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2

3 **Title:** The effect of ethnicity on the vascular responses to cold exposure of the  
4 extremities

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1 **Abstract**

2

3 Purpose

4 Cold injuries are more prevalent in individuals of African descent (AFD). Therefore,  
5 we investigated the effect of extremity cooling on skin blood flow (SkBF) and  
6 temperature ( $T_{sk}$ ) between ethnic groups.

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8 Methods

9 Thirty males (10 Caucasian (CAU), 10 Asian (ASN), 10 AFD) undertook three tests  
10 in 30°C air whilst digit  $T_{sk}$  and SkBF were measured: i) vasomotor threshold (VT) test  
11 - arm immersed in 35°C water progressively cooled to 10°C and rewarmed to 35°C  
12 to identify vasoconstriction and vasodilatation. ii) Cold-induced vasodilatation (CIVD)  
13 test - hand immersed in 8°C water for 30 minutes followed by spontaneous warming.  
14 iii) cold sensitivity (CS) test - foot immersed in 15°C water for two minutes followed  
15 by spontaneous warming. Cold sensory thresholds of the forearm and finger were  
16 also assessed.

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18 Results

19 In the VT test, vasoconstriction and vasodilatation occurred at a warmer finger  $T_{sk}$  in  
20 AFD during cooling (21.2(4.4)°C vs. 17.0(3.1)°C,  $P=0.034$ ) and warming (22.0(7.9)°C  
21 vs. 12.1(4.1)°C,  $P=0.002$ ) compared with CAU. In the CIVD test, average SkBF  
22 during immersion was greater in CAU (42(24)%) than ASN (25(8)%,  $P=0.036$ ) and  
23 AFD (24(13)%,  $P=0.023$ ). Following immersion, SkBF was higher and rewarming  
24 faster in CAU (3.2(0.4)°C·min<sup>-1</sup>) compared with AFD (2.5(0.7)°C·min<sup>-1</sup>,  $P=0.037$ ) but  
25 neither group differed from ASN (3.0(0.6)°C·min<sup>-1</sup>). Responses to the CS test and  
26 cold sensory thresholds were similar between groups.

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28 Conclusion

29 AFD experienced a more intense protracted finger vasoconstriction than CAU during  
30 hand immersion whilst ASN experienced an intermediate response. This greater  
31 sensitivity to cold may explain why AFD are more susceptible to cold injuries.

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1 **Keywords:** non-freezing cold injury; cold water immersion; skin blood flow; skin  
2 temperature; ethnicity

3

4 **Abbreviations**

5 AFD: African descent

6 ANOVA: Analysis of variance

7 ASN: Asian

8 CAU: Caucasian

9 CIVD: Cold-induced vasodilatation

10 CS: Cold sensitivity

11 IQR: Interquartile range

12 LDU: Laser Doppler units

13 Mdn: Median

14 SD: Standard deviation

15 SKBF: Skin blood flow

16  $T_{db}$ : Dry bulb temperature

17  $T_{sk}$ : Skin temperature

18  $T_{wb}$ : Wet bulb temperature

19 VT: Vasomotor threshold

20 WBGT: Wet bulb globe temperature

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## 1 Introduction

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3 Non-freezing cold injury (NFCI) occurs mainly in the feet or hands following  
4 prolonged exposure to tissue temperatures above freezing and up to about 20°C  
5 (Ungley and Blackwood 1942). NFCI covers a range of clinical syndromes including  
6 “trench foot”, “immersion foot” and “shelter limb” (Ungley et al. 1945). Prolonged  
7 cooling of the extremities is associated with vasoconstriction (Allwood and Burry  
8 1954) which may cause tissue hypoxia (Irwin 1996) and subsequently endothelial  
9 damage and loss of nitric oxide dependent endothelial function (Endrich et al. 1990;  
10 Jia and Pollock 1997; Stephens et al. 2009). Long term symptoms of NFCI often  
11 include pain, numbness, hyperhidrosis as well cold sensitivity of the injured limb  
12 (Ungley et al. 1945). This cold sensitivity is characterised by protracted  
13 vasoconstriction following a cold stimulus (e.g. cold water immersion) at a level that  
14 does not produce a lasting vasoconstriction in an uninjured individual (Ungley et al.  
15 1945; Golden et al. 2013).

16

17 NFCI has impacted upon military operations throughout history and was the largest  
18 cause of non-combatative casualties during the Falkland Isles conflict (Golden et al.  
19 2013). Non-military populations are also at risk of receiving a cold injury in particular  
20 those employed in agricultural and fishery work (Conway and Husberg 1999;  
21 Mäkinen et al. 2009; Ikäheimo and Hassi 2011), as well as those undertaking  
22 recreational activities such as skiing (Ikäheimo and Hassi 2011; Russell et al. 2013)  
23 and mountaineering (Hashmi et al. 1998). Epidemiological studies have indicated  
24 ethnicity as a factor that impacts on the incidence of NFCI, with individuals of African  
25 descent (AFD) more likely to develop NFCI compared with Caucasian individuals  
26 (CAU) (Miller and Bjornson 1962; Taylor 1992; Conway and Husberg 1999; DeGroot  
27 et al. 2003; Burgess and Macfarlane 2009). Within the British Army, under similar  
28 conditions of training, clothing and nutrition, AFD are 30.3 times more likely to  
29 experience cold injuries compared to their CAU counterparts (Burgess and  
30 Macfarlane 2009). Likewise, during Army field training in Alaska, AFD accounted for  
31 approximately 50% of cold injuries experienced whilst only representing 10% of the  
32 troops in training (Meehan 1955). Supporting this, during peacetime United States  
33 (U.S.) military training, 220 cases of cold injuries, including NFCI, were recorded  
34 within a 52 month period with AFD accounting for 140 cases (63.6%) although only

1 representing 28.1% of the total U.S. military (Taylor 1992). The reason for the  
2 disproportional incidence of cold injuries in AFD compared with CAU may be  
3 differences in skin blood flow (SkBF) and therefore skin temperature ( $T_{sk}$ ) during cold  
4 exposure and subsequent rewarming.

5  
6 Immersion of an extremity into cold water ( $<15^{\circ}\text{C}$ ) results in vasoconstriction  
7 followed by cyclical vasodilatation (Lewis 1930), with greater CIVD with colder  
8 temperatures. This cold-induced vasodilatation (CIVD), usually demonstrated in the  
9 finger, is thought to maintain manual performance and prevent frostbite in individuals  
10 who are normothermic (Daanen 2003). Therefore the CIVD test is sometimes used  
11 to determine the susceptibility of individuals to cold injuries (Yoshimura and Iida  
12 1950; Daanen and van der Struijs 2005). The CIVD response appears to be blunted  
13 in AFD compared with CAU (Meehan 1955; Lampietro et al. 1959; Newman 1969;  
14 Jackson et al. 1989), whereas Asian individuals (ASN) may (Hirai et al. 1970) or may  
15 not (Little et al. 1971) have a pronounced CIVD response compared with CAU. If  
16 CIVD is protective, this could indicate that AFD are at greater risk of suffering a cold  
17 injury.

18  
19 As well as the CIVD test there are various cold challenges that can be applied to the  
20 extremities to study vascular responses. A cold sensitivity (CS) test has previously  
21 been utilised to help in the diagnosis of NFCI (Eglin et al. 2013). This test examines  
22 both the SkBF and  $T_{sk}$  response to a two minute immersion of the foot in  $15^{\circ}\text{C}$  water  
23 whilst in a warm ( $30^{\circ}\text{C}$ ) room. The rationale being that a lower starting  $T_{sk}$  and a  
24 slower rate of rewarming are indicative of cold sensitivity and NFCI. With cooling and  
25 rewarming the point at which vasoconstriction and vasodilatation occur can be  
26 identified. An earlier onset of maximal vasoconstriction during cooling and a later  
27 vasodilatation during rewarming results in larger and more protracted tissue cooling  
28 ("dose" of cold) and, presumably, a greater risk of NFCI. In addition, altered thermal  
29 sensation to cold stimuli may also increase the risk of NFCI by influencing  
30 behavioural thermoregulation. One previous investigation reported that white British  
31 males had a similar cold sensory threshold on their volar forearm as south Asian  
32 males (Watson et al. 2005). However, no previous research has investigated forearm  
33 or finger cold sensory thresholds between CAU, ASN and AFD groups.

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1 No previous research has investigated the vascular responses of the hands, as well  
2 as the feet, to cold exposures between ethnic groups utilising techniques such as  
3 laser Doppler flowmetry. Differences in the vascular responses to cold exposure  
4 between ethnic groups could underpin the increased susceptibility to NFCI in AFD.  
5 The aim of the present study was to investigate SkBF and  $T_{sk}$  responses in different  
6 ethnic groups during hand and foot cold water immersions. It was hypothesised that  
7 during local cold water immersion and subsequent rewarming AFD would experience  
8 lower SkBF and  $T_{sk}$  compared with CAU and ASN. To test the hypothesis that AFD  
9 may have an altered thermal perception to cold, cold sensory thresholds of the finger  
10 and forearm were also assessed.

11

## 12 **Methods**

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### 14 **Participants**

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16 Thirty male volunteer participants were recruited: ten CAU, ten ASN and ten AFD,  
17 their physical characteristics are presented in Table 1. All participants were non-  
18 smokers, were free from any vascular or blood disorders including hypertension,  
19 diabetes and Raynaud's phenomenon, with no history of either freezing or non-  
20 freezing cold injuries. Participants' history of cold exposure was ascertained by  
21 questionnaire with each ethnic group reporting similar exposure to cold. Ethnicity  
22 was determined by self-classification and all participants were UK residents at the  
23 time of testing. All CAU were born in the UK apart from one. Three AFD were born in  
24 the UK with the other seven participants residing in the UK for an average of eleven  
25 years with a minimum of seven years. Seven ASN were born in the UK with the  
26 remaining three ASN residing in the UK for an average of three years with a  
27 minimum of two years. Prior to testing, participants were asked to refrain from  
28 consuming alcohol for 24 hours and participating in exercise or consuming caffeine  
29 for 12 hours. The study received ethical and scientific approval from the University of  
30 Portsmouth Science Faculty Ethics Committee. Written, informed consent was  
31 obtained from each participant prior to their involvement in the study.

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1 Experimental procedures and measurements

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3 Participants were required to attend the laboratory on two occasions to undertake  
4 three cold water immersions and a sensory threshold test (described below). On one  
5 visit participants completed a CS test of the right foot followed by a CIVD test of the  
6 right hand. On the other visit participants first completed a sensory threshold test  
7 followed by a vasomotor threshold (VT) test of the right hand and forearm. The two  
8 laboratory visits were randomised and separated by at least 24 hours.  
9 Anthropometric measurements were collected on a separate visit. Body surface area  
10 was estimated using the equation by DuBois and DuBois (1916) and hand and  
11 forearm volume was calculated by water displacement. Environmental temperature  
12 was recorded throughout each test using an Eltek 1000 series data logger (Grant  
13 Instruments, UK) and expressed as dry bulb ( $T_{db}$ ), wet bulb ( $T_{wb}$ ) and wet bulb globe  
14 temperature (WBGT).

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16 Sensory threshold test

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18 Participants were tested for their sensitivity in detecting a cold temperature stimulus  
19 using a thermal sensitivity tester (Physitemp Instruments Ltd., USA) in a climate  
20 controlled chamber (mean (SD)  $T_{db}$ : 26.0(0.5)°C,  $T_{wb}$ : 19.6(4.9)°C, WBGT:  
21 21.5(3.4)°C). The protocol used in the present study was the same as we have used  
22 previously (Golja et al. 2003). An adaptable thermal plate was placed on the volar  
23 side of the right forearm approximately 5 cm from the elbow joint and then separately  
24 on the middle finger pad for the assessment of thermal sensitivity. The arm was  
25 rested on a padded table throughout and participants were asked not to move their  
26 forearm or fingers for the duration of the test. Participants were instructed that a cold  
27 stimulus would be presented to the skin through the thermal plate. Immediately after  
28 the presentation of the cold stimulus participants were instructed to report whether  
29 they perceived a change in the starting temperature of the plate (30°C). After each  
30 temperature change the plate was returned to the starting temperature. If the  
31 participant perceived a cold stimulus the subsequent stimulus was of a smaller  
32 magnitude. In the event that the cold stimulus was not perceived the subsequent  
33 stimulus was of a greater magnitude. Sham stimuli were intermittently provided  
34 whereby no stimulus was presented. Final cold sensory threshold was calculated as

1 the temperature preceding the point at which the cold stimulus was not perceived on  
2 three consecutive occasions.

3

#### 4 Cold sensitivity test

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6 The protocol used in the present study was the same as we have used previously  
7 (Eglin et al. 2013). Participants entered a climate controlled chamber ( $T_{db}$ :  
8  $30.1(0.7)^{\circ}\text{C}$ ,  $T_{wb}$ :  $19.9(1.8)^{\circ}\text{C}$ , WBGT:  $22.9(1.3)^{\circ}\text{C}$ ), removed their socks and shoes,  
9 and rested in a recumbent position for 15 minutes. Following this, participants cycled  
10 on an electronically braked ergometer (Tunturi T4 Alpha 150, Tunturi Fitness B.V.,  
11 Netherlands) for 12 minutes at an external workload of 50W. Participants then rested  
12 in a recumbent position for an additional five minutes. During this rest period, a laser  
13 Doppler probe (VP1T / 7, Moor Instruments, UK) was applied to the Great toe on the  
14 right foot using double sided adhesive rings to measure SkBF using a laser Doppler  
15 flowmetry monitor (moorVMS-LDF, Moor Instruments, UK). Laser Doppler data were  
16 recorded using a data acquisition system and software (Powerlab and LabChart 7,  
17 AD Instruments, New Zealand). Prior to immersion at least one minute of SkBF data  
18 were collected. Participants then placed their right foot into a plastic bag and then  
19 immersed it up to the point of the mid-malleoli into a stirred water bath maintained at  
20  $15.0(0.2)^{\circ}\text{C}$  (Grant Instruments, UK) for two minutes. Following the two minute  
21 immersion period, participants removed their foot from the water bath and plastic bag  
22 and continued to rest in a recumbent position for a further ten minutes to allow  
23 spontaneous rewarming. The right Great toe  $T_{sk}$  was measured with an infrared  
24 camera (A320G, FLIR systems, UK) distally to the laser Doppler probe and recorded  
25 immediately pre and post immersion and every minute during the rewarming period.  
26 The camera was pointed at the volar aspect of the feet and was placed 1 meter  
27 away from the participant.

28

#### 29 Cold-induced vasodilatation test

30

31 The protocol used in the present study was adopted from previous investigations  
32 (Cheung and Mekjavic 2007; Reynolds et al. 2007). Following the CS test,  
33 participants rested for a further ten minutes in the same climate controlled chamber  
34 ( $T_{db}$ :  $30.1(0.7)^{\circ}\text{C}$ ,  $T_{wb}$ :  $20.3(1.8)^{\circ}\text{C}$ , WBGT:  $23.2(1.3)^{\circ}\text{C}$ ). A laser Doppler probe and



1 skin thermistor (Type EUS-U, Grant Instruments, UK) were applied to the middle and  
2 index finger pad on the right hand respectively, using a single piece of adhesive  
3 tape.  $T_{sk}$  was measured using a squirrel 1200 series data logger (Grant Instruments,  
4 UK) and recorded every 5 seconds. Following the ten minute rest period, participants  
5 placed their right hand into a plastic bag and immersed their hand up to the styloid  
6 process in a water bath maintained at  $35.1(0.3)^{\circ}\text{C}$  for five minutes whilst SkBF and  
7  $T_{sk}$  data were recorded. Following this, participants removed their hand, still within  
8 the plastic bag, and immediately placed it in a mechanically stirred water bath  
9 maintained at  $7.9(0.1)^{\circ}\text{C}$  for a further 30 minutes. Participants then removed their  
10 hand from the water bath and plastic bag to allow spontaneous rewarming of the  
11 hand for ten minutes whilst resting their arm on an arm rest at the same height as  
12 during immersion. The CIVD cycle during cold water immersion was assessed from  
13 the  $T_{sk}$  measurements and included: average finger  $T_{sk}$ , minimum finger  $T_{sk}$ , number  
14 of CIVD waves, onset time of first CIVD wave, finger  $T_{sk}$  prior to onset of first CIVD  
15 wave and maximum finger  $T_{sk}$  during the first CIVD wave and amplitude of CIVD  
16 wave (Fig. 1). For the SkBF assessment during hand immersion, average, minimum  
17 and area under the curve [from normalised values](#) were calculated. SkBF was further  
18 analysed by splitting the data into quartiles (<25%, 25-49%, 50-74% and >75% of  
19 the SkBF observed during immersion in  $35^{\circ}\text{C}$  water) and calculating the time spent  
20 within each quartile.

21

22 *[Insert Fig. 1 here]*

23

24 Vasomotor threshold test

25

26 Participants sat in a climate controlled chamber ( $T_{db}$ :  $30.1(0.6)^{\circ}\text{C}$ ,  $T_{wb}$ :  $18.8(1.9)^{\circ}\text{C}$ ,  
27 WBGT:  $22.2(1.4)^{\circ}\text{C}$ ) for 20 minutes. A laser Doppler probe and thermistor were  
28 applied to the middle and index finger pad on the right hand, [respectively, and](#)  
29 covered by one layer of transparent film dressing (Tegaderm Film Dressing, 3M,  
30 USA). Following the 20 minute rest period, participants immersed their right hand  
31 and forearm approximately 5 cm above the medial epicondyle of the humerus in a  
32 water bath maintained at  $35.1(0.2)^{\circ}\text{C}$  for ten minutes. After ten minutes of immersion  
33 the water temperature was gradually reduced to  $10.0(0.1)^{\circ}\text{C}$  at a rate of  
34  $0.8(0.02)^{\circ}\text{C}\cdot\text{min}^{-1}$ . Following this, the water bath was reheated at a rate of

1 1.0(0.04)°C·min<sup>-1</sup> which resulted in a cooling and rewarming cycle of approximately  
2 57 minutes. Once the water reached 35°C the experiment ended. Finger T<sub>sk</sub> was  
3 sampled every five seconds to provide calculation of one minute averages to match  
4 SkBF recordings (see below in *data analyses*). Three repeat tests of the VT test  
5 were conducted on nine CAU to assess within participant reproducibility of the onset  
6 of maximal vasoconstriction and vasodilatation on three visits, each separated by at  
7 least one week. Onset of maximal vasoconstriction was defined as the start of  
8 maximal vasoconstriction identifiable by eye. Coefficient of variation and intra-class  
9 correlation analysis indicated that this definition was reproducible (7%, 0.61,  
10 respectively). Vasodilatation was defined as the start of an increase in SkBF rising  
11 toward baseline levels. Statistical analysis yielded a coefficient of variation of 12%  
12 and intra-class correlation of 0.52. Although the authors accept the definition of the  
13 onset of maximal vasoconstriction as reproducible, the vasodilatation definition was  
14 outside the criteria for preferable limits of reproducibility (coefficient of variation: <  
15 20%, intra-class correlation: > 0.60; Abbink et al. 2001; Harris et al. 2007; Roustit et  
16 al. 2010). Despite this, the test was applied to all ethnic groups and vasoconstriction  
17 and vasodilatation were calculated using the above definitions.

18

## 19 Data analyses

20

21 The assumption that different laser Doppler units (LDU) between or within  
22 participants is indicative of different SkBF across a microcirculation bed can be  
23 somewhat misleading as laser Doppler measurement is, in part, dependent on  
24 placement of the probe as there is a complex underlying microvascular anatomy  
25 (Braverman et al. 1990). Therefore, LDU normalisation can be used so that relative  
26 comparisons can be made either within individuals or between groups thus reducing  
27 any confounding effects of any slight differences in probe placement on LDU which  
28 should give a more accurate dynamic change in SkBF. Although every effort was  
29 made to position the probe on the same skin site between participants, normalisation  
30 was used within the present study. SkBF was calculated as minute averages,  
31 normalised and expressed as percentage change from that recorded prior to  
32 exposure of the extremity to cold water (set at 100%). SkBF during the foot  
33 immersion in the CS test was averaged between 30 to 90 seconds to avoid any  
34 movement artefact from placing the foot into the water.

1 Statistical analyses were conducted using IBM SPSS for Windows version 20 (IBM  
2 SPSS Statistics, USA). Testing for normality of the data was assessed with a  
3 Shapiro-Wilk test. An  $\alpha$  value of 0.05 was used to determine statistical significance. If  
4 the data were parametric then an analysis of variance (ANOVA) was performed to  
5 establish significant differences with *post hoc* analysis conducted, where  
6 appropriate, by independent samples *t*-test with Bonferroni adjustments applied.  
7 When the assumptions of parametric tests were not met, a Kruskal-Wallis test was  
8 utilised with *post-hoc* analysis conducted, where appropriate, using a Mann-Whitney  
9 U test. Parametric data in text is presented in mean and SD. Non-parametric data is  
10 presented as median (Mdn) and interquartile range (IQR). Figures shown are  
11 presented as mean and SD. Effect sizes, where appropriate, were calculated and  
12 are denoted by *d* for parametric data and *r* for non-parametric data.

13

## 14 **Results**

15

16 There were no significant differences for the participant characteristics between  
17 ethnic groups, except for height with pairwise comparisons indicating CAU were  
18 significantly taller than AFD (Table 1,  $P = 0.024$ ). Absolute SkBF expressed as LDU  
19 prior to extremity immersion in cold water was not significantly different between  
20 ethnic groups for the CS test, CIVD test, or VT test (Table 2,  $P > 0.05$ ).

21

22 *[Insert Table 1 here]*

23

24 *[Insert Table 2 here]*

25

### 26 Sensory threshold test

27

28 Cold sensory thresholds were not significantly different between ethnic groups for  
29 the forearm (CAU ( $n=10$ ): 0.3(0.2) $^{\circ}$ C, ASN ( $n=10$ ): 0.3(0.2) $^{\circ}$ C, AFD ( $n=10$ ):  
30 0.5(0.3) $^{\circ}$ C,  $P > 0.05$ ) or finger pad (Mdn (IQR) CAU ( $n=10$ ): 0.6(0.9) $^{\circ}$ C, ASN ( $n=10$ ):  
31 1.0(2.6) $^{\circ}$ C, AFD ( $n=9$ ): 1.5(1.7) $^{\circ}$ C,  $P > 0.05$ ).

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1 Cold sensitivity test

2

3 Fig. 2a and 2b shows the Great toe  $T_{sk}$  and SkBF responses to the CS test during  
4 the immersion and rewarm period. Great toe  $T_{sk}$  was similar between ethnic groups  
5 prior to immersion (Mdn (IQR) CAU: 34.6(1.2)°C, ASN: 35.3(1.4)°C, AFD:  
6 35.5(1.4)°C,  $P > 0.05$ ). No significant differences were observed between ethnic  
7 groups for Great toe  $T_{sk}$  immediately post-immersion or during subsequent  
8 rewarming ( $P > 0.05$ ). Additionally, Great toe SkBF during the foot immersion period  
9 was not significantly different between ethnic groups ( $P > 0.05$ ). However, during  
10 rewarming, Great toe SkBF was significantly lower in AFD compared with CAU (Fig.  
11 2b) at minute five (Mdn (IQR) 90(15)% vs. 130(59)%,  $P = 0.008$ ,  $r = 0.68$ ) and six  
12 (Mdn (IQR) 92(17)% vs. 130(42)%,  $P = 0.031$ ,  $r = 0.57$ ).

13

14 *[Insert Fig. 2 here]*

15

16 Cold-induced vasodilatation test

17

18 Fig. 3a and 3b shows the index finger  $T_{sk}$  and middle finger SkBF responses to both  
19 the immersion and rewarm period. Finger  $T_{sk}$  was similar between ethnic groups  
20 prior to immersion (Mdn (IQR) CAU: 35.6(0.4)°C, ASN: 35.8(0.5)°C, AFD:  
21 35.9(0.2)°C,  $P > 0.05$ ) and during hand immersion in 8°C water except for the final  
22 minute of immersion (Mdn (IQR) CAU: 10.4(2.3)°C vs. AFD: 9.3(1.7)°C,  $P = 0.047$ ,  $r$   
23  $= 0.54$ ). During rewarming AFD experienced a lower finger  $T_{sk}$  compared with CAU  
24 up to the sixth minute of rewarming (Fig. 3a,  $P = 0.006 - 0.038$ ,  $r = 0.56 - 0.69$ ) which  
25 represented a slower rate of finger rewarm in AFD compared with CAU  
26 ( $2.5(0.7)^\circ\text{C}\cdot\text{min}^{-1}$  vs.  $3.2(0.4)^\circ\text{C}\cdot\text{min}^{-1}$ ,  $P = 0.037$ ,  $d = 1.22$ ) whilst neither group  
27 differed from ASN ( $3.0(0.6)^\circ\text{C}\cdot\text{min}^{-1}$ ,  $P > 0.05$ ). Additionally, AFD experienced a  
28 lower finger  $T_{sk}$  compared with ASN at the tenth minute of rewarm (Mdn (IQR)  
29  $29.3(10.9)^\circ\text{C}$  vs.  $33.6(1.6)^\circ\text{C}$ ,  $P = 0.029$ ,  $r = 0.58$ ).

30

31 Finger SkBF was lower in AFD compared with CAU at various time points throughout  
32 hand immersion in 8°C water and subsequent rewarming (Fig. 3b,  $P = 0.003 - 0.047$ ,  
33  $r = 0.54 - 0.74$ ). ASN also experienced a lower finger SkBF compared with CAU at  
34 the 29<sup>th</sup> minute of hand immersion in cold water ( $P = 0.021$ ,  $r = 0.60$ ).

1 Average finger SkBF during hand immersion was significantly greater in CAU  
2 compared with ASN ( $P = 0.036$ ,  $r = 0.56$ ) and AFD ( $P = 0.023$ ,  $r = 0.60$ ; Table 3).  
3 Additionally, AFD had a significantly lower minimum SkBF compared with CAU ( $P =$   
4  $0.033$ ,  $r = 0.57$ ) but neither group differed from ASN ( $P > 0.05$ ; Table 3). The area  
5 under the curve for finger SkBF during hand immersion was significantly lower in  
6 ASN ( $P = 0.036$ ,  $r = 0.56$ ) and AFD ( $P = 0.023$ ,  $r = 0.60$ ) compared with CAU (Table  
7 3). During rewarming, the area under the curve for finger SkBF was significantly  
8 lower in AFD compared with CAU (Table 3,  $P = 0.002$ ,  $r = 0.76$ ) whilst ASN did not  
9 differ from either group ( $P > 0.05$ ).

10

11 *[Insert Table 3 here]*

12

13 During hand immersion, CAU spent a longer period of time in the 25-49% quartile of  
14 finger SkBF compared with AFD (Fig 4a,  $P = 0.036$ ,  $r = 0.56$ ) and a shorter period of  
15 time in the <25% quartile compared with both AFD (Fig 4a,  $P = 0.004$ ,  $r = 0.71$ ) and  
16 ASN (Fig 4a,  $P = 0.033$ ,  $r = 0.57$ ). ASN also spent a shorter period of time in the 50-  
17 74% quartile compared with CAU (Fig 4a,  $P = 0.017$ ,  $r = 0.62$ ). During rewarming,  
18 following hand immersion, CAU experienced a longer period of time in the >75%  
19 quartile compared with AFD (Fig 4b,  $P = 0.004$ ,  $r = 0.72$ ) and, similarly to the hand  
20 immersion period, a shorter period of time in the <25% quartile compared with AFD  
21 (Fig 4b,  $P = 0.001$ ,  $r = 0.79$ ). ASN also spent a shorter period of time in the <25%  
22 quartile compared with AFD (Fig 4b,  $P = 0.038$ ,  $r = 0.56$ ).

23

24 *[Insert Fig. 3 here]*

25

26 *[Insert Fig. 4 here]*

27

28 Table 4 shows the CIVD variables between ethnic groups. Minimum finger  $T_{sk}$  was  
29 significantly different between ethnic groups with AFD experiencing a significantly  
30 lower minimum finger  $T_{sk}$  compared with ASN ( $P = 0.029$ ,  $d = 1.09$ ) and, although  
31 lower, was not statistically significant compared to CAU ( $P = 0.061$ ,  $d = 1.44$ ).  
32 Although the number of CIVD waves did not differ between ethnic groups, nine CAU  
33 experienced at least one CIVD whilst only six AFD and ASN experienced the same

1 number of waves. Furthermore, three CAU experienced two CIVD waves whilst this  
2 occurred in only one AFD and two ASN.

3

4 *[Insert Table 4 here]*

5

6 Vasomotor threshold test

7

8 Finger  $T_{sk}$  was similar between groups prior to cooling (CAU: 35.55(0.21)°C, ASN:  
9 35.45(0.15)°C, AFD: 35.40(0.19)°C,  $P > 0.05$ ). The onset of maximal  
10 vasoconstriction was significantly different between ethnic groups with AFD  
11 experiencing vasoconstriction at a warmer finger  $T_{sk}$  compared with CAU during  
12 water cooling (Fig. 5, 21.2(4.4)°C vs. 17.0(3.1)°C,  $P = 0.034$ ,  $d = 1.11$ ). However, the  
13 onset of maximal vasoconstriction for ASN was intermediate and did not differ from  
14 either CAU or AFD (17.7(2.4)°C,  $P > 0.05$ ). Two ASN were not included in the  
15 analysis for the onset of maximal vasoconstriction as their onset points could not be  
16 established. The onset of vasodilatation was also significantly different between  
17 ethnic groups with AFD vasodilating at a significantly warmer finger  $T_{sk}$  compared  
18 with CAU during water warming (Fig. 5, Mdn (IQR) 22.(7.9)°C vs. 12.1(4.1)°C,  $P =$   
19 0.002,  $r = 0.76$ ). The onset of vasodilatation for ASN did not differ from either CAU or  
20 AFD (18.4(9.0)°C,  $P > 0.05$ ).

21

22 *[Insert Fig. 5 here]*

23

## 24 **Discussion**

25

26 The primary findings from this study were that, during hand immersion in cold water,  
27 AFD experienced a more intense and protracted finger vasoconstriction than CAU,  
28 vasoconstriction also occurred at a warmer finger  $T_{sk}$  in AFD compared with CAU  
29 (Fig. 3, 4 and 5). The differences in the vascular responses during the CIVD test led  
30 to AFD experiencing a slower rewarm of finger  $T_{sk}$  (Fig. 3). However, no significant  
31 physiological differences were demonstrated during the milder CS test on the foot  
32 (Fig. 2).

33

1 Previous investigations have demonstrated that AFD experience different CIVD  
2 patterns compared with CAU (Meehan 1955; Iampietro et al. 1959; Newman 1969;  
3 Jackson et al. 1989), however the present study did not observe any differences  
4 between AFD and CAU. Minimum finger  $T_{sk}$  did not go as low in ASN compared with  
5 AFD but neither group differed from CAU (Table 4). This does not support previous  
6 work (Hirai et al. 1970) which suggested ASN have a greater protection against cold  
7 exposure compared with CAU; perhaps responses from ASN in that study may have  
8 been influenced by factors such as the number of years in the residing country and  
9 seasonal variation rather than ethnic differences.

10

11 At various times throughout hand immersion and subsequent rewarming, SkBF was  
12 significantly lower in AFD compared with CAU which led to a slower rewarming  
13 response in AFD (Fig. 3). Further analysis revealed that during hand immersion AFD  
14 and ASN spent a greater period of time in the lowest SkBF quartile (*i.e.*, <25%)  
15 compared with CAU (Fig. 4a) and during subsequent rewarming AFD spent less time  
16 in the highest SkBF quartile (*i.e.*, >75%) compared with CAU (Fig. 4b). This data  
17 indicates that whilst CIVD may provide protection against cold injuries (Yoshimura  
18 and Iida 1950; Daanen and van der Struijs 2005) the relative SkBF experienced  
19 during hand immersion and subsequent rewarming may, in part, also determine an  
20 individual's susceptibility to cold injuries. The present study and the higher number of  
21 AFD who suffer NFCI compared with CAU provide some support for this idea (Miller  
22 and Bjornson 1962; Taylor 1992; Conway and Husberg 1999; DeGroot et al. 2003;  
23 Burgess and Macfarlane 2009).

24

25 The lower SkBF observed in AFD during hand immersion could be a result of  
26 augmented vasoconstriction, attenuated vasodilatation or a combination of the two.  
27 Plasma endothelin-1, a potent vasoconstrictor released from endothelial cells  
28 (Yanagisawa et al. 1988), has been shown to potentiate the sympathetic  
29 vasoconstrictor response to cooling (García-Villalón et al. 1997); this may be due to  
30 the increased vasoconstrictor effect of noradrenaline in the presence of endothelin-1  
31 (Yang et al. 1990). Endothelin-1 in AFD may (Evans et al. 1996; Treiber et al. 2000)  
32 or may not (Hong et al. 2006; Cooper et al. 2009) be increased at rest compared  
33 with CAU; however, in response to forehead cooling AFD exhibit a higher level of  
34 plasma endothelin-1 compared with CAU (Treiber et al. 2000) although AFD started

1 the test with greater levels of endothelin-1 compared with CAU. Whilst plasma  
2 noradrenaline at rest does not differ between AFD and CAU (Hohn et al. 1983) an  
3 increased sensitivity to intra-arterial administration of phenylephrine, an  $\alpha_1$ -  
4 adrenoceptor agonist, has been previously reported in AFD resulting in greater  
5 cutaneous vasoconstriction compared with CAU (Stein et al. 2000). Vasoactive  
6 substances that induce vasodilatation have previously been shown to be attenuated  
7 in AFD compared with CAU (Lang et al. 1995; Stein et al. 1997; Stein et al. 2000).  
8 Despite this, the endothelium-independent dilatation caused by sublingual  
9 nitroglycerin (Perregaux et al. 2000) and intra-arterial infusion of sodium  
10 nitroprusside (Kahn et al. 2002) does not differ between AFD and CAU; this raises  
11 questions over the precise mechanisms responsible for the vascular differences  
12 between ethnic groups. Differences in the sensitivity to vasoactive substances  
13 between AFD and CAU affecting both vasodilatation and vasoconstriction capacity  
14 may play a role in the more intense level of vasoconstriction observed in response to  
15 local extremity cooling which may contribute to the increase prevalence of NFCI in  
16 AFD. No previous investigations have studied the control of SkBF between CAU and  
17 AFD in areas of the body that are affected by NFCI (*i.e.*, fingers and toes). We are  
18 currently investigating the vascular responses to locally applied vasoactive agents in  
19 the extremities to identify the mechanisms controlling SkBF in both CAU and AFD.

20

21 The VT test provided an insight into the onset of maximal vasoconstriction and  
22 vasodilatation during progressively cooling and subsequently rewarming water. The  
23 results from the present study show that AFD experienced a longer period of time  
24 under a vasoconstrictor tone (Fig. 5). None of the test conditions in the present study  
25 were severe enough to elicit NFCI, but if a repeated cold exposure and / or a longer  
26 duration of cold stimuli were elicited then AFD may be at greater risk of NFCI due to  
27 this longer period of vasoconstriction (Ungley et al. 1945; Jia and Pollock 1997;  
28 Stephens et al. 2009).

29

30 Another possible cause for the increased risk of cold injuries in AFD may have been  
31 a diminished thermal sensation of cold and consequent behavioural response.  
32 However, in support of a previous study comparing white British and south Asian  
33 individuals (Watson et al. 2005), the present study found no significant differences



1 between groups in cold sensory thresholds in the forearm. The present study  
2 extends this further by reporting no ethnic differences in the finger pad.

3

4 The CS test is used to identify cold sensitivity and aid in the diagnosis of NFCI, with  
5 a lower pre-immersion toe or finger  $T_{sk}$  and slower rate of rewarming indicating cold  
6 sensitivity (Eglin et al. 2013). CAU experienced a higher Great toe SkBF at minute  
7 five and six during rewarming compared with AFD, but these statistical differences  
8 are not considered physiologically significant firstly because the differences in Great  
9 toe SkBF between AFD and CAU did not result in significant differences in Great toe  
10  $T_{sk}$ , and secondly, by minute five Great toe SkBF for AFD had almost returned to  
11 baseline values (*i.e.*, 100%). The insignificant physiological differences between  
12 ethnic groups in the CS test may be due to a number of factors: the gentle exercise  
13 prior to foot immersion in the CS test is undertaken to promote a vasodilated state  
14 and minimise any central vasoconstrictor response on the local response to the foot  
15 immersion. Whilst this has been shown to increase the reproducibility of the test  
16 (Eglin et al. 2005) it may have masked any underlying ethnic differences.  
17 Additionally, a two minute immersion period and 15°C water used in the CS test may  
18 not have been a strong enough cold stimulus to elicit differences between ethnic  
19 groups as were observed in the CIVD and VT test which involved a greater cold  
20 stimulus (longer immersion time and lower water temperature). These latter tests are  
21 probably more representative of the conditions which cause NFCI in fingers and  
22 toes.

23

24 Possible methodological differences between the present study and previous CIVD  
25 studies may help explain why we did not observe any differences in the CIVD  
26 variables assessed from  $T_{sk}$  (Table 4). Water temperatures between 0°C and 5°C  
27 have been used previously, with the addition of one investigation immersing  
28 participants' extremities for 45 minutes (Iampietro et al. 1959) compared with 30  
29 minutes in the present study. Thus, the cold water immersion in the previous  
30 investigations would have provided a greater sympathetic stimulus perhaps enabling  
31 differentiation of the CIVD variable responses between ethnic groups.

32

33 It is concluded that on cooling, AFD experience a greater vasoconstrictor response  
34 and also rewarm later and slower than CAU. As a consequence, the peripheral

1 tissues, in particular the fingers, of AFD experience a greater “dose” of cold which  
2 may make them more likely to acquire a NFCI during exposure to cold.

3

#### 4 **Acknowledgements**

5

6 The authors wish to thank the participants for volunteering for the study and Geoff  
7 Long and Danny White for their technical support.

8

#### 9 **Ethical standards**

10

11 This study complied with The Declaration of Helsinki, as adopted at the 18<sup>th</sup> World  
12 Medical Association (WMA) General Assembly, Helsinki, Finland, 1964 and last  
13 amended at the 64<sup>th</sup> World Medical Association General Assembly, Brazil 2013. This  
14 study complied with the Council of Europe (2005). Additional Protocol to the  
15 convention on human rights and biomedicine concerning biomedical research.  
16 European Treaty Series No. 195, Strasbourg 25 January 2005. Additionally, the  
17 study received ethical and scientific approval from the Science Faculty Ethics  
18 Committee, prior to recruitment of volunteer participants, who gave informed written  
19 consent.

20

#### 21 **Conflict of Interest**

22

23 The authors declare that they have no conflict of interest.

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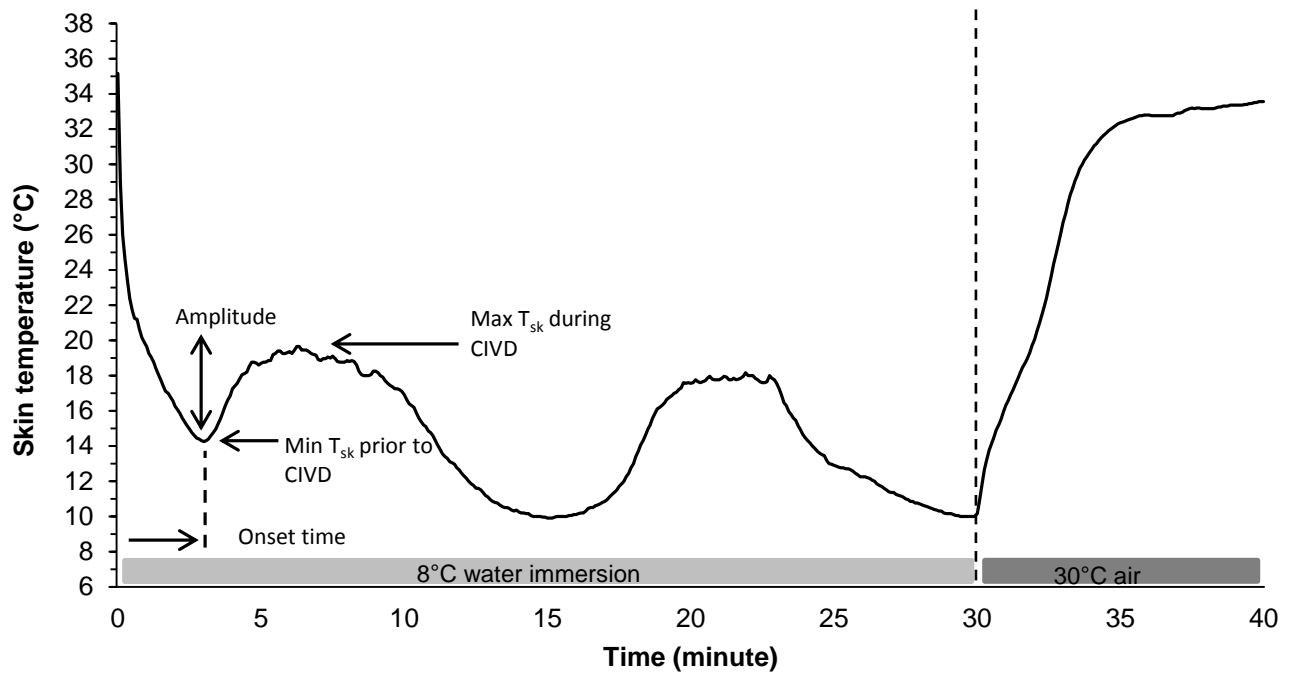
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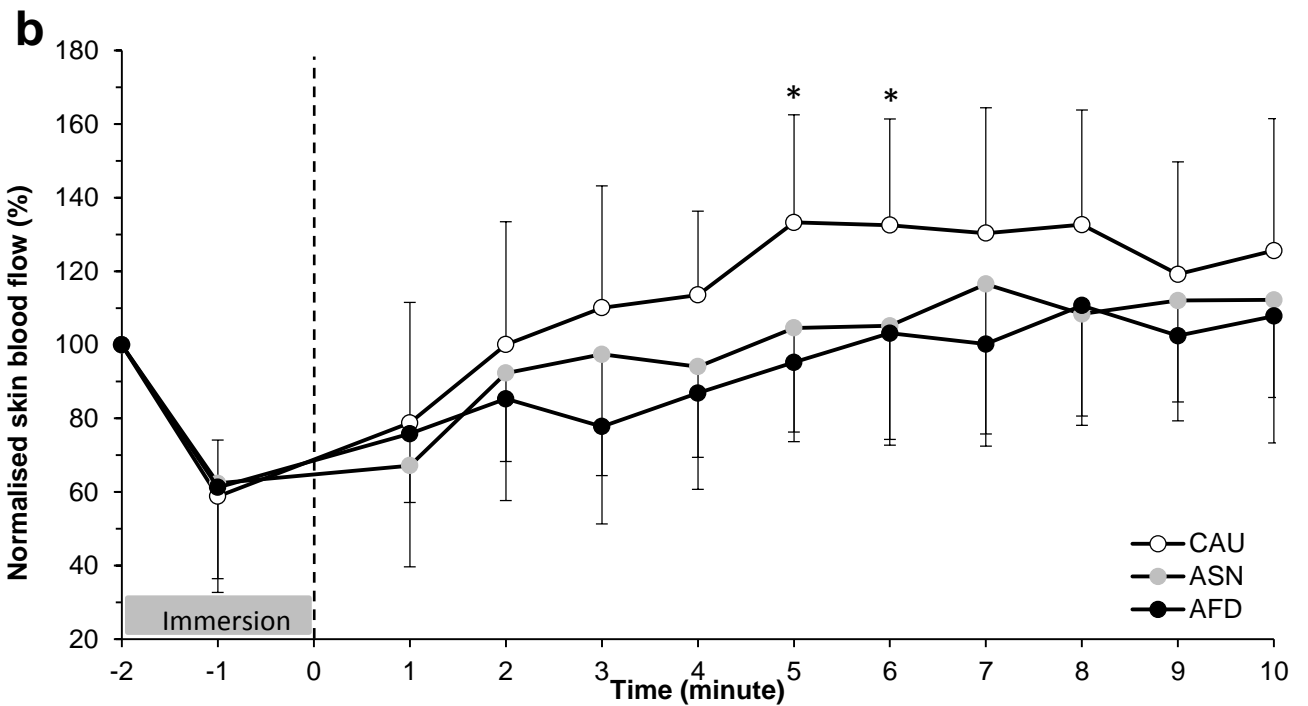
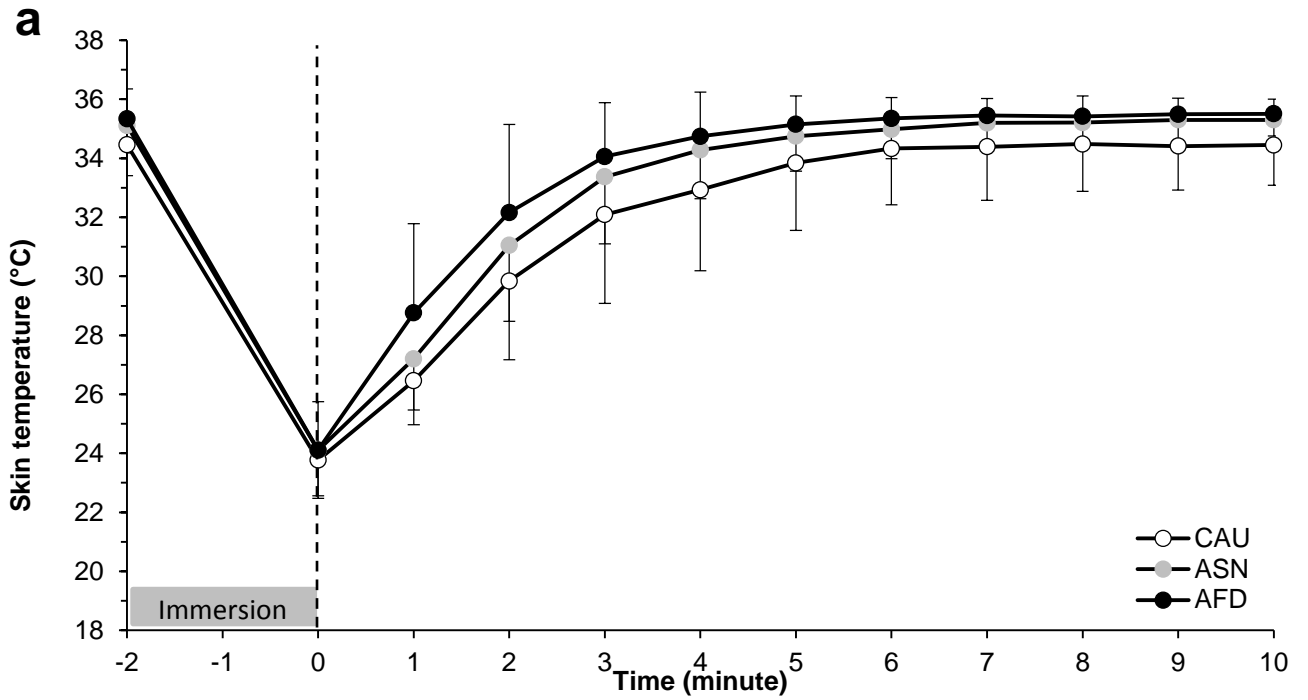
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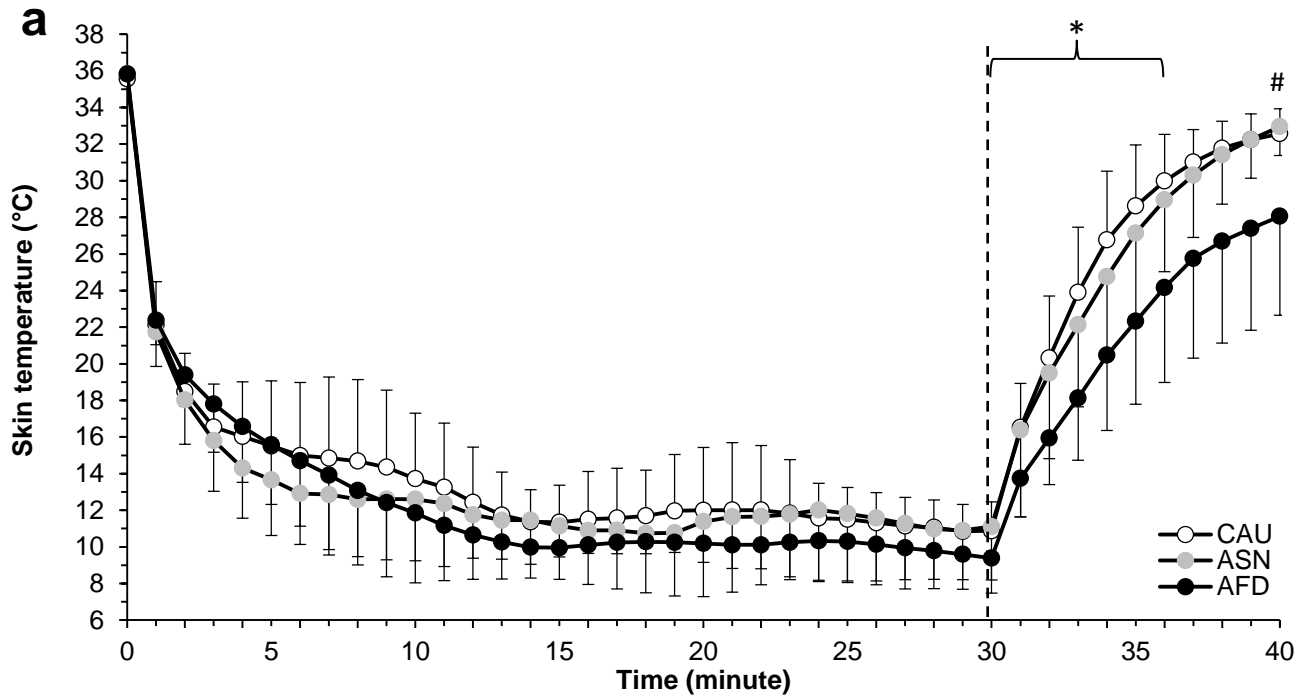
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**Fig. 1** Schematic of the variables for cold-induced vasodilation analysis. Typical trace of finger skin temperature during hand immersion and subsequent rewarming. Vertical dashed line represents start of rewarming in air

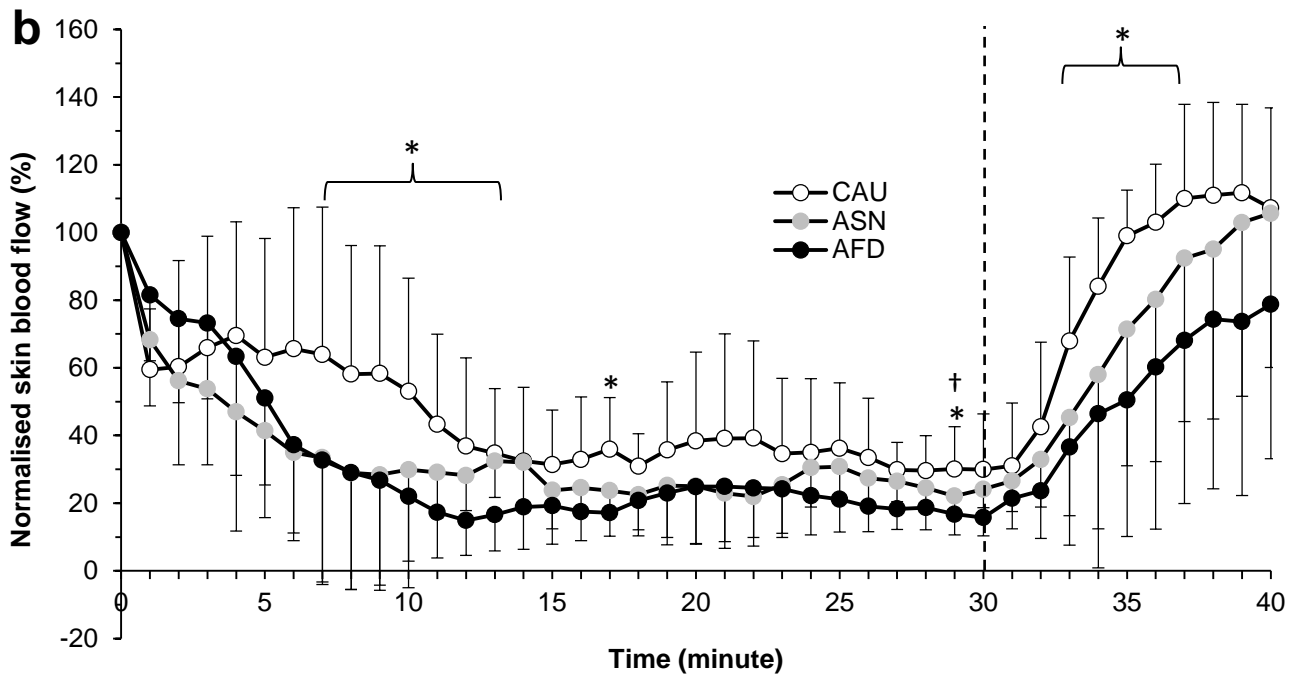


**Fig. 2** Mean (SD) Great toe skin temperature (a) and normalised skin blood flow (b) during foot immersion in 15°C water and subsequent rewarming in 30°C air during the cold sensitivity test. \* Significant difference between CAU and AFD,  $P < 0.05$ ,  $n=10$  for each group. Vertical dashed line represents start of rewarm





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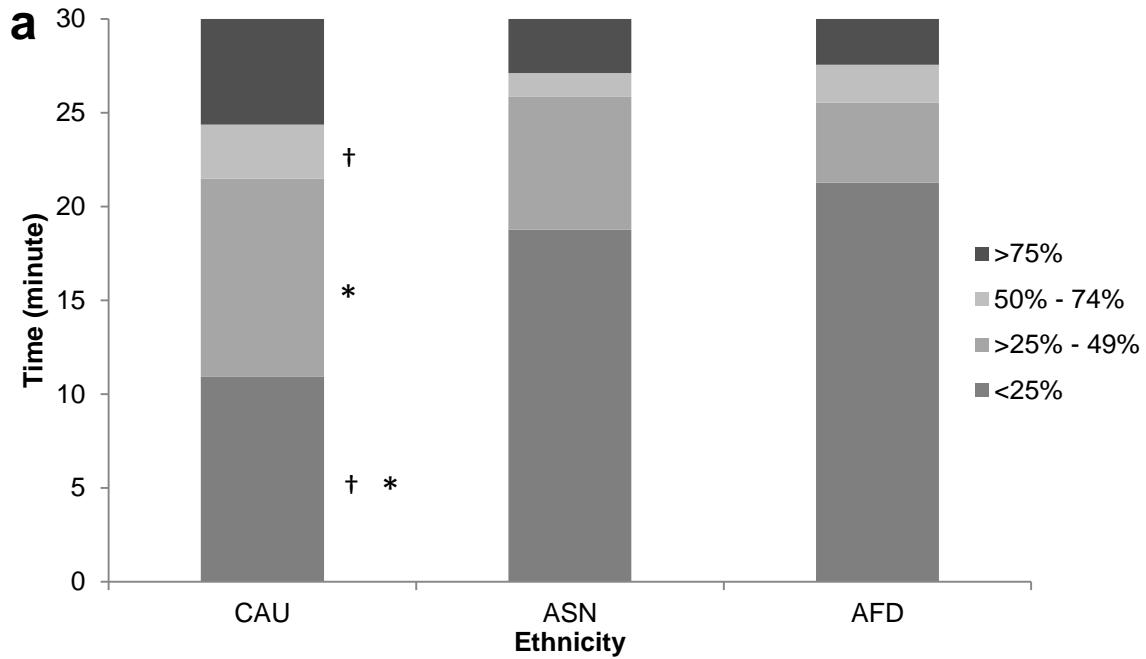
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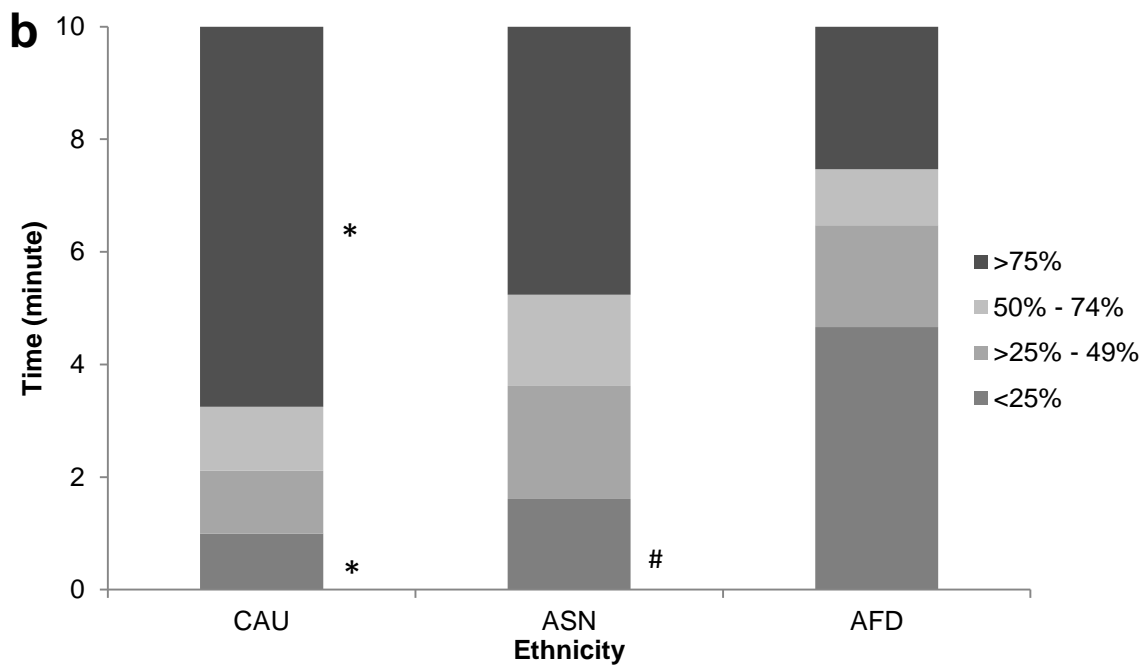
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**Fig. 3** Mean (SD) finger skin temperature (a) and normalised skin blood flow (b) during hand immersion in 8°C water and subsequent rewarming in 30°C air during the cold-induced vasodilatation test. \* Significant difference between CAU and AFD,  $P < 0.05$ ; † significant difference between CAU and ASN,  $P < 0.05$ ; # significant difference between ASN and AFD,  $P < 0.05$ ,  $n=10$  for each group. Vertical dashed line represents start of rewarming

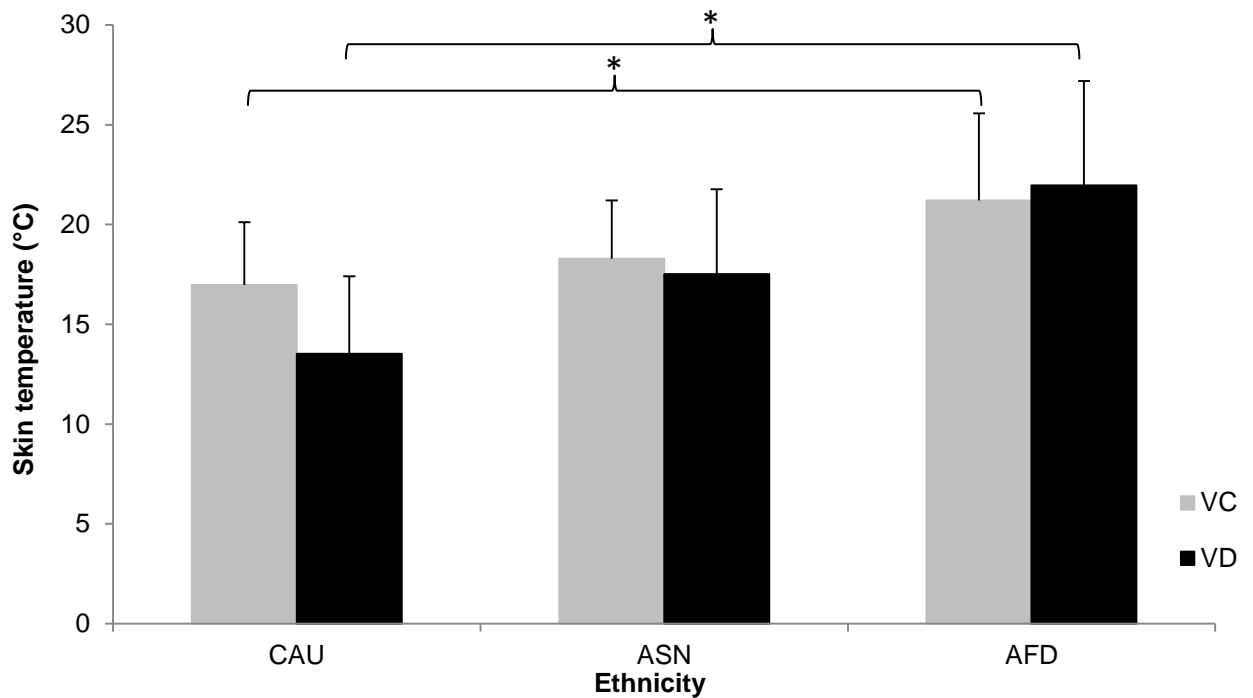


1



2

3 **Fig. 4** Mean time spent in each quartile of skin blood flow during (a) 30 minute of  
 4 immersion in 8°C water and (b) 10 minute of rewarming in 30°C air during the cold-  
 5 induced vasodilatation test. *Note:* Skin blood flow percentage calculated relative to  
 6 values obtained during immersion in 35°C water (100%). \*Significant difference  
 7 between CAU and AFD,  $P < 0.05$ ; †significant difference between CAU and ASN,  $P <$   
 8 0.05; #significant difference between ASN and AFD,  $P < 0.05$ .  $n=10$  for each group.



1  
 2 **Fig. 5** Mean (SD) finger skin temperature (°C) at which vasoconstriction (VC) and  
 3 vasodilatation (VD) occurred during hand and arm cooling and rewarming  
 4 respectively. \*Significant difference between CAU and AFD,  $P < 0.05$ .  $n=10$  for each  
 5 group except for ASN vasoconstriction only ( $n=8$ )  
 6

7 **Table 1.** Mean (SD) physical characteristics for each ethnic group

Variable	CAU	ASN	AFD
Age (years)	20 (1)	20 (1)	20 (1)
Height (m)	1.8 (0.1)*	1.8 (0.1)	1.7 (0.1)*
Body mass (kg)	78.5 (13.4)	73.5 (10.8)	78.3 (17.3)
Body surface area (m <sup>2</sup> )	2.0 (0.1)	1.9 (0.1)	1.9 (0.2)
Body mass/body surface area (kg·m <sup>2</sup> )	39.1 (4.2)	38.5 (3.1)	40.3 (4.5)
Hand length (cm)	19.9 (0.7)	20.2 (0.8)	20.0 (1.3)
Forearm length (cm)	28.4 (2.1)	27.4 (1.6)	28.3 (1.7)
Forearm girth (cm)	27.4 (2.3)	27.1 (1.5)	28.2 (2.1)
Hand and forearm volume (L)	1.7 (0.3)	1.5 (0.2)	1.6 (0.2)

8 *Note:*  $n=10$  for each group. \*Significant difference between CAU and AFD,  $P < 0.05$   
 9

1 **Table 2.** Mean (SD) skin blood flow expressed as absolute laser Doppler units at  
2 rest in air at 30°C prior to the cold sensitivity (CS) test and in 35°C water prior to the  
3 cold-induced vasodilatation (CIVD) and vasomotor threshold (VT) tests

<b>Test</b>	<b>Site</b>	<b>CAU</b>	<b>ASN</b>	<b>AFD</b>
CS test (LDU)	Great toe	177 (55)	239 (89)	251 (92)
CIVD test (LDU)	Finger pad	261 (87)	292 (108)	321 (104)
VT test (LDU)	Finger pad	260 (63)	272 (33)	318 (78)

4 *Note: n=10 for each group*

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1 **Table 3.** Median (IQR) skin blood flow variables during immersion in 8°C water and  
 2 subsequent rewarming in 30°C air in the cold-induced vasodilatation test

<b>Variable</b>	<b>CAU</b>	<b>ASN</b>	<b>AFD</b>
Average SkBF during immersion (%)	42 (24)* <sup>†</sup>	25 (8)	24 (13)
Minimum SkBF during immersion (%)	8 (5)*	6 (3)	5 (4)
AUC during immersion	75779 (43111)* <sup>†</sup>	45535 (14122)	43288 (23694)
AUC during rewarming	46039 (20861)*	37566 (12326)	25380 (22332)

3 *Note:*  $n=10$  for each group. AUC = area under curve. \* Significant difference between

4 CAU and AFD,  $P < 0.05$ ; <sup>†</sup>significant difference between CAU and ASN,  $P < 0.05$

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1 **Table 4.** Mean (SD) or Median (IQR) cold-induced vasodilatation variables (as  
 2 described in Fig. 1) during immersion in 8°C water in the cold-induced vasodilatation  
 3 test

<b>Variable</b>	<b>CAU (n=10)</b>	<b>ASN (n=10)</b>	<b>AFD (n=10)</b>
Average T <sub>sk</sub> (°C) <sup>+</sup>	13.02 (2.90)	11.97 (2.01)	11.50 (2.29)
Minimum T <sub>sk</sub> (°C)	9.69 (0.55)	9.81 (1.09) <sup>#</sup>	8.84 (0.63) <sup>#</sup>
Number of waves <sup>+</sup>	1.00 (1.00)	1.00 (1.25)	1.00 (1.00)
	<b>CAU (n=9)</b>	<b>ASN (n=6)</b>	<b>AFD (n=6)</b>
Onset time of CIVD (minute)	11.35 (7.67)	9.56 (4.84)	16.89 (7.01)
T <sub>sk</sub> prior to onset of CIVD (°C) <sup>+</sup>	10.95 (6.10)	10.28 (4.24)	9.10 (2.72)
Max T <sub>sk</sub> during CIVD (°C)	15.57 (4.28)	15.65 (3.74)	13.44 (4.04)
Amplitude (°C)	3.36 (1.62)	3.83 (1.45)	3.08 (1.68)

4 *Note:* <sup>+</sup> = Median (IQR), <sup>#</sup>Significant difference between ASN and AFD, *P* < 0.05

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