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

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Abstract

Purpose

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

Methods

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

Results

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

Conclusion

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

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

Abbreviations

AFD: African descent
ANOVA: Analysis of variance
ASN: Asian
CAU: Caucasian
CIVD: Cold-induced vasodilatation
CS: Cold sensitivity
IQR: Interquartile range
LDU: Laser Doppler units
Mdn: Median
SD: Standard deviation
SkBF: Skin blood flow
T_{db}: Dry bulb temperature
T_{sk}: Skin temperature
T_{wb}: Wet bulb temperature
VT: Vasomotor threshold
WBGT: Wet bulb globe temperature

Introduction

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

NFCI has impacted upon military operations throughout history and was the largest cause of non-combative casualties during the Falkland Isles conflict (Golden et al. 2013). Non-military populations are also at risk of receiving a cold injury in particular those employed in agricultural and fishery work (Conway and Husberg 1999; Mäkinen et al. 2009; Ikäheimo and Hassi 2011), as well as those undertaking recreational activities such as skiing (Ikäheimo and Hassi 2011; Russell et al. 2013) and mountaineering (Hashmi et al. 1998). Epidemiological studies have indicated ethnicity as a factor that impacts on the incidence of NFCI, with individuals of African descent (AFD) more likely to develop NFCI compared with Caucasian individuals (CAU) (Miller and Bjornson 1962; Taylor 1992; Conway and Husberg 1999; DeGroot et al. 2003; Burgess and Macfarlane 2009). Within the British Army, under similar conditions of training, clothing and nutrition, AFD are 30.3 times more likely to experience cold injuries compared to their CAU counterparts (Burgess and Macfarlane 2009). Likewise, during Army field training in Alaska, AFD accounted for approximately 50% of cold injuries experienced whilst only representing 10% of the troops in training (Meehan 1955). Supporting this, during peacetime United States (U.S.) military training, 220 cases of cold injuries, including NFCI, were recorded within a 52 month period with AFD accounting for 140 cases (63.6%) although only representing 28.1% of the total U.S. military (Taylor 1992). The reason for the disproportional incidence of cold injuries in AFD compared with CAU may be differences in skin blood flow (SkBF) and therefore skin temperature (T_{sk}) during cold exposure and subsequent rewarming.

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

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

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

Methods

Participants

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

Experimental procedures and measurements

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

Sensory threshold test

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

Cold sensitivity test

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

Cold-induced vasodilatation test

The protocol used in the present study was adopted from previous investigations (Cheung and Mekjavic 2007; Reynolds et al. 2007). Following the CS test, participants rested for a further ten minutes in the same climate controlled chamber (T_{db}: 30.1(0.7)°C, T_{wb}: 20.3(1.8)°C, WBGT: 23.2(1.3)°C). A laser Doppler probe and skin thermistor (Type EUS-U, Grant Instruments, UK) were applied to the middle and index finger pad on the right hand respectively, using a single piece of adhesive tape. T_{sk} was measured using a squirrel 1200 series data logger (Grant Instruments, UK) and recorded every 5 seconds. Following the ten minute rest period, participants placed their right hand into a plastic bag and immersed their hand up to the styloid process in a water bath maintained at 35.1(0.3)°C for five minutes whilst SkBF and T_{sk} data were recorded. Following this, participants removed their hand, still within the plastic bag, and immediately placed it in a mechanically stirred water bath maintained at 7.9(0.1)°C for a further 30 minutes. Participants then removed their hand from the water bath and plastic bag to allow spontaneous rewarming of the hand for ten minutes whilst resting their arm on an arm rest at the same height as during immersion. The CIVD cycle during cold water immersion was assessed from the T_{sk} measurements and included: average finger T_{sk}, minimum finger T_{sk}, number of CIVD waves, onset time of first CIVD wave, finger T_{sk} prior to onset of first CIVD wave and maximum finger T_{sk} during the first CIVD wave and amplitude of CIVD wave (Fig. 1). For the SkBF assessment during hand immersion, average, minimum and area under the curve from normalised values were calculated. SkBF was further analysed by splitting the data into quartiles (<25%, 25-49%, 50-74% and >75% of the SkBF observed during immersion in 35°C water) and calculating the time spent within each quartile.

[Insert Fig. 1 here]

Vasomotor threshold test

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

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

Data analyses

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

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

Results

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

[Insert Table 1 here]

[Insert Table 2 here]

Sensory threshold test

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

Cold sensitivity test

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

[Insert Fig. 2 here]

Cold-induced vasodilatation test

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

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

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

[Insert Table 3 here]

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

[Insert Fig. 3 here]

[Insert Fig. 4 here]

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

[Insert Table 4 here]

Vasomotor threshold test

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

[Insert Fig. 5 here]

Discussion

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

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

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

The lower SkBF observed in AFD during hand immersion could be a result of augmented vasoconstriction, attenuated vasodilatation or a combination of the two. Plasma endothelin-1, a potent vasoconstrictor released from endothelial cells (Yanagisawa et al. 1988), has been shown to potentiate the sympathetic vasoconstrictor response to cooling (García-Villalón et al. 1997); this may be due to the increased vasoconstrictor effect of noradrenaline in the presence of endothelin-1 (Yang et al. 1990). Endothelin-1 in AFD may (Evans et al. 1996; Treiber et al. 2000) or may not (Hong et al. 2006; Cooper et al. 2009) be increased at rest compared with CAU; however, in response to forehead cooling AFD exhibit a higher level of plasma endothelin-1 compared with CAU (Treiber et al. 2000) although AFD started the test with greater levels of endothelin-1 compared with CAU. Whilst plasma noradrenaline at rest does not differ between AFD and CAU (Hohn et al. 1983) an increased sensitivity to intra-arterial administration of phenylephrine, an α_1-adrenoceptor agonist, has been previously reported in AFD resulting in greater cutaneous vasoconstriction compared with CAU (Stein et al. 2000). Vasoactive substances that induce vasodilatation have previously been shown to be attenuated in AFD compared with CAU (Lang et al. 1995; Stein et al. 1997; Stein et al. 2000). Despite this, the endothelium-independent dilatation caused by sublingual nitroglycerin (Perregaux et al. 2000) and intra-arterial infusion of sodium nitroprusside (Kahn et al. 2002) does not differ between AFD and CAU; this raises questions over the precise mechanisms responsible for the vascular differences between ethnic groups. Differences in the sensitivity to vasoactive substances between AFD and CAU affecting both vasodilatation and vasoconstriction capacity may play a role in the more intense level of vasoconstriction observed in response to local extremity cooling which may contribute to the increase prevalence of NFCI in AFD. No previous investigations have studied the control of SkBF between CAU and AFD in areas of the body that are affected by NFCI (*i.e.,* fingers and toes). We are currently investigating the vascular responses to locally applied vasoactive agents in the extremities to identify the mechanisms controlling SkBF in both CAU and AFD.

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

Another possible cause for the increased risk of cold injuries in AFD may have been a diminished thermal sensation of cold and consequent behavioural response. However, in support of a previous study comparing white British and south Asian individuals (Watson et al. 2005), the present study found no significant differences between groups in cold sensory thresholds in the forearm. The present study extends this further by reporting no ethnic differences in the finger pad.

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

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

It is concluded that on cooling, AFD experience a greater vasoconstrictor response and also rewarm later and slower than CAU. As a consequence, the peripheral tissues, in particular the fingers, of AFD experience a greater "dose" of cold which may make them more likely to acquire a NFCI during exposure to cold.

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Ethical standards

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

Conflict of Interest

The authors declare that they have no conflict of interest.

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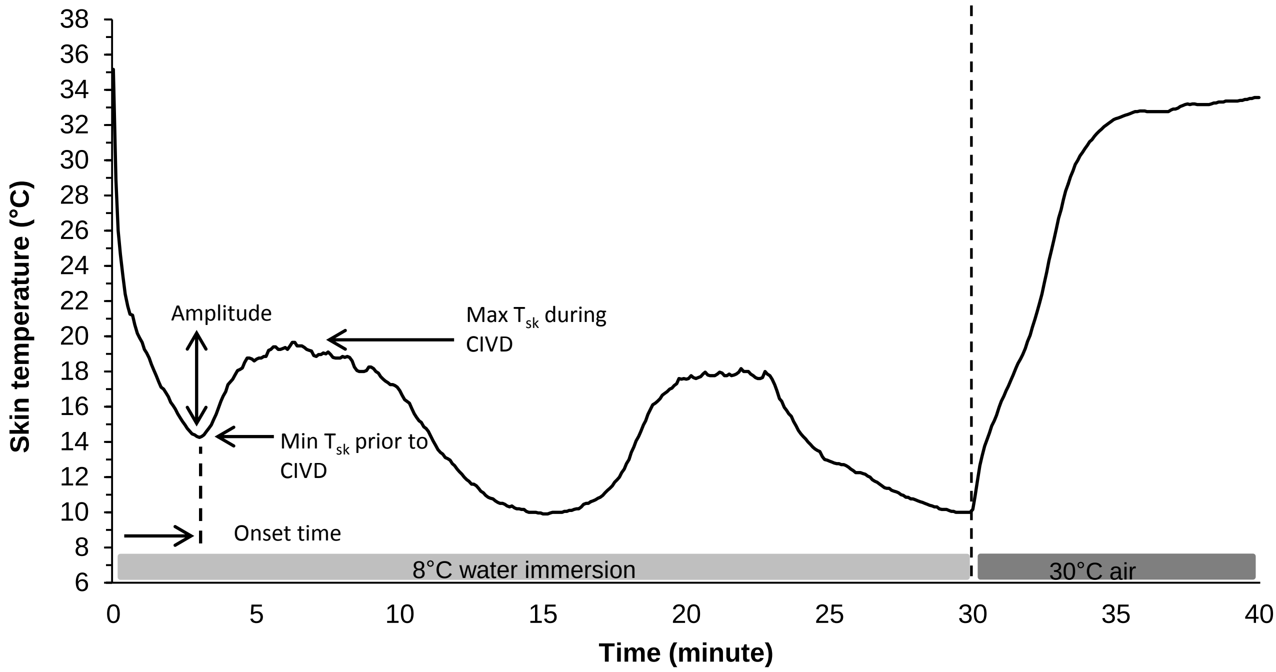

Fig. 1 Schematic of the variables for cold-induced vasodilatation analysis. Typical trace of finger skin temperature during hand immersion and subsequent rewarming. Vertical dashed line represents start of rewarming in air

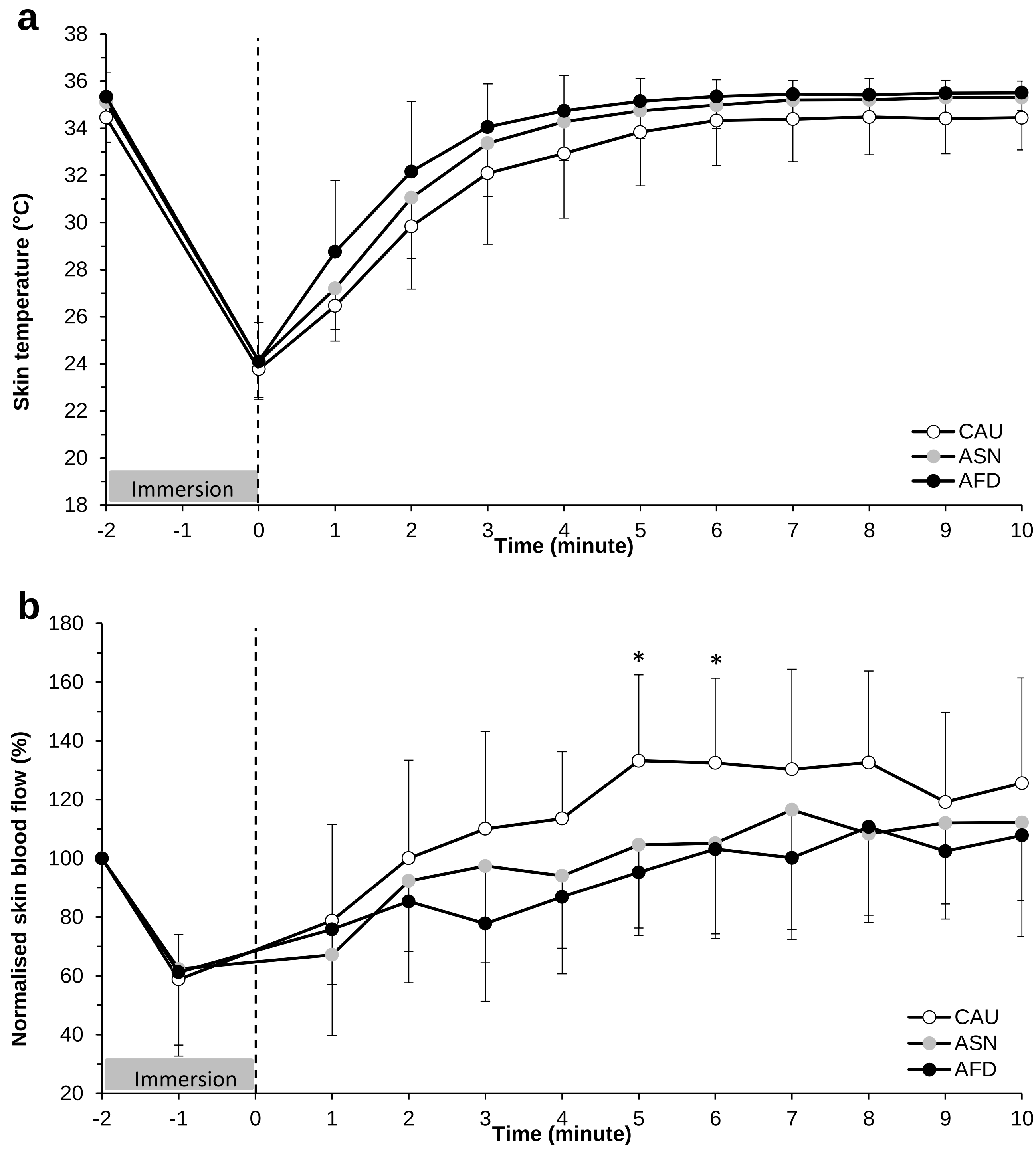

Fig. 2 Mean (SD) Great toe skin temperature (a) and normalised skin blood flow (b) during foot immersion in 15°C water and subsequent rewarming in 30°C air during the cold sensitivity test. *Significant difference between CAU and AFD, $P < 0.05$, n=10 for each group. Vertical dashed line represents start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

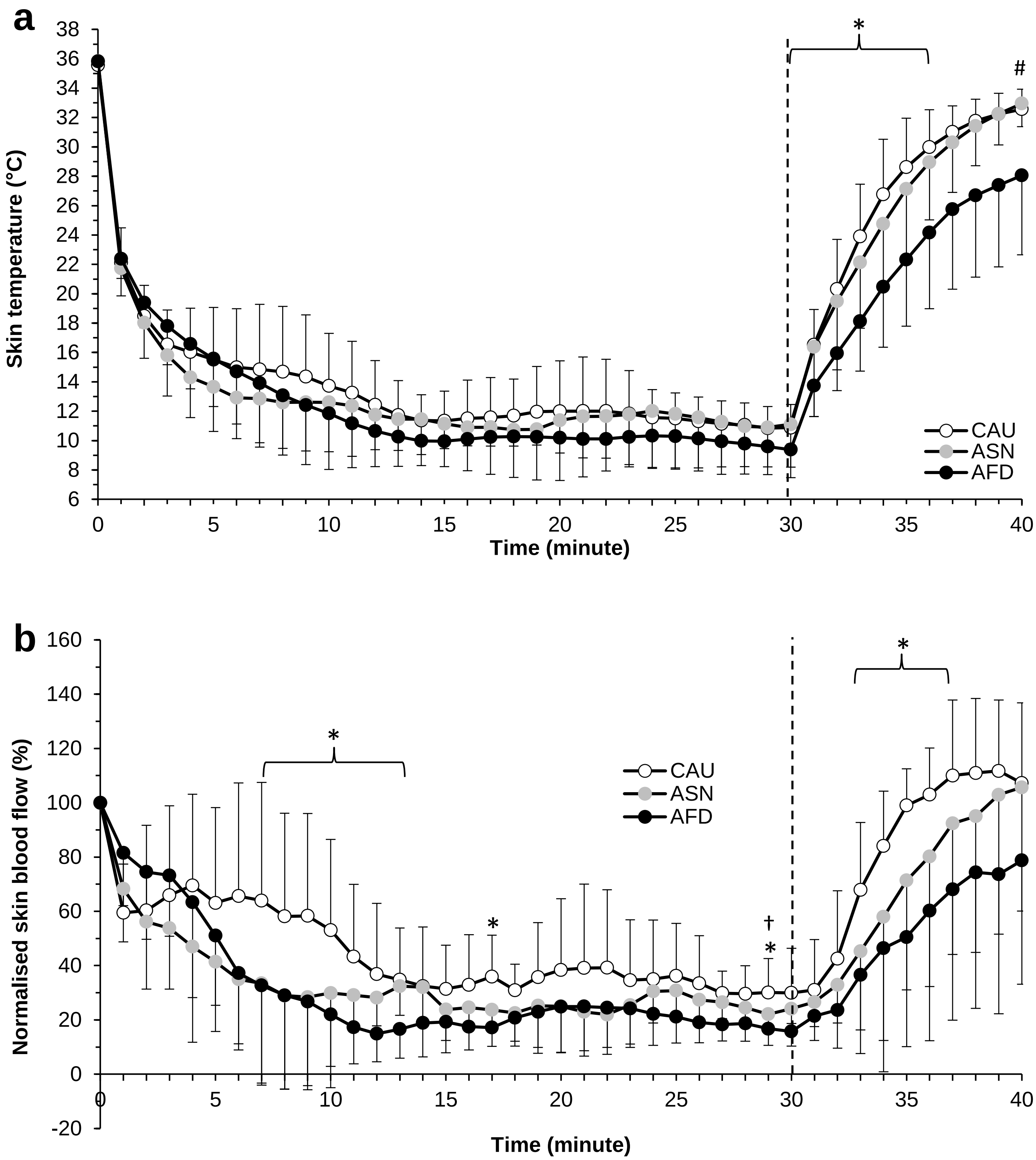

Fig. 3 Mean (SD) finger skin temperature (a) and normalised skin blood flow (b) during hand immersion in 8°C water and subsequent rewarming in 30°C air during the cold-induced vasodilatation test. *Significant difference between CAU and AFD, $P < 0.05$; †significant difference between CAU and ASN, $P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

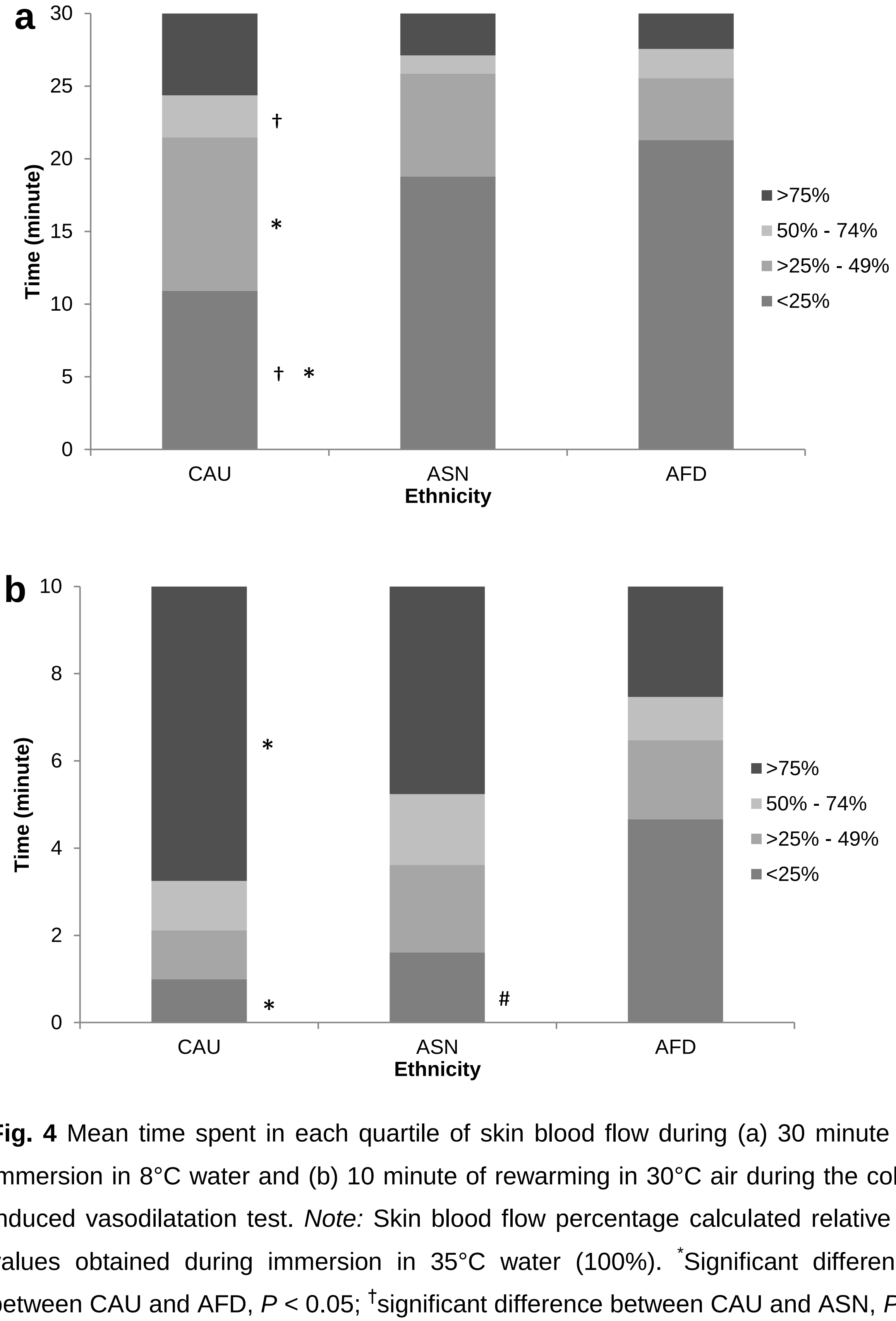

Fig. 4 Mean time spent in each quartile of skin blood flow during (a) 30 minute of immersion in 8°C water and (b) 10 minute of rewarming in 30°C air during the cold-induced vasodilatation test. *Note:* Skin blood flow percentage calculated relative to values obtained during immersion in 35°C water (100%). *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.

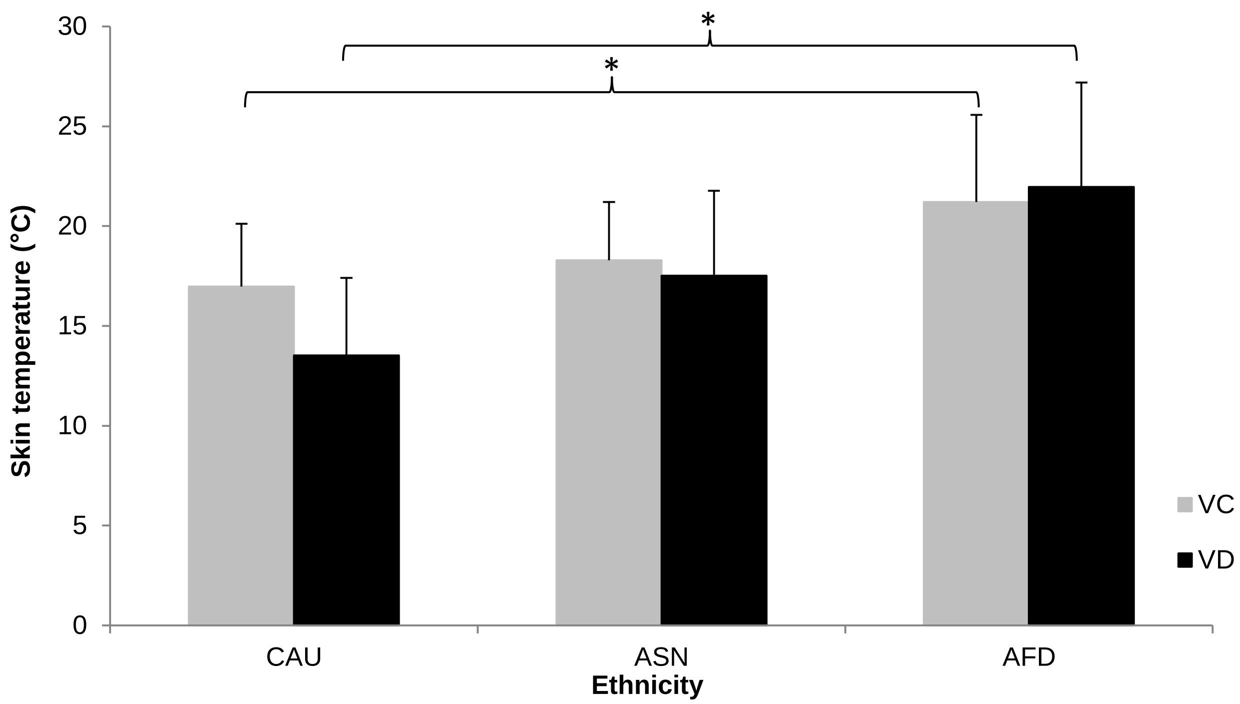

Fig. 5 Mean (SD) finger skin temperature (°C) at which vasoconstriction (VC) and vasodilatation (VD) occurred during hand and arm cooling and rewarming respectively. *Significant difference between CAU and AFD, $P < 0.05$. n=10 for each group except for ASN vasoconstriction only (n=8)

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^2)	2.0 (0.1)	1.9 (0.1)	1.9 (0.2)
Body mass/body surface area ($kg \cdot m^2$)	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)

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

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

Test	**Site**	**CAU**	**ASN**	**AFD**
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)

Note: n=10 for each group

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

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

Note: n=10 for each group. AUC = area under curve. *Significant difference between CAU and AFD, $P < 0.05$; †significant difference between CAU and ASN, $P < 0.05$

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

Variable	**CAU** (*n*=10)	**ASN** (*n*=10)	**AFD** (*n*=10)
Average T_{sk} (°C)[+]	13.02 (2.90)	11.97 (2.01)	11.50 (2.29)
Minimum T_{sk} (°C)	9.69 (0.55)	9.81 (1.09)[#]	8.84 (0.63)[#]
Number of waves[+]	1.00 (1.00)	1.00 (1.25)	1.00 (1.00)
	CAU (*n*=9)	**ASN** (*n*=6)	**AFD** (*n*=6)
Onset time of CIVD (minute)	11.35 (7.67)	9.56 (4.84)	16.89 (7.01)
T_{sk} prior to onset of CIVD (°C)[+]	10.95 (6.10)	10.28 (4.24)	9.10 (2.72)
Max T_{sk} 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)

Note: [+] = Median (IQR), [#]Significant difference between ASN and AFD, $P < 0.05$