2016), the phenotype appears to be less severe than that reported by Ungley

in the 1940s (Kuht et al., 2019). The increased popularity of outdoor recreational activities has meant that the civilian population is also at risk. The 'dose' (magnitude and duration) of cold exposure required to induce NFCI is not known but is likely to vary between individuals (Burgess & Macfarlane, 2009) and also to be dependent on activity (Kuht et al., 2019). In addition, subclinical forms of NFCI may also exist in individuals frequently exposed to cold conditions for short durations during recreational activities such as windsurfing, surfing and open water swimming (Eglin, 2011; Eglin et al., 2017).

The pathophysiology of NFCI is poorly understood but is thought to include both vascular (Eglin et al., 2013) and neural dysfunction (Vale et al., 2017). However, to date, the existing literature has not included appropriate control groups who have been exposed to cold conditions but have not received a cold injury. In order to investigate the mechanisms underpinning NFCI, it is important to characterize the responses of individuals with varying previous exposure to cold to determine whether cold exposure *per se* can alter neural and vascular function.

Cold sensitivity is present in ~70% of NFCI cases (Francis & Oakley, 1996). Even in the 'normal' uninjured population, some individuals may have a degree of cold sensitivity as a result of their recreational activities (e.g. windsurfers), although they have not been diagnosed with a NFCI (Eglin, 2011). The cold sensitivity may be a result of compromised vasodilatation, because glyceryl trinitrate, an endothelium-independent nitric oxide donor, was found to increase the rate of rewarming after a mild cold challenge in individuals with cold sensitivity (Hope, Eglin, Golden, & Tipton, 2014). In addition, individuals of African or Caribbean origin, who are more susceptible to NFCI than their Caucasian counterparts (Burgess & Macfarlane, 2009), have been shown to have a reduced vasodilatory response to ACh (Maley, House, Tipton, & Eglin, 2015). These studies indicate that the underlying mechanism of the cold sensitivity associated with NFCI is endothelial dysfunction.

Sensory thermal thresholds (STTs) are impaired in individuals with NFCI (Oakley & Lloyd, 1990; Vale et al., 2017). These changes in thermal sensation may be long lasting, if not permanent (Oakley & Lloyd, 1990). If individuals who demonstrate cold sensitivity in the absence of a cold injury diagnosis have a subclinical condition, it is postulated that they might also show reduced thermal sensitivity.

Therefore, in this study we investigated: (i) the effect of cold exposure experienced during recreational activities on peripheral vascular function and STT; and (ii) whether subclinical NFCI (cold sensitivity) was accompanied by endothelial dysfunction and impaired STT. Our first hypothesis was that prior cold exposure would be negatively correlated with peripheral vascular function (i.e. greater cold exposure would be associated with a lower skin blood flow response to transdermal delivery of ACh) and positively correlated with STT (i.e. greater cold exposure would be associated with higher STT, indicating poorer thermal sensitivity). Our second hypothesis was that cold-sensitive individuals would have impaired endothelial function and STT compared with age- and sex-matched control subjects.

New Findings

- **What is the central question of this study?**
  Does recreational cold exposure result in cold sensitivity and is this associated with endothelial dysfunction and impaired sensory thermal thresholds?
- **What is the main finding and its importance?**
  Previous cold exposure was correlated with cold sensitivity of the foot, which might indicate the development of a subclinical non-freezing cold injury. Endothelial function and thermal detection were not impaired in cold-sensitive individuals; therefore, further research is required to understand the pathophysiology of subclinical and clinical forms of non-freezing cold injury.

2 | METHODS

2.1 | Ethical approval

The protocol was approved by a local research ethics committee (SFEC 2016-031), and all volunteers gave informed, written consent before participation. The study conformed to standards set out in the *Declaration of Helsinki* (2013), except for registration in a database.

2.2 | Participants

A total of 27 healthy volunteers (15 men and 12 women) with a range of previous cold exposure participated in the study (Table 1). The greatest cold exposure reported was by a frequent open water swimmer, who also completed an ice mile without a wetsuit. Intermediate cold exposure included participants who reported regularly undertaking short sea swims or dips or who undertook water sports, such as dingy sailing or kite surfing, throughout the year. Participants who reported frequently undertaking outdoor activities such as cross-country running, mountain biking, football or rugby were considered minimally cold exposed. Participants who reported undertaking no regular outdoor activities in the last 2 years were considered non-cold exposed. None of the participants had been diagnosed previously with NFCI, and all participants had normal responses to the Douleur Neuropathique en 4 (DN4) questionnaire and Ipswich touch test, indicating that they were free from neuropathies.

Participants were classified as being either cold sensitive (CS; 10 men and five women) or normal (control; five men and seven women) based on the rewarming profile of their foot during the cold-sensitivity test (CST; detailed below). An *a priori* power calculation based on previously reported maximal responses to iontophoresis of ACh on the foot in Caucasian and African individuals (Maley, House, Tipton, & Eglin, 2017) and cold and warm STTs in men and women (Golja, Tipton, & Mekjavic, 2003) revealed that between five and 10 participants would be required in each group for a power of 0.8 and α of 0.05. From the pool of 27 participants, two groups of participants, CS and control,

TABLE 1 Mean (SD) physical characteristics of the participants

Characteristic	All ($n = 27$)	Men ($n = 15$)	Women ($n = 12$)
Age (years)	43.4 (12.2)	41.5 (12.8)	45.8 (11.3)
Height (cm)	175.2 (79.8)	182.1 (5.7)	166.7 (4.3)
Mass (kg)	79.8 (16.8)	88.8 (12.4)	68.6 (15.1)
Body mass index (kg m^{-2})	25.8 (2.4)	26.8 (3.8)	24.6 (5.2)
Sum of four skinfolds (mm)	59.8 (26.9)	61.2 (30.0)	58.0 (23.6)
Foot volume (l)	0.915 (0.220)	1.016 (0.171)	0.790 (0.217)
Hand volume (l)	0.43 (0.11)	0.48 (0.11)	0.36 (0.07)
Physical activity (MET min $week^{-1}$)	5108 (3715)	4459 (2669)	5919 (4718)

TABLE 2 Mean (SD) physical characteristics of matched control and cold-sensitive (CS) groups

Characteristic	Control ($n = 9$)	CS ($n = 9$)	Statistics (d.f. = 16)
Sex	5 men, 4 women	5 men, 4 women	
Age (years)	42.6 (13.3)	43.7 (11.9)	$t = 0.1863$; $P = 0.855$
Height (cm)	175.4 (10.0)	172.5 (7.9)	$t = 0.3237$; $P = 0.750$
Mass (kg)	77.8 (18.3)	80.2 (13.2)	$t = 0.6783$; $P = 0.507$
Body mass index (kg m^{-2})	25.1 (5.3)	26.9 (3.7)	$t = 0.7898$; $P = 0.441$
Sum of four skinfolds (mm)	55.4 (24.7)	63.2 (25.9)	$t = 0.6541$; $P = 0.522$
Foot volume (l)	0.94 (0.2)	0.84 (0.2)	$t = 1.023$; $P = 0.321$
Hand volume (l)	0.39 (0.08)	0.43 (0.07)	$t = 0.8865$; $P = 0.389$
Physical activity (MET min $week^{-1}$)	4935 (4048)	4426 (3824)	$U = 36$; $P = 0.703$
Cold exposure ranking (1–27)	18.2 (6.3)	11.6 (8.5)	$U = 22$; $P = 0.114$

were formed, who were closely matched for age, sex, physical activity and anthropometry (Table 2; $n = 9$ in each group). In addition, they undertook the testing at the same time of day.

Participants undertook three tests. In all but two cases (owing to the availability of the participants to attend the laboratory), these were conducted on the same day in the following order: STT, endothelial function and CST. Testing was conducted in Portsmouth, UK between June and July 2016, when the mean outdoor temperature was 18.3 (1.8)°C.

On arrival at the laboratory, the height and mass of participants were measured using a stadiometer (Bodycare, Leicester, UK) and digital weighing scales (770, Seca, Hamburg, Germany), respectively. Skinfold thickness was measured using skinfold callipers at the biceps, triceps, subscapular and suprailliac. Hand and foot volume were calculated using a water-displacement method by immersing the foot to the most prominent part of the external malleolus and the hand to the styloid process of the ulna. Sensitivity to touch on the toe pads and finger pads was assessed using the Ipswich touch test (Sharma, Kerry, Atkins, & Rayman, 2014). Female volunteers were also asked about their menstrual cycle to determine whether they were in the follicular or luteal phase or whether they were peri- or postmenopausal. However, the phase of the menstrual cycle was not controlled for because reproductive hormone status does not affect the responses to local cooling (Charkoudian, Stephens, Pirkle, Kosiba, & Johnson, 1999; Lunt & Tipton, 2014), thermal perception (Lunt & Tipton, 2014; Söderberg, Sundström Poromaa, Nyberg, Bäckström, & Nordh, 2006) or iontophoresis of ACh (Ketel et al., 2009).

Current physical activity was assessed using the International Physical Activity Questionnaire (Craig et al., 2003). Estimated physical activity was calculated by multiplying the reported duration of vigorous activity by eight, moderate activity by four and walking duration by 3.3 over the previous 7 day period to give MET-minutes per week (Craig et al., 2003). The DN4 questionnaire (Bouhassira et al., 2005) was used to identify whether participants had neuropathic pain. Cold exposure was assessed with a cold exposure questionnaire used previously (Appendix 1; Eglin et al., 2017), which asked the participants to recall their previous cold exposure through school, work and leisure activities throughout their life (before 12, 12–18 and after 18 years of age). For each of these phases, the participants were asked where they lived (geographically) and whether they had participated in any sports or activities that took place in cold/wet conditions, giving details including the type of activity, when this occurred (e.g. June 2014–present), frequency, duration and estimated water/air temperature. In addition, they were asked how they rated their whole body and hands/feet to cope with the cold (worse than average/average/better than average) and whether they had experienced any symptoms (numbness, swelling, redness, tenderness or tingling) after being exposed to cold/wet conditions. Finally, they were asked whether they thought they had either Raynaud's phenomenon or NFCI and whether this had been diagnosed medically and, if undiagnosed, to describe

their symptoms. Given the reliability of recalling cold exposure might vary between individuals, rankings of cold exposure were estimated by examining the participants' reports of their cold exposure history in the previous 2 years, taking into consideration the frequency, duration and severity (air temperature and water temperature) of their exposure. This ranking [from 1 (greatest cold exposure) to 27 (least cold exposure)] was initially completed by two researchers (C.M.E. and H.M.) independently, after which any discrepancies in ordering were settled by a third researcher (J.T.C.). Cold sensitivity was determined from the results of the CST (see section 2.5).

2.3 | Sensory thermal threshold test

Warm and cold STTs of the hand and foot were assessed using a thermal sensitivity tester (Physitemp Instruments Ltd, Clifton, NJ, USA) at an environmental temperature of 23.6 (0.5)°C, as previously described (Golja et al., 2003; Maley, Eglin, House, & Tipton, 2014). The participant placed their middle three fingers of the left hand on top of a thermal plate (5.1 cm × 4.4 cm) for determination of warm followed by cold STT. Participants were instructed that a warm stimulus would be presented to the skin through the thermal plate. Immediately after the presentation of the warm stimulus, participants were instructed to report whether they perceived a change in the resting temperature of the plate. After each temperature change, the plate was returned to the adapting temperature of 30°C. If the participant perceived a warm stimulus, the subsequent stimulus was of a smaller magnitude. In the event that the stimulus was not perceived, the subsequent stimulus was of a greater magnitude. Sham stimuli were intermittently initiated whereby no stimulus was presented. The final warm sensory threshold was calculated as the temperature preceding the point at which the warm stimulus was not perceived on three consecutive occasions. The same process was repeated for determination of the cold STT, with an adapting temperature of 30°C. The thermal plate was then inverted and mounted on a guiding system and placed on the dorsal aspect of the left foot for measurement of warm followed by cold STT. The order of testing was not randomized because pilot testing showed that cooling (during the cold STT) could influence the warm STT and reduce sensitivity. Skin temperature adjacent to the test site was measured using a skin thermistor (Grant Instruments, Cambridge, UK) and recorded every minute on a data logger (Squirrel; Grants Instruments).

2.4 | Endothelial function; response to acetylcholine

After a 20 min acclimation period to the ambient conditions [23.6 (0.5)°C], ACh was delivered transdermally using iontophoresis to three sites in the following order: the volar aspect of the left forearm, the middle phalanx of the middle finger of the left hand and the dorsal aspect of the left foot, as described previously (Eglin et al., 2017). Acetylcholine (Sigma Chemicals, Aldrich) was diluted with sterile water for injection to achieve a concentration of 1% w/v. The iontophoresis protocol consisted of four pulses of 25 μA followed by one pulse of 50 μA, one of 100 μA, one of 150 μA and a final pulse of 200 μA applied for 20 s, with 60 s intervals between each pulse, during which no current was applied. After an interval of 5 min, the protocol was repeated on the next skin site.

Skin blood flow was measured using a laser Doppler probe (VP1T/7; Moor Instruments, Axminster, UK) connected to a laser Doppler perfusion monitor (moorVMS-LDF, Moor Instruments, Axminster, UK). Flux data from the laser Doppler and iontophoresis controller were recorded using a data-acquisition system and software (Powerlab and LabChart 7; AD Instruments, Colorado Springs, New Zealand). The laser Doppler probe was placed into the iontophoresis chamber on the forearm, finger and dorsal foot and on the contralateral forearm/finger/foot. Skin blood flow was reported as the cutaneous vascular conductance (CVC), which was calculated by dividing flux by mean arterial pressure calculated from brachial blood pressure measurements (Minimon 7137 Plus; Kontron Instruments, UK) taken before and after each iontophoresis protocol. The average skin blood flow in response to iontophoresis of ACh was calculated over the final 20 s of the interval between successive pulses and between 40 and 60 s after the final pulse (Maley et al., 2017).

The skin temperature adjacent to the iontophoresis site was measured using a skin thermistor (Grant Instruments) and recorded on a data logger (Grant Instruments).

2.5 | Cold sensitivity test

The CST used in the present study has been described in detail elsewhere (Eglin et al., 2013; Maley et al., 2017; Shepherd et al., 2019). Participants entered a climatic chamber controlled at an air temperature of 30.6 (0.3)°C, removed their shoes and socks, and rested in a seated position for 15 min. They then donned their shoes and exercised on a cycle ergometer (874E; Monark, Vansbro, Sweden) for 12 min at an external work rate of 50 W. After the 12 min cycling, the participant then rested in a recumbent position for 5 min while baseline skin temperature and skin blood flow were recorded.

The left foot of the participant was then placed in a plastic bag (to keep it dry) and immersed in a water bath stirred and maintained at 14.9 (0.1)°C to the point of their mid-malleoli for 2 min. After the immersion period, the plastic bag was removed and rewarming monitored for 10 min while the participant remained resting in a supine position. The participant then placed their left hand in a plastic bag and immersed it to the level of the wrist in water at 14.9 (0.1)°C for 2 min. After the immersion period, the plastic bag was removed and rewarming monitored for 10 min while the participant remained resting in a supine position.

Skin temperature was measured using an infrared camera (A320G; FLIR Systems, High Wycombe, UK) according to the guidelines described previously (Moreira et al., 2017). The camera was positioned 1.0 m away and pointed at the sole of the foot or palm of the hand, and the temperature of the toe/finger pads was recorded using a spot analysis function on the Flir software before immersion and during the rewarming period. A mean toe/finger skin temperature of <32°C before immersion and after 5 min of rewarming was classified as being cold sensitive (Eglin et al., 2017; Hope et al., 2014). Within our

laboratory, the coefficient of variation for the CST for finger and toe skin temperature is 2.7 and 8.7%, respectively (Eglin et al., 2017).

Skin blood flow was measured using a laser Doppler probe (VP1T/7; Moor Instruments, Axminster, UK) placed on the big toe pads during foot immersion and on the pads of the thumbs during hand immersion. Skin blood flow was calculated using minute averages and expressed as CVC (flux/mean arterial pressure).

Thermal sensation and comfort of the immersed foot/hand were measured using 20 cm visual analog scales (from 0, extremely cold to 20, extremely hot; and from 0„ very comfortable to 20 extremely uncomfortable) and recorded before immersion, during immersion and every 2 min of the rewarming period. Pain sensation in the immersed foot/hand was recorded using a 0–10 pain scale (Ferreira-Valente, Pais-Ribeiro, & Jensen, 2011) at the same time points.

During each test, environmental conditions adjacent to the participant were measured using a wet bulb globe temperature meter (Grant Instruments) and recorded every 5 min.

2.6 | Data analysis

The assumption of normal distribution of data was assessed using descriptive methods (skewness, outliers and distribution plots) and inferential statistics (Shapiro–Wilk test). Where appropriate, effect sizes were calculated using Cohen's *d*, with 0.2 being considered a small effect size, 0.6 a moderate effect size, 1.2 a large effect size and 2.0 a very large effect size (Hopkins, Marshall, Batterham, & Hanin, 2009).

Hand and foot skin temperature during the STTs were compared between groups using Student's unpaired *t* test and the Mann–Whitney *U* test, respectively. Warm and cold STTs of the foot and hand were compared between groups using the Mann–Whitney *U* test.

Forearm, finger and foot skin temperature during iontophoresis were compared between CS and control groups using Student's unpaired *t* tests. All the participants were able to receive the maximal current (200 μA) during iontophoresis on the forearm. Owing to high skin resistance or leaking of the iontophoresis chamber (in individuals with very narrow fingers), only 20 of 27 (74%) and 16 of 27 (59%) received the full current on the fingers and foot, respectively. Therefore, to include as many data sets as possible the CVC response up to 100 μA was analysed for calculation of area under the curve (AUC) and maximal CVC. Baseline CVC, maximal CVC and AUC were compared between groups at each site using Student's unpaired *t* tests, with Bonferroni corrections for multiple comparisons.

Average toe and finger skin temperature and minute averages of big toe and thumb skin CVC during the CST were compared between CS and control groups before, immediately after immersion and at 5 and 10 min of the rewarming period, using a mixed-model ANOVA [group (two factors) × time (three factors)] followed by Bonferonni-corrected multiple comparison tests. Thermal comfort and sensation were compared between groups at the following time points: before immersion, during immersion, immediately after immersion, at minute 2 of rewarming and the average response over minutes 4–10 of rewarming, using a mixed-model ANOVA.

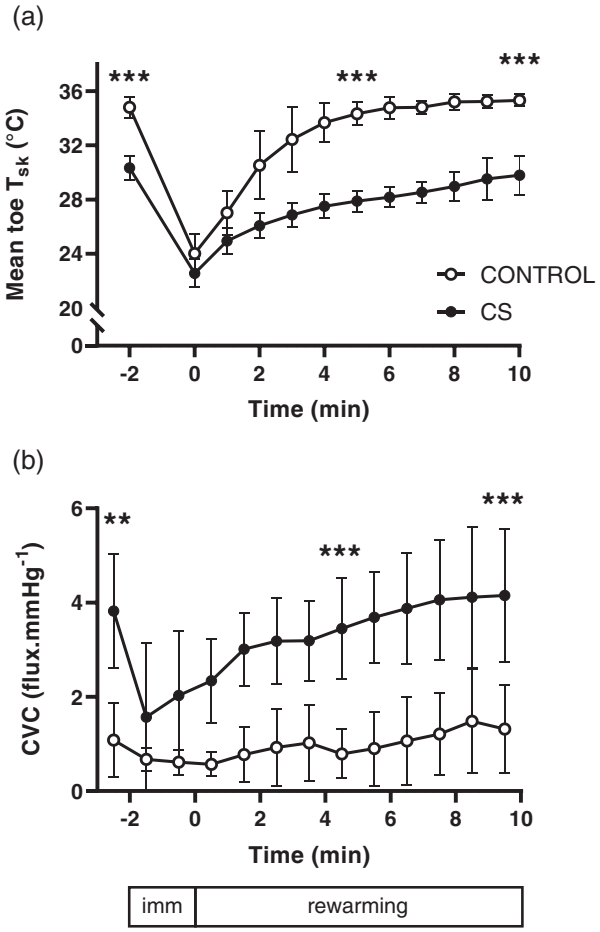

FIGURE 1 Mean (SD) skin temperature (T_{sk}) of the toes (a) and cutaneous vascular conductance (CVC) in the big toe (b) before, during (imm) and after foot immersion (rewarming) in water at 15°C for control ($n = 9$) and cold-sensitive (CS; $n = 9$) groups. $^{**}P < 0.01$ and $^{***}P < 0.001$ between CS and control group

Correlations between cold ranking by participants and their responses to STT, iontophoresis and CST were investigated using Spearman's rank correlations. A correlation coefficient of ≥0.7 was considered strong, 0.4–0.69 moderate and <0.4 weak (Dancey & Reidy, 2017).

Data were analysed using GraphPad Prism v.8.0.0 (GraphPad Prism Inc., San Diego, CA, USA). An α level of 0.05 was considered statistically significant. Data are presented as the mean (SD) or as the median and 25th and 75th percentiles unless otherwise stated.

3 | RESULTS

3.1 | Cold sensitivity test

Mean toe skin temperature was significantly lower in CS compared with control subjects ($F_{1,48} = 151.8$, $P < 0.001$; Figure 1a) before immersion and at 5 and 10 min of rewarming (all $P < 0.001$), but not immediately after foot immersion ($P = 0.1038$, $d = 1.41$), although there was a large effect size. This was associated with a reduced

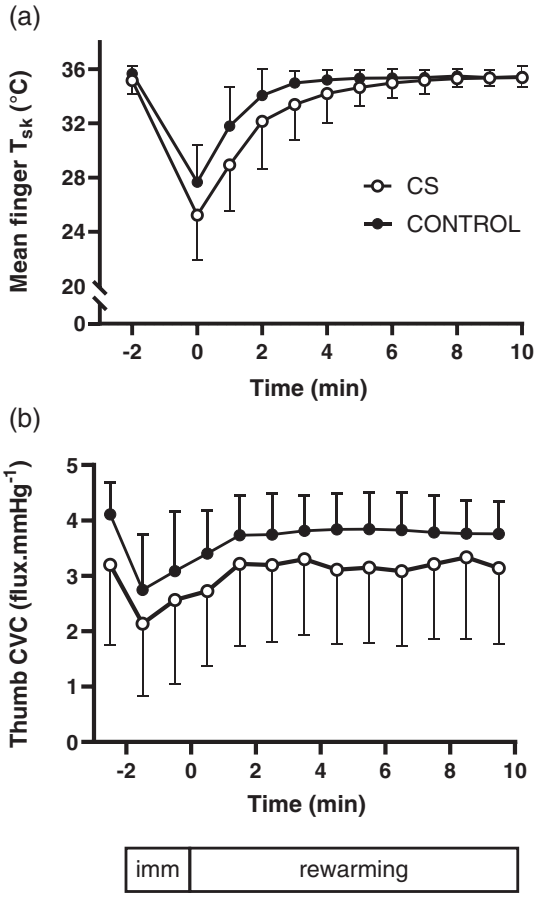

FIGURE 2 Mean (SD) skin temperature (T_{sk}) of the fingers (a) and cutaneous vascular conductance (CVC) in the thumb (b) before, during (imm) and after hand immersion (rewarming) in water at 15°C for control ($n = 9$) and cold-sensitive (CS; $n = 9$) groups

CVC in the big toe in the CS group ($F_{1,16}$ = 31.91, $P < 0.001$; Figure 1b) before immersion and during the rewarming period. During immersion there was a tendency towards a decrease in CVC in the CS group, which, although not statistically significant, had a very large effect size ($P = 0.057$, $d = 3.31$). In contrast, no difference in mean finger skin temperature or thumb CVC was observed between groups ($F_{1,16} = 2.69$, $P = 0.121$ and $F_{1,16} = 2.05$, $P = 0.172$, respectively) during the hand CST (Figure 2).

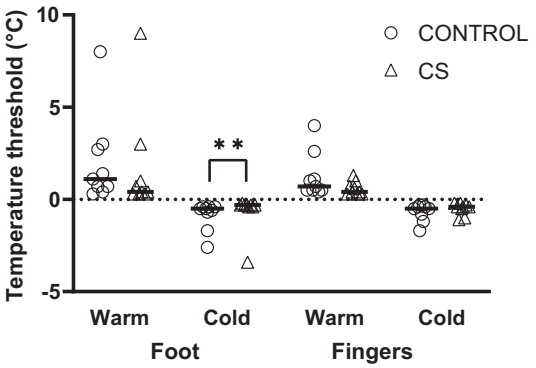

FIGURE 3 Median (individual data points) for warm and cold temperature thresholds of the foot and fingers of control ($n = 9$) and cold-sensitive (CS; $n = 9$) groups. $^{**}P < 0.01$

Thermal sensation and thermal comfort were similar between groups before, during and after foot and hand immersion (Table 3). During foot immersion, four control participants and two CS participants reported mild pain. Three of these control participants and both CS participants also reported mild pain during hand immersion. Only one individual in the control group reported mild pain during the rewarming period (hand only).

3.2 | Sensory thermal thresholds

Skin temperature of the fingers and foot did not differ between groups during the STT tests [fingers: control, 31.33 (1.52)°C and CS, 30.35 (1.81)°C, $P > 0.05$; and foot: control, 29.41 (1.52)°C and CS, 28.95 (2.06)°C; $P > 0.05$]. The CS subjects had a significantly lower sensory threshold to cold stimuli in their feet compared with control subjects [median (interquartile range): 0.3 (0.3–0.4) *versus* 0.5 (0.4–0.7)°C; $U = 12$, $P = 0.0093$, $d = 0.23$), but warm sensory thresholds were similar. In the fingers, both cold and warm STTs were similar between groups (Figure 3).

3.3 | Endothelial function

Skin temperature was similar between groups at the forearm [control, 30.49 (0.83)°C and CS, 30.47 (1.36)°C], finger [control, 29.98 (1.77)°C and CS, 28.91 (2.45)°C] and foot [control, 27.55 (1.47)°C and CS,

TABLE 3 Mean (SD) thermal sensation and thermal comfort of the foot and hand before, during and after immersion for the control ($n = 9$) and cold-sensitive (CS; $n = 9$) groups

	Foot				Hand			
	Thermal sensation		Thermal comfort		Thermal sensation		Thermal comfort	
Time point	Control	CS	Control	CS	Control	CS	Control	CS
Baseline	13.4 (2.5)	11.6 (3.3)	14.5 (4.5)	13.1 (4.0)	14.8 (2.5)	13.7 (2.8)	14.4 (3.8)	14.1 (4.3)
Immersion	5.4 (1.8)	6.1 (1.5)	10.6 (4.5)	11.4 (3.7)	4.6 (1.7)	5.4 (1.6)	9.3 (4.6)	11.9 (5.2)
0 min rewarming	9.1 (2.9)	8.4 (2.4)	10.7 (3.3)	13.1 (3.6)	8.4 (1.4)	8.4 (2.2)	12.0 (2.4)	13.4 (3.0)
2 min rewarming	9.1 (2.4)	9.2 (1.5)	13.5 (2.9)	14.1 (3.5)	10.8 (2.0)	9.7 (0.5)	14.1 (3.6)	14.5 (2.4)
4–10 min rewarming	12.7 (2.3)	10.5 (1.6)	15.3 (4.1)	14.9 (2.9)	13.3 (2.7)	12.3 (2.3)	15.4 (3.8)	15.3 (2.6)

Ratings for thermal sensation and thermal comfort are as follows: 0 = extremely cold/uncomfortable; 10 = neutral; 20 = extremely hot/comfortable.

TABLE 4 Mean (SD) skin blood flow responses to iontophoresis of acetylcholine in the forearm, finger and foot of control and cold-sensitive (CS) participants

Blood flow (flux mmHg^{-1})		Forearm($n = 9$)	Finger($n = 8$)	Foot($n = 8$)
	Maximal current	200 μA	100 μA	100 μA
Baseline	Control	0.13 (0.07)	0.60 (0.45)	0.11 (0.07)
	CS	0.11 (0.06)	0.41 (0.21)	0.09 (0.07)
Maximum	Control	2.10 (0.65)	1.89 (0.85)	0.66 (0.56)
	CS	2.29 (0.88)	1.21 (0.50)	0.71 (0.53)
AUC	Control	9.53 (4.09)	6.34 (3.70)	2.03 (1.82)
	CS	11.07 (5.32)	4.00 (1.89)	1.94 (1.20)

Average maximum cutaneous vascular conductance (CVC) and area under the curve (AUC) are given for both groups.

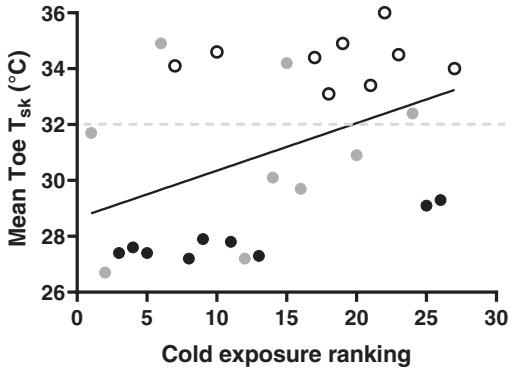

FIGURE 4 Correlation between cold exposure ranking and mean skin temperature (T_{sk}) of the toe pads after 5 min of rewarming during the cold sensitivity test. Each point represents an individual data point: filled circles, CS group; open circles, control group; and grey circles, not matched and therefore not included in the between-group analyses. The dotted line indicates the cut-off skin temperature before immersion and after 5 min of rewarming (32°C) for classification of an individual as cold sensitive

26.83 (1.55)°C], as was baseline CVC at each site (Table 4). No differences in the skin blood flow responses to ACh in the forearm, finger or foot were observed between groups (Table 4).

3.4 | Correlations

A moderate correlation was found between cold exposure ranking and mean toe skin temperature after 5 and 10 min of rewarming ($r = 0.4083$, $P = 0.0345$ and $r = 0.4189$, $P = 0.03$, respectively; Figure 4). No significant correlations were observed with any other measures taken during the cold sensitivity, STT or endothelial function tests.

4 | DISCUSSION

This is the first study to examine systematically the effect of recreational cold exposure on vascular function and sensory thermal thresholds. A moderate correlation was observed between cold exposure rank and toe skin temperature during rewarming after foot immersion. No other significant correlations were identified with cold exposure ranking, and therefore our first hypothesis is accepted, in part. Contrary to our second hypothesis, we did not observe any physiologically meaningful differences in endothelium-dependent vasodilatation or detection of warm or cold stimuli between the CS and control groups.

The peripheral vascular responses and sensory thermal thresholds were examined in 27 individuals with a wide range of previous cold exposure. Although some of these participants were frequently exposed to very cold environments during their leisure activities (winter sea swimming), none of the participants had NFCI or neuropathic pain. As expected, individuals with greater exposure to cold showed a greater degree of cold sensitivity, having lower skin temperatures during the rewarming phase of the CST; however, this was only a moderate correlation (Figure 4). Interestingly, the participant with the greatest cold exposure in the last 2 years rewarmed relatively quickly after the 2 min foot immersion, whereas other participants with apparently limited cold exposure rewarmed slowly (Figure 4). This might, in part, be attributable to self-selection, with those who are more 'cold tolerant' being more likely to participate in recreational activities involving cold exposure.

The situational risk factors that predispose individuals to NFCI during cold exposure include feeling generally cold and having static duties (Kuht et al., 2019). In addition, repeated hand immersions into water at 8°C result in an attenuation of the cold-induced vasodilatation response and lower skin temperature (Daanen, Koedam, & Cheung, 2012; Geurts, Sleivert, & Cheung, 2005; Mekjavic, Dobnikar, Kounalakis, Musizza, & Cheung, 2008). Although the cold-exposed participants in the present study reported cold extremities, they were all undertaking physical activity during their cold exposures and, in many cases, strenuous exercise. Exercise, particularly involving the whole body, during cold exposure might therefore protect peripheral vascular function. Indeed, both exercise training (Keramidas, Musizza, Kounalakis, & Mekjavic, 2010) and a slightly elevated body temperature are known to augment the cold-induced vasodilatation response (Daanen, Van de Linde, Romet, & Ducharme, 1997). In addition, although cold sensitivity is a common long-term symptom of NFCI (Francis & Oakley, 1996), the severity of the cold sensitivity is variable and not related to the severity of NFCI (Eglin et al., 2013).

Cold exposure ranking was not correlated with the responses to either ACh or STT. This indicates that previous cold exposure, which

does not result in cold injury, does not compromise endothelium-dependent vasodilatation or alter the detection of warm or cool stimuli. However, there are limitations associated with the cold exposure ranking, which relied on accurate recall, because retrospective self-reporting is limited by recall bias and might not be well suited to address how behaviour changes over time and across contexts (Shiffman, Stone, & Hufford, 2008). In addition, our subjective judgement of the cold exposure of the participants might augment this bias. The ranking was based on the previous 2 years of cold exposure, because it was thought that this would provide the most accurate recall. However, significant cold exposure earlier in life might have altered vascular function regardless of the current level of cold exposure. Some patients with NFCI still have cold sensitivity many years after their initial injurious cold exposure (Francis & Oakley, 1996).

This study has confirmed the differing response of the feet and hands to a cold challenge (Figure 1; Cheung, 2015; Eglin et al., 2017; Norrbrand, Kölegård, Keramidas, Mekjavic, & Eiken, 2017) despite the fact they are both likely to be exposed to the same environment, especially during swimming. In addition, endothelium-dependent vasodilatation was greater in the fingers compared with the foot (Table 2), but STTs were similar between sites. The reduced endothelium-dependent vasodilatory capacity in the foot might underpin the increased susceptibility of the feet to NFCI (DeGroot et al., 2003; Golden et al., 2013; Kuht et al., 2019). However, dependency and wet and tight footwear are also likely to be factors (Golden et al., 2013; Kuht et al., 2019).

Despite having a lower toe skin temperature, the CS group reported similar thermal sensation and thermal comfort to the control group throughout the CST (Table 3). In addition, endothelium-dependent vasodilatation was not compromised in the CS group (Table 4). Contrary to our second hypothesis, CS subjects appeared to be more sensitive to detecting cold stimuli in their feet than their control counterparts (Figure 3). However, the magnitude of this difference was small (0.2°C), as was the effect size (0.23), and therefore we did not consider this significant in practical terms. In contrast, patients with NFCI have a reduced ability to detect warm and cold stimuli (Vale et al., 2017).

The low toe skin temperatures and slow rate of rewarming in CS individuals might be attributable to impaired vasodilatory capacity, because their response to the CST can be augmented by administration of glyceryl trinitrate (Hope et al., 2014) but not through dietary nitrate supplementation (Eglin et al., 2017). In the present study, the response to transdermal delivery of ACh was similar between groups (Table 4), indicating that endothelium-dependent vasodilatation was not compromised in the CS group. This does not preclude the possibility that vascular function assessed using other techniques would not be altered with CS. Therefore, further research using other methods for assessment of peripheral vascular function in CS individuals, such as the vascular response to postocclusive reactive hyperaemia, local cutaneous heating and transdermal delivery of other vasoactive substances, is required. This study also highlights the need to compare neural and vascular function in patients with NFCI with control individuals who have had a similar cold exposure but who have not received an NFCI.

In conclusion, a moderate correlation was observed between previous cold exposure and cold sensitivity in the foot. Despite having lower toe skin temperatures and slower rates of rewarming, CS individuals did not have impaired endothelial function or thermal detection compared with carefully matched control individuals.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

The experiments were undertaken at the Extreme Environments Laboratory in the School of Sport, Health and Exercise Science, University of Portsmouth. C.M.E., J.T.C. and H.M. conceived and designed the research and conducted experiments and data analysis. All authors contributed to interpretation of the data. The first draft of the manuscript was prepared by C.M.E., and all authors commented on versions of the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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APPENDIX 1: COLD EXPOSURE QUESTIONNAIRE

The purpose of this questionnaire is to build as much information as we can about the cold exposure, which you have experienced. Please read the questions carefully, and answer as fully as you can. Where a space is given, please enter the information there. Where alternative answers are given (e.g. Yes/No), please cross out the ones which do not apply. If you have any questions, or are uncertain about your answers, please ask for assistance.

Please do not complete this section:

Participant ID _____

Date of completion of this questionnaire_____

Dates of Thermal Thresholds _____

Iontophoresis _____

CST_____

Please complete this section:

1. Date of Birth date____ month_____year _____

2a. Have you ever smoked regularly for longer than 1 month? Yes/No

b. If yes, do you still smoke? Yes/No

c. If you have smoked, please give details of your smoking habits:

d. How much? (type, quantity)

e. When did you start? (year)

f. If you have stopped, when did you stop? (year)

3. Ethnic group: Caucasian/Afro-Caribbean/Asian/Japanese/Chinese/Other____

4. Place of birth (country, region/county/city)

5. Where did you spend the first 3 years of your life? (country, region, county, city)

6a. Where did you attend school before the age of 12? (country, region/county/city)

b. Before the age of 12, did you engage in any sports or activities, which took place in cold/wet conditions? Yes/No

If yes, please give details:

7a. Where did you attend school after the age of 12? (country, region, county, city)

b. After the age of 12, did you engage in any sports or activities which took place in cold/wet conditions? Yes/No

If yes, please give details:

c. Did you engage in scouting or similar adventurous activities which took place in cold/wet conditions? Yes/No

If yes, please give details:

8a. Do/did you attend college/university? Yes/No

If yes, please answer the following questions. If no, please go on to Question 9.

b. Where? (country, region, county, city)

c. Do/did you engage in any sports or activities which take place in cold/wet conditions at college/university? Yes/No

If yes, please give details:

9. Have you ever engaged in any sports or activities which take place in cold/wet conditions? Yes/No

If yes, please give details:

Activity:

When do/did you do this activity e.g. *June 2014 - present*:

How frequently (times per week):

Duration (minutes per session):

Water and/or air temperature (°C):

10a. Do you have a full time/part time job? Yes/No

b. If yes, has it exposed you to any of the following:

Cold	Yes/No
Vibration or vibrating tools	Yes/No
Chemicals (poisonous, hazardous, etc.)	Yes/No

If yes to any of these, please give further details (duration of exposure, protective clothing, etc.)

11. Have you engaged in any hobby or pursuit which has exposed you to the following:

Cold	Yes/No
Vibration or vibrating tools	Yes/No
Chemicals (poisonous, hazardous, etc.)	Yes/No

If yes to any of these, please give further details (duration of exposure, protective clothing, etc.)

12a. Are you in good general health? Yes/No

If no, please list any significant or chronic conditions from which you suffer:

b. Are you taking any drugs or medication, whether prescribed by a doctor or bought from a chemist? Yes/No

If yes, please list:

13a. How would you rate your body's ability to cope with the cold:

Worse than average/average/Better than average

b. Compared to your peers, in Winter, do you tend to wear:

More clothes/about the same amount of clothes/less clothes

c. How would you rate the ability of your *feet/hands* to cope with the cold:

Worse than average/average/Better than average

d. Have your feet/hands ever gone numb in the cold or wet? Yes/No

If yes, please give details:

e. Have your feet/hands ever been swollen, red and tender or tingled after they have been exposed to the cold/wet? Yes/No

If yes, please give details:

f. Does anyone in your family suffer from cold hands or feet? Yes/No

If yes, please give details:

14. Do you think you have Raynaud's Phenomenon? Yes/No

If yes, has this been diagnosed? Yes/No

If it has been diagnosed, when was this?

If it hasn't been diagnosed, briefly describe your symptoms.

15. Do you think you have a non-freezing cold injury? Yes/No

If yes, has this been diagnosed? Yes/No

If it has been diagnosed, when was this?

If it hasn't been diagnosed, briefly describe your symptoms.

16. Please give any other information which you think may be relevant or of interest:

Thank you for completing this questionnaire