

1 **TITLE:** Vascular responses of the extremities to transdermal application of
2 vasoactive agents in Caucasian and African descent individuals

3

4 **AUTHORS:** Matthew J. Maley, James R. House, Michael J. Tipton and Clare M.
5 Eglin

6

7 **ADDRESS FOR CORRESPONDENCE:**

8 Matthew J. Maley

9 Extreme Environments Laboratory

10 Department of Sport and Exercise Science

11 University of Portsmouth

12 Portsmouth, PO1 2ER, UK

13 Email: matthew.maley@port.ac.uk

14 Telephone: +44 23 9284 6395

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

1 **ABSTRACT**

2

3 *Purpose*

4 Individuals of African descent (AFD) are more susceptible to non-freezing cold
5 injury than Caucasians (CAU) which may be due, in part, to differences in the
6 control of skin blood flow. We investigated the skin blood flow responses to
7 transdermal application of vasoactive agents.

8

9 *Methods*

10 Twenty four young males (12 CAU and 12 AFD) undertook three tests in which
11 iontophoresis was used to apply acetylcholine (ACh 1 w/v %), sodium
12 nitroprusside (SNP 0.01 w/v %) and noradrenaline (NA 0.5 mM) to the skin. The
13 skin sites tested were: volar forearm, non-glabrous finger and toe, and glabrous
14 finger (pad) and toe (pad).

15

16 *Results*

17 In response to SNP on the forearm, AFD had less vasodilatation for a given
18 current application than CAU ($P = 0.027$ to 0.004). ACh evoked less
19 vasodilatation in AFD for a given application current in the non-glabrous finger
20 and toe compared with CAU ($P = 0.043$ to 0.014) with a lower maximum
21 vasodilatation in the non-glabrous finger (Median [interquartile], AFD $n=11$,
22 $41[234]\%$, CAU $n=12$, $351[451]\%$, $P = 0.011$) and non-glabrous toe (Median
23 [interquartile], AFD $n=9$, $116[318]\%$, CAU $n=12$, $484[720]\%$, $P = 0.018$). ACh
24 and SNP did not elicit vasodilatation in the glabrous skin sites of either group.
25 There were no ethnic differences in response to NA.

26

27 *Conclusion*

28 AFD have an attenuated endothelium-dependent vasodilatation in non-glabrous
29 sites of the fingers and toes compared with CAU. This may contribute to lower
30 skin temperature following cold exposure and the increased risk of cold injuries
31 experienced by AFD.

32

1 **KEY WORDS:** ethnicity; vasodilatation; vasoconstriction; glabrous; non-
2 glabrous

3

4 **ABBREVIATIONS**

5 ACh Acetylcholine

6 AFD African descent

7 AVA Arteriovenous anastomoses

8 CAU Caucasian

9 ED50 Half-maximal effective dose

10 IQR Interquartile range

11 MAP Mean arterial pressure

12 Mdn Median

13 NA Noradrenaline

14 NFCI Non-freezing cold injury

15 SNP Sodium nitroprusside

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

1 INTRODUCTION

2

3 The feet and hands of individuals who experience local cold tissue
4 temperatures (0 °C to 20 °C) for prolonged periods are at risk of non-freezing
5 cold injury (NFCI) (Ungley and Blackwood 1942). Although the feet are at the
6 greatest risk of suffering a NFCI, various peripheral regions including hand, face
7 and ears are also susceptible (Ungley et al. 1945). Daanen and van der Struijs
8 (2005) showed that out of 57 individuals who suffered a NFCI during winter
9 military operations, 72 % occurred in the feet, 25 % in the hands with the
10 remaining injuries occurring on the head. In the UK military, the proportion of
11 patients seen with NFCI of the hands was 41 % in 2007 seen by the Institute of
12 Naval Medicine's Cold Injury Clinic (Oakley 2009). Symptoms of this injury may
13 last for many years and often include pain, numbness and hyperhidrosis which,
14 combined with cold hypersensitivity of the injured limb, can lead to increased
15 susceptibility to further cold injuries (Ungley et al. 1945; Golden et al. 2013).
16 This type of injury is a concern for those involved in fishery and agricultural
17 work, military operations as well as those participating recreational activities in
18 the cold (e.g. skiing, mountaineering) (Hashmi et al. 1998; Mäkinen et al. 2009;
19 Russell et al. 2013). Additionally, NFCI has come close to deciding the outcome
20 of military conflicts (Golden et al. 2013) and has had financial implications in the
21 form of occupational claims for compensation.

22

23 There are various predisposing factors for cold injuries, these include: high
24 altitude (Hashmi et al. 1998); age (> 62 years) (Koutsavlis and Kosatsky 2003;
25 Sawada 2005); gender (females) (Army Medical Surveillance Activity 2013),
26 nicotine (Cleophas et al. 1982; Waeber et al. 1984) and caffeine consumption
27 (Kim et al. 2013). Ethnicity is also a risk factor; individuals of black African
28 descent (AFD) are more susceptible than Caucasian (CAU) individuals to NFCI
29 (Miller and Bjornson 1962; Taylor 1992; Candler and Ivey 1997; Conway and
30 Husberg 1999; DeGroot et al. 2003; Burgess and Macfarlane 2009). It is
31 thought that sustained skin blood flow in the extremities in low environmental
32 temperatures can prevent local cold injuries such as NFCI (Lewis 1930; Wilson

1 and Goldman 1970; Daanen and van der Struijs 2005). We have previously
2 observed that during hand immersion in cold water (8 °C) for 30 minutes and
3 subsequent rewarming of dry skin in 30 °C air, AFD experience greater
4 vasoconstriction and also rewarm later and more slowly than CAU (Maley et al.
5 2014). Furthermore, during hand and forearm cooling and rewarming the onset
6 of finger vasoconstriction and vasodilatation occurs at a warmer skin
7 temperature in AFD compared with CAU, resulting in a greater 'dose of cold'
8 (Maley et al. 2014).

9
10 Taken together, it appears that the greater susceptibility of AFD to NFCI may be
11 due to differences in the control of skin blood flow between these two ethnic
12 groups. It is important to investigate both glabrous and non-glabrous extremity
13 skin sites as NFCI occurs at both sites, but the control of skin blood flow
14 appears to differ between these sites. The control of skin blood flow is intricate.
15 Sympathetic vasoconstrictor and vasodilator nerves innervate all areas of non-
16 glabrous (hairy) skin, whereas glabrous (hairless) skin is thought to be
17 innervated solely by vasoconstrictor nerves (Sarnoff and Simeone 1947;
18 Johnson et al. 1995) although this has been contested (Lundberg et al. 1989).
19 An important feature of the control of skin blood flow in glabrous skin is the
20 existence of arteriovenous anastomoses (AVA), which are thick muscular, low
21 resistance vessels that allow high flow rates directly from arterioles to venules
22 (Grant 1930; Grant and Bland 1931; Clark 1938). Glabrous regions, such as the
23 finger tips, have up to 236 AVA per cm², whereas no AVA are found in non-
24 glabrous regions of the hand or foot, or the volar surface of the forearm (Grant
25 and Bland 1931).

26
27 The control of skin blood flow has been examined using agents that influence
28 endothelium-dependent or independent vasodilatation, as well as
29 vasoconstriction. Previous investigations have reported that AFD experience an
30 attenuated response to endothelium-dependent vasodilators (e.g. methacholine
31 or acetylcholine) compared with CAU in the forearm circulation (Stein et al.
32 1997; Jones et al. 1999) although this was not supported by Kahn *et al.* (2002).

1 Blood flow responses to an endothelium-independent vasodilator (e.g. sodium
2 nitroprusside or glyceryltrinitrate) appears to be mixed with some observations
3 showing similar responses between AFD and CAU (Kahn et al. 2002; Melikian
4 et al. 2007), whilst others have shown a lower blood flow response in AFD
5 compared with CAU (Stein et al. 1997; Cardillo et al. 1999). The vascular
6 responses to vasoconstrictor agents (e.g. angiotensin II or phenylephrine) has
7 not been as extensively studied, but responses may (Stein et al. 2000), or may
8 not (Jones et al. 1999), differ between ethnic groups.

9

10 The skin blood flow responses to local application of vasoactive agents in CAU
11 and AFD in skin sites susceptible to NFCI are not known. Therefore, the aim of
12 the present study was to examine endothelium-dependent and independent
13 dilatation by using acetylcholine (ACh) and sodium nitroprusside (SNP),
14 respectively. We also investigated any potential vasoconstrictor differences by
15 utilising noradrenaline (NA). Both non-glabrous and glabrous skin sites on the
16 fingers and toes as well as the forearm were tested. It was hypothesised that
17 AFD would experience an attenuated response to ACh and SNP and an
18 exaggerated response to NA compared with CAU.

19

20 **METHODS**

21

22 *Participants*

23

24 12 CAU and 12 AFD male volunteers participated in the study. All participants
25 were non-smokers, were free from any vascular or blood disorders including
26 hypertension, sickle cell disease, diabetes and Raynaud's phenomenon, with no
27 history of either freezing or non-freezing cold injuries. Participants' history of
28 cold exposure was ascertained by questionnaire with each ethnic group
29 reporting similar exposure to cold. Ethnicity was determined by self-
30 classification and all participants were UK residents at the time of testing. All
31 CAU were born in the UK. Four AFD participants were born in the UK whilst
32 eight had resided in the UK for an average of six years; four were born in

1 Zimbabwe, two were born in Nigeria, one was born in Ghana and one was born
2 in Italy. Prior to testing, participants were asked to refrain from consuming
3 alcohol for 24 hours and participating in exercise or consuming caffeine for 12
4 hours.

5

6 *Experimental procedures and measurements*

7

8 Participants attended the laboratory on three occasions separated by at least 24
9 hours where they received local transdermal application of either ACh, SNP or
10 NA using iontophoresis in a balanced order. The technique of iontophoresis has
11 been described previously (Morris and Shore 1996; Roustit et al. 2014). Briefly,
12 iontophoresis is a non-invasive method of transdermal drug delivery which
13 transfers charged molecules using a low-intensity electric current into and
14 through the skin to a depth of approximately 2 to 4 mm (Anderson et al. 2003).

15

16 All experiments were carried out in a quiet temperature controlled chamber.
17 Environmental (dry bulb) temperature was maintained at 23 °C for ACh and
18 SNP protocols. The NA protocol was conducted at a (dry bulb) temperature of
19 24 °C. Pilot testing demonstrated that participants at rest in these environmental
20 temperatures were within the “vasomotor zone” (i.e. neither fully
21 vasoconstricted nor vasodilated) and provided the ideal baseline to observe
22 vasodilatation and vasoconstriction respectively. Each participant was supine
23 throughout the experiment. Blood pressure, from the contralateral arm used for
24 iontophoresis, was recorded pre and post each application of iontophoresis and
25 measured using an automated monitor (Minimon 7137 Plus, Kontron
26 Instruments, UK). All participants rested for 20 minutes in a supine position to
27 allow skin temperature and skin blood flow to stabilise before application of
28 iontophoresis.

29

30 Iontophoresis was applied to the left side of the body to the volar aspect of the
31 forearm, non-glabrous region of the middle finger, non-glabrous region of the
32 Great toe, glabrous region of the middle finger pad and the glabrous region of

1 the Great toe pad. The order of sites tested was balanced. Each skin site was
2 cleaned with deionised water prior to iontophoresis.

3
4 In preliminary studies we observed that iontophoresis was difficult to conduct on
5 AFD as it appeared that they had greater skin resistance which limited the
6 current that could be applied. Approximately 25 μ A could be consistently
7 delivered to both AFD and CAU participants. We therefore adapted our protocol
8 based on previous investigations using intermittent pulses of similar duration
9 (Morris and Shore 1996; Hendry and Marshall 2004; Easter and Marshall 2005).

10
11 Iontophoresis was performed using both an anode and cathode connected to a
12 battery powered iontophoresis controller (MIC2, Moor Instruments, UK). The
13 iontophoresis chamber, which is a small Perspex ring (MIC-ION1R-P1, Moor
14 Instruments, UK) with an inner diameter of 8 mm, was filled with approximately
15 0.2 mL of the relevant drug solution. A laser Doppler probe (VP1T / 7, Moor
16 Instruments, UK), utilised to measure skin temperature and skin blood flow, was
17 placed into the Perspex ring and connected to a laser Doppler flowmetry
18 monitor (moorVMS-LDF, Moor Instruments, UK). Laser Doppler and
19 iontophoresis data were recorded using a data acquisition system and software
20 (Powerlab and LabChart 7, AD Instruments, New Zealand).

21 22 *Protocols*

23 *Acetylcholine (ACh)*

24
25 ACh was used at the anode with the cathode placed proximally to the site of
26 interest. The protocol consisted of six pulses of 25 μ A followed by one pulse of
27 50 μ A and one of 100 μ A for 20 seconds all separated by 60 second intervals in
28 which no current was applied. After an interval of five minutes the protocol was
29 repeated on the next skin site.

30 31 *Sodium nitroprusside (SNP)*

1 SNP was used at the cathode with the anode placed proximally to the site of
2 interest. The protocol consisted of six pulses of 25 μ A followed by one pulse of
3 50 μ A and one of 100 μ A for 20 seconds all separated by 120 second intervals
4 in which no current was applied as the dilator response to SNP takes longer to
5 develop than ACh (Ramsay et al. 2002; Hendry and Marshall 2004). After an
6 interval of five minutes the protocol was repeated on the next skin site.

7 8 *Noradrenaline (NA)*

9
10 NA was used at the anode with the cathode placed proximally to the site of
11 interest. The protocol consisted of six pulses of 25 μ A followed by one pulse of
12 50 μ A and one of 100 μ A for 30 seconds all separated by 60 second intervals in
13 which no current was applied. After an interval of five minutes the protocol was
14 repeated on the next skin site.

15 16 *Drugs*

17
18 ACh was obtained as Miochol-E (Bausch & Lomb, Surrey, UK) and prepared
19 immediately prior to use to a concentration of 1 w/v %. SNP (Rottapharm
20 Madaus, Barcelona, Spain) was dissolved into water for injection to a
21 concentration of 0.01 w/v %. NA (Hospira, Leamington Spa, UK) was diluted
22 into water for injection to a concentration of 0.5 mM. As SNP and NA are
23 photosensitive, all solutions were wrapped in foil and stored in the dark prior to
24 use, with stock solutions being used within eight hours.

25
26 Pilot studies using the vehicle for each drug on the skin sites used for the main
27 protocol showed that the only skin site to show an increase in skin blood flow
28 was the forearm when the cathode was used (for SNP only); this is addressed
29 within the discussion section.

30 31 *Data analyses*

1 Due to high skin resistance it was not possible to deliver all of the current pulses
2 in each skin site for all participants; this occurred more in the AFD participants.
3 As a consequence the number of participants contributing to the mean data
4 decreased as the cumulative current increased (see Fig. 1 and 2). Participants
5 who were able to receive the iontophoresis charge were included in the data
6 analysis. Analysis of the skin blood flow data showed the results were similar if
7 only those who completed the protocol were included compared to inclusion of
8 all participants until they were not able to receive the desired current. Therefore,
9 as we wanted to include as many participants as possible in the analysis we
10 included all participants until they were not able to receive the desired current.
11 As blood pressure remained constant throughout the study (see Table 1), skin
12 blood flow is expressed in laser Doppler units rather than cutaneous vascular
13 conductance (flux/mean arterial pressure). Responses evoked in the cutaneous
14 circulation by iontophoresis were expressed as percentage change from that
15 prior to iontophoresis in the resting condition (averaged over 20 seconds and
16 set at 0 %). For responses to ACh, average skin blood flow was calculated over
17 the final 20 seconds of the interval between successive pulses and between 40
18 to 60 seconds after the final pulse. For SNP, average skin blood flow was
19 calculated over the final 30 seconds of the interval between successive pulses
20 and at 90 to 120 seconds after the final pulse. Responses to NA are shorter-
21 lasting (Hendry and Marshall 2004) therefore the minimum skin blood flow was
22 identified between each pulse and between 0 to 60 seconds after the final
23 pulse.

24

25 Statistical analyses were conducted using IBM SPSS for Windows version 20
26 (IBM SPSS Statistics, USA). An α value of 0.05 was used to determine
27 statistical significance. An independent samples *t*-test was utilised to compare
28 participant characteristics and blood pressures between ethnic groups. A paired
29 samples *t*-test was utilised to assess within-group change in blood pressure
30 from pre to post iontophoresis. The skin blood flow data were not normally
31 distributed therefore group comparisons were conducted utilising a Mann-
32 Whitney *U* test. Half-maximal effective dose (ED50) expressed as 95 %

1 confidence intervals was calculated using GraphPad (Version 5, USA). Maximal
2 skin blood flow (for ACh and SNP) and minimum skin blood flow (for NA) was
3 calculated for each participant and compared between ethnic groups. The point
4 at which the skin blood flow was at a maximum or minimum point was not
5 always identified following the final pulse, therefore maximum skin blood flow
6 was taken from wherever it was highest and minimum skin blood flow was taken
7 from whenever it was lowest. Parametric data in text are presented as mean
8 (SD). Non-parametric data are presented as median (interquartile range - IQR).
9 Data displayed in figures are presented as mean (SD). Effect sizes, where
10 appropriate, were calculated using Cohen's d for parametric data (denoted by d
11 in text) and Rosenthal's r for non-parametric data (denoted by r in text).

12

13 **RESULTS**

14

15 Both ethnic groups were of similar age (CAU: 21[3] years, AFD: 21[2] years),
16 height (CAU: 1.8[0.1] m, AFD: 1.8[0.1] m) and mass (CAU: 73.4[10.7] kg, AFD:
17 73.6[12.2] kg). Blood pressure did not differ between ethnic groups prior to
18 iontophoresis at each skin site (average over 5 recordings) for ACh, SNP and
19 NA (Table 1). Blood pressure did not differ between ethnic groups post
20 iontophoresis, or within group's pre to post iontophoresis.

21

22 *[Insert Table 1 here]*

23

24 Prior to iontophoresis local skin blood flow (Table 2) and skin temperature
25 (Table 3) did not differ between ethnic groups at any skin site except that AFD
26 had a lower resting skin blood flow at the glabrous toe compared to CAU prior
27 to SNP application ($P = 0.014$) however this did not translate to a difference in
28 skin temperature (Table 3). As expected, resting skin blood flow was higher in
29 the glabrous skin regions compared to the non-glabrous regions and this was
30 more marked in the fingers (Table 2). Not all participants could receive the first
31 pulse of iontophoresis due to high skin resistance; therefore only those who

1 successfully received the first pulse were included in the analyses (Tables 2
2 and 3).

3

4 *[Insert Table 2 here]*

5

6 *[Insert Table 3 here]*

7

8 *Skin blood flow responses to acetylcholine (ACh)*

9

10 Dose response curves to ACh were achieved in the non-glabrous regions
11 (finger, toe and forearm; Fig. 1a, 1c and 1e respectively) but not the glabrous
12 regions (finger and toe pads) where the skin blood flow response remained
13 unchanged (Fig. 1b and 1d respectively). In the non-glabrous finger, AFD
14 demonstrated less vasodilatation for a given current (Fig. 1a, $P = 0.043 - 0.014$,
15 $r = 0.48 - 0.52$), a lower maximum vasodilatation (Mdn [IQR], AFD $n = 11$,
16 $41[234]$ %, CAU $n = 12$, $351[451]$ %, $P = 0.011$, $r = 0.53$) and a greater ED50
17 (95% confidence intervals, AFD = $136 \mu\text{A} - 223 \mu\text{A}$, CAU = $40 \mu\text{A} - 117 \mu\text{A}$, P
18 < 0.001) compared with CAU. In the non-glabrous toe, again AFD demonstrated
19 less vasodilatation for a given current (Fig. 1c, $P = 0.024 - 0.023$, $r = 0.50 -$
20 0.68) and a lower maximum vasodilatation (Mdn [IQR], AFD $n = 9$, $116[318]$ %, CAU
21 $n = 12$, $484[720]$ %, $P = 0.018$, $r = 0.51$) compared with CAU, however
22 ED50 was similar. There were no skin blood flow differences between groups
23 for the forearm (Fig. 1e).

24

25 *[Insert Fig. 1 here]*

26

27 *Skin blood flow responses to sodium nitroprusside (SNP)*

28

29 Dose response curves to SNP were observed in the forearm skin site for both
30 groups (Fig. 2e). Dose response curves were also obtained for the non-
31 glabrous finger and toe for CAU but not AFD (Fig. 2a and 2c). No dose
32 response curves were achieved in the glabrous sites (finger and toe) for CAU or

1 AFD (Fig. 2b and 2d). In the forearm skin site AFD demonstrated less
2 vasodilatation for a given current (Fig. 2e, $P = 0.027 - 0.004$, $r = 0.46 - 0.58$)
3 and a greater ED50 (95% confidence intervals, AFD = 130 μ A – 167 μ A, CAU =
4 80 μ A – 107 μ A, $P < 0.001$) compared with CAU. Following the final pulse on
5 the glabrous toe skin site AFD had a smaller skin blood flow response
6 compared with CAU ($P = 0.018$, $r = 0.61$).

7

8 *[Insert Fig. 2 here]*

9

10 *Skin blood flow responses to noradrenaline (NA)*

11

12 Vasoconstriction in response to NA was achieved in the forearm, non-glabrous
13 finger and glabrous toe. Vasoconstriction was also achieved in the glabrous
14 finger and non-glabrous toe of AFD but not CAU; however there were no skin
15 blood flow differences at any skin site between ethnic groups. There were no
16 ethnic differences for minimum skin blood flow for any skin site (Table 4).

17

18 *[Insert Table 4 here]*

19

20 The responses to transdermal delivery of ACh, SNP and NA to the various skin
21 regions in the two ethnic groups are summarised in Table 5.

22

23 *[Insert Table 5 here]*

24

25 **DISCUSSION**

26

27 In the present study, comparing young male CAU and AFD participants, we
28 observed a lower increase in skin blood flow for a given current in response to
29 ACh in the non-glabrous finger and non-glabrous toe in AFD (Fig. 1a and 1c,
30 respectively); however these differences were not repeated with SNP (Fig. 2).
31 Furthermore, there were no differences between ethnic groups in response to
32 NA at any skin site. These findings allow us to partly accept our hypothesis

1 regarding ACh and SNP whilst we reject our hypothesis for NA. This is in
2 contrast to other studies which have investigated whole arm or systemic
3 responses between ethnic groups (Jones et al. 1999; Cardillo et al. 1999;
4 Rosenbaum et al. 2002). We observed a lower endothelium-dependent
5 vasodilatation (Fig. 1) in AFD than CAU in the non-glabrous finger and non-
6 glabrous toe skin sites although responses at the forearm did not differ between
7 groups. The non-glabrous toe skin blood flow responses for CAU displays an
8 initial increase in skin blood flow, followed by a decrease and a subsequent
9 increase. Looking at the individual data it appears that this pattern is primarily
10 driven by two CAU who had an early exaggerated response to ACh where as all
11 other CAU demonstrated a linear increase in skin blood flow. We are unsure
12 why two CAU demonstrated such an early exaggerated response to ACh.
13 Although we observed a significantly lower skin blood flow response in the non-
14 glabrous toe skin site between CAU and AFD following the final pulse, only
15 three AFD were remaining at that data point thus caution should be exercised
16 when considering this time point.

17

18 Our results from the forearm skin site, displaying similar skin blood flow
19 responses, appear to support some previous findings (Kahn et al. 2002) but not
20 all (Jones et al. 1999; Cardillo et al. 1999). In the present study, whilst the
21 forearm microcirculation appears to respond similarly in CAU and AFD to
22 iontophoresis of ACh, it seems that the control of the microcirculation of the
23 fingers and toes in the non-glabrous skin sites differs between CAU and AFD;
24 this may play a role in the increased susceptibility of AFD to cold injuries.

25

26 The present study supports the findings that a difference exists in response to
27 SNP at the forearm (Stein et al. 1997; Cardillo et al. 1998; Cardillo et al. 1999;
28 Gainer et al. 2001; Rosenbaum et al. 2002). Reduced vasodilatation in AFD
29 compared with CAU in response to SNP would suggest an altered function at
30 the vascular smooth muscle cells. However, an electric-induced hyperaemic
31 response (galvanic response) is prominent at the cathode site where SNP is
32 used (Morris and Shore 1996). The increase in skin blood flow observed upon

1 electrical stimulation has been shown to recruit mechano-insensitive C-units
2 (Schmelz et al. 2000) which release vasodilators calcitonin gene related peptide
3 with smaller contributions from substance P (Sauerstein et al. 2000). We
4 attempted to attenuate this response by using a low current (i.e. 25 μ A) in short
5 durations (i.e. 20 seconds). During pilot testing we observed an increase in skin
6 blood flow (~2100 % increase from rest following the final pulse, $n = 5$) in the
7 forearm when water for injection was used at the cathode whereas the other
8 skin sites did not demonstrate any significant increase in skin blood flow. This is
9 therefore a limitation of the iontophoresis technique in that the responses to
10 iontophoresis of SNP in the forearm circulation may, in part, be influenced by a
11 sensory component / galvanic response (Morris and Shore 1996), but the
12 responses to SNP in other skin sites appear to be nitric oxide driven.
13 Responses to SNP at other sites did not differ between groups except following
14 the final pulse on the glabrous toe (Fig. 2d). We are unsure why this difference
15 between groups occurred but feel that we cannot comment on the possible
16 physiological implications of this due to the low number of participants
17 remaining in the AFD group ($n = 3$) at this time / current application.

18

19 It was expected that NA would cause a greater decrease in skin blood flow in
20 AFD compared with CAU; this was not the case in the present study. Previous
21 research has showed that CAU and AFD do not appear to differ in response to
22 intra-arterial infusion of angiotensin II (Jones et al. 1999); others have shown
23 that intra-arterial infusion of phenylephrine causes a greater vasoconstrictor
24 response in AFD than CAU (Stein et al. 2000). Both vasoactive agents appear
25 to exert their vasoconstrictor effects by modulating intracellular calcium levels
26 but the reason for the discrepancy between studies may be due to different
27 mechanism of action; angiotensin II acts on the endothelium as well as the
28 smooth muscle cell, whereas phenylephrine, an α_1 receptor agonist, acts upon
29 the smooth muscle cell (Pueyo and Michel 1997; Rang et al. 2012). NA is able
30 to activate α (vasoconstrictor) as well as β (vasodilatory) adrenergic receptors
31 (Bylund et al. 1994; Rang et al. 2012) which may mask an increased sensitivity
32 in α receptors in AFD as previously reported (Stein et al. 2000). Human

1 vascular smooth muscle contains several types of α receptors with the α_{2c}
2 subtype appearing to become active under conditions such as skin cooling
3 (Chotani et al. 2004). In support of this, previous studies have shown
4 vasoconstriction responses to local cooling is governed primarily by α_2
5 receptors (Ekenvall et al. 1988), more specifically α_{2c} subtype (Chotani et al.
6 2000). These specific receptors are translocated from the Golgi to the vascular
7 smooth muscle cell surface facilitated by Rho kinase (Bailey et al. 2004; Honda
8 et al. 2007). During forearm skin cooling, cutaneous vascular conductance is
9 significantly attenuated following Rho kinase inhibition which may be as a result
10 of a reduced translocation of α_{2c} receptors to the vascular smooth muscle cell
11 surface (Thompson-Torgerson et al. 2007). We previously observed a lower
12 skin blood flow in AFD during hand cooling compared with CAU (Maley et al.
13 2014); whether there are ethnic differences in α_{2c} receptor subtype is not
14 known.

15

16 Whilst the present study provides evidence of an attenuated response to ACh in
17 AFD, the precise mechanism(s) controlling this response is not known. ACh
18 binds to muscarinic receptors on the endothelial surface and produces
19 mediators to effect vasodilatation (Furchgott 1983; Komori and Suzuki 1987;
20 Shore 1996; Shibasaki et al. 2002). These mediators include prostanoids,
21 produced by the cyclooxygenase (COX) enzyme, which are subsequently
22 metabolised to various prostaglandins, including prostacyclin, a known
23 vasodilator (Duffy et al. 1998; Parkington et al. 2004; Félétou 2011). Previous
24 investigations have demonstrated that responses to ACh in the forearm
25 circulation are predominantly mediated through prostanoid-dependent
26 pathways, and an attenuated vasodilatation is observed in the forearm
27 circulation with COX inhibition compared with a control (Noon et al. 1998;
28 Holowatz et al. 2005). However, there are further increases in non-glabrous
29 finger skin blood flow in response to ACh when COX is inhibited in young
30 healthy males (Hendry and Marshall 2004). Thus, it appears that there may be
31 a greater contribution of prostanoid vasoconstrictor products in the finger
32 compared with the forearm. From this, it may be that the balance between

1 vasoconstrictor and vasodilator COX products may differ between groups. Even
2 though responses to SNP, a nitric oxide donor, is similar between groups this
3 does not rule out possible differences in nitric oxide bioavailability affecting ACh
4 induced vasodilatation. Evidence of a reduced nitric oxide bioavailability in AFD
5 compared with CAU has been previously reported and is attributable to an
6 increased superoxide production (Kalinowski et al. 2004; Mata-Greenwood and
7 Chen 2008; Fearheller et al. 2011); this type of oxidative stress and diminished
8 nitric oxide bioavailability has been attributed to vascular disorders such as
9 hypertension (Griendling and FitzGerald 2003) as well as a reduced nitric oxide-
10 dependent vasodilatation in aged skin (Holowatz and Kenney 2010).

11

12 Although the present study examined skin blood flow responses to vasoactive
13 agents in individuals at rest at environmental temperature of 23 °C to 24 °C, we
14 have previously reported that AFD experience an attenuated vasodilatation
15 compared with CAU during rewarming following hand cooling (Maley et al.
16 2014). Low perfusion of tissue during exposure to cold and upon rewarming can
17 be a factor leading to environmental injuries such as NFCI (Endrich et al. 1982;
18 Jia and Pollock 1997; Jia and Pollock 1998). From the present study, as NA
19 responses were similar between ethnic groups it is suggested that the skin
20 blood flow differences observed during the previous study may, in part, be due
21 to differences in the reactivity of the endothelium in AFD compared with CAU
22 which may help explain why this group is more susceptible to freezing and non-
23 freezing cold injuries. The precise mechanism underpinning differences in
24 rewarming times following local cold exposure between AFD and CAU such as
25 that seen previously (Maley et al., 2014) has not yet been fully elucidated. Clues
26 come from findings such as those of Hope et al. (2014) who reported that the
27 nitric oxide donor glyceryl trinitrate improves rewarming times following foot
28 cooling in individuals with cold sensitivity (sub-clinical NFCI). However, we did
29 not observe any differences in skin temperature between uninjured AFD and
30 CAU to the same foot cooling protocol (Maley et al. 2014) therefore, in support
31 of the present study, the role of nitric oxide in CAU and AFD appears similar in
32 response to short duration (2 minute) foot cooling and subsequent rewarming.

1

2 It is concluded that young healthy AFD have an attenuated endothelium-
3 dependent vasodilatation, compared with CAU, in non-glabrous sites of the
4 fingers and toes. This may help explain why AFD are more susceptible to cold
5 injuries which occur predominantly in the peripheries.

6

7 **ACKNOWLEDGEMENTS**

8 The authors wish to thank the participants for volunteering for the study.

9

10 **ETHICAL STANDARDS**

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

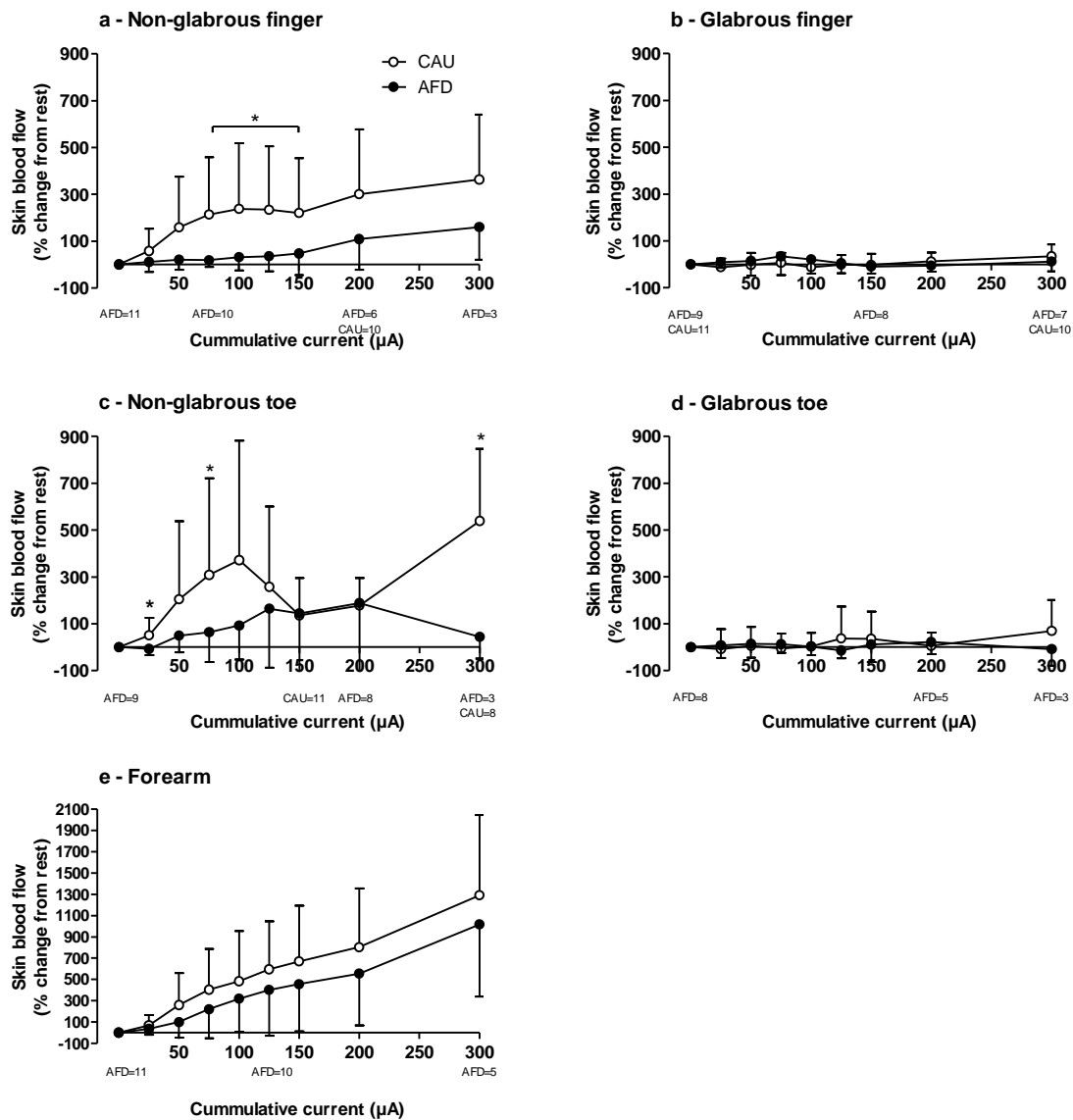
20

21 **CONFLICT OF INTEREST**

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

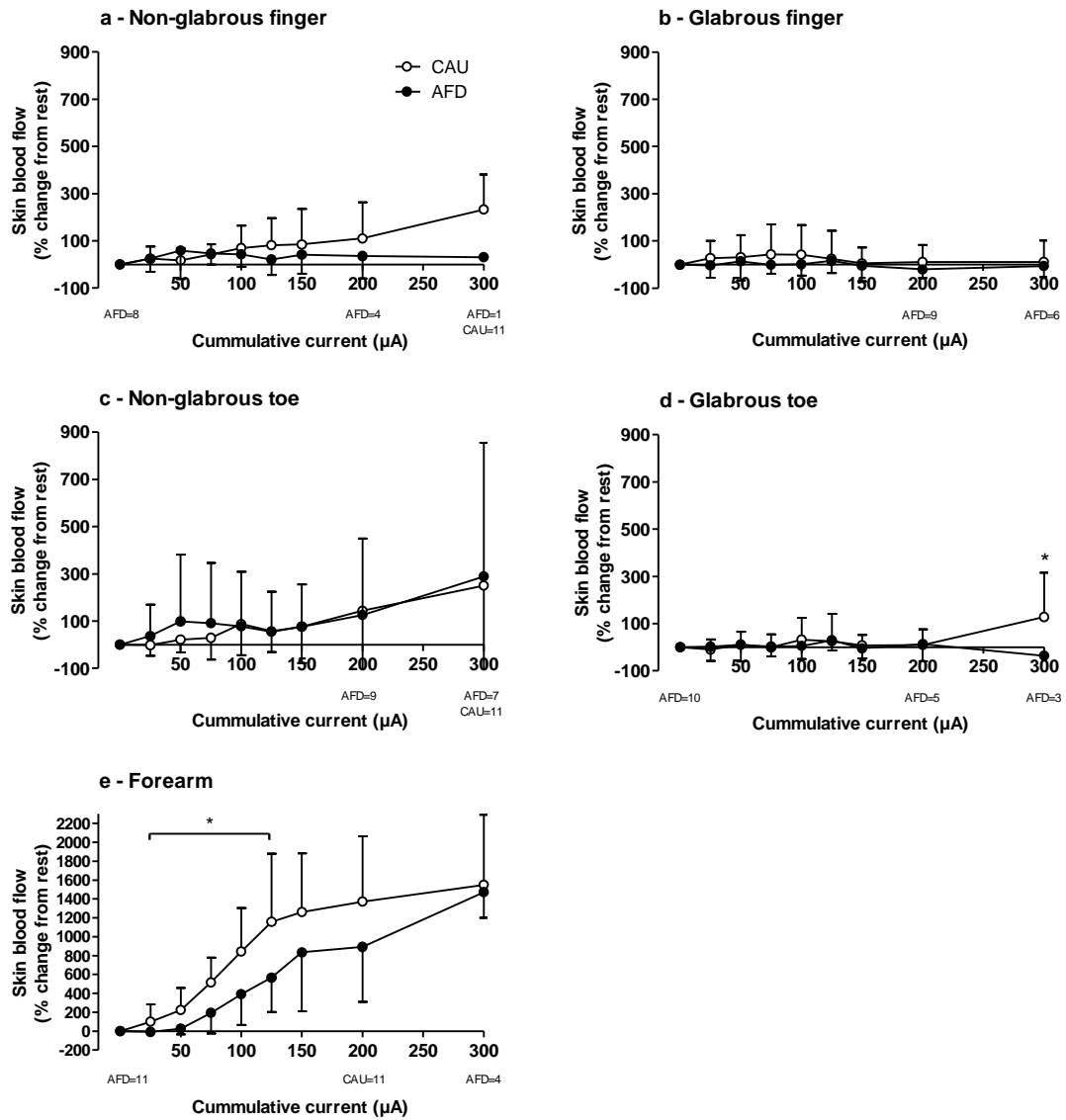
23

24



1
2 **Fig. 1** Mean (SD) skin blood flow responses to iontophoresis of acetylcholine in
3 the non-glabrous finger (a), glabrous finger (b), non-glabrous toe (c) and
4 glabrous toe (d) and forearm (e). n = 12, unless stated. *Significant difference
5 between CAU and AFD, P < 0.05. Note scale change for Fig 1e.

6
7
8



1
 2 **Fig. 2** Mean (SD) skin blood flow responses to iontophoresis of sodium
 3 nitroprusside in the non-glabrous finger (a), glabrous finger (b), non-glabrous
 4 toe (c) and glabrous toe (d) and forearm (e). n = 12, unless stated. *Significant
 5 difference between CAU and AFD, P < 0.05. Note scale change for Fig 2e.

6
 7
 8
 9

1 Table 1 Mean (SD) blood pressure prior to iontophoresis ($n = 24$)

Variable	Blood Pressure (mmHg)					
	Acetylcholine		Sodium nitroprusside		Noradrenaline	
	CAU	AFD	CAU	AFD	CAU	AFD
Systolic	120(11)	121(9)	119(9)	124(9)	120(7)	123(9)
Diastolic	60(6)	64(8)	62(5)	65(8)	62(7)	63(5)
MAP	80(6)	83(8)	81(5)	85(8)	81(6)	83(6)

2 MAP = Mean Arterial Pressure.

3

4

5

1 Table 2 Median (IQR) local skin blood flow (laser Doppler units) prior to
 2 iontophoresis at each skin site

Site	Laser Doppler Units					
	Acetylcholine		Sodium nitroprusside		Noradrenaline	
	CAU	AFD	CAU	AFD	CAU	AFD
Forearm	15(10) <i>n</i> =12	18(16) <i>n</i> =11	16(8) <i>n</i> =12	15(6) <i>n</i> =11	17(17) <i>n</i> =11	11(14) <i>n</i> =11
NGF	58(24) <i>n</i> =12	53(21) <i>n</i> =11	61(31) <i>n</i> =12	36(117) <i>n</i> =8	55(35) <i>n</i> =12	59(102) <i>n</i> =11
GF	399(182) <i>n</i> =11	326(370) <i>n</i> =9	341(249) <i>n</i> =12	255(293) <i>n</i> =12	247(283) <i>n</i> =11	122(462) <i>n</i> =11
NGT	18(37) <i>n</i> =12	11(34) <i>n</i> =9	20(22) <i>n</i> =12	11(8) <i>n</i> =12	11 (27) <i>n</i> =12	16(18) <i>n</i> =11
GT	38(124) <i>n</i> =12	13(53) <i>n</i> =8	44(61)* <i>n</i> =12	15(24) <i>n</i> =10	71(163) <i>n</i> =11	41(94) <i>n</i> =9

3 *Significant difference between groups ($P < 0.05$). NGF = Non-glabrous finger, GF = Glabrous
 4 finger, NGT = Non-glabrous toe, GT = Glabrous toe.

5
 6
 7
 8

1 Table 3 Median (IQR) local skin temperature prior to iontophoresis at each skin
 2 site

Skin Temperature (°C)						
Site	Acetylcholine		Sodium nitroprusside		Noradrenaline	
	CAU	AFD	CAU	AFD	CAU	AFD
Forearm	28.9(1.2) <i>n</i> =12	28.4(1.1) <i>n</i> =11	28.8(0.6) <i>n</i> =12	27.7(1.6) <i>n</i> =11	29.4(1.9) <i>n</i> =11	29.2(1.4) <i>n</i> =11
NGF	29.1(1.9) <i>n</i> =12	28.7(1.2) <i>n</i> =11	29.6(2.7) <i>n</i> =12	27.3(2.0) <i>n</i> =8	30.2(0.8) <i>n</i> =12	29.5(2.4) <i>n</i> =11
GF	29.7(2.0) <i>n</i> =11	30.5(4.8) <i>n</i> =9	30.2(3.6) <i>n</i> =12	28.8(4.8) <i>n</i> =12	30.4(1.9) <i>n</i> =11	30.2(3.3) <i>n</i> =11
NGT	26.6(3.3) <i>n</i> =12	27.1(3.2) <i>n</i> =9	26.6(2.4) <i>n</i> =12	27.0(3.5) <i>n</i> =12	27.6(3.4) <i>n</i> =12	27.6(3.4) <i>n</i> =11
GT	26.3(3.9) <i>n</i> =12	25.5(2.7) <i>n</i> =8	25.4(4.4) <i>n</i> =12	24.3(2.9) <i>n</i> =10	28.2(2.4) <i>n</i> =11	26.9(3.4) <i>n</i> =9

3 NGF = Non-glabrous finger, GF = Glabrous finger, NGT = Non-glabrous toe, GT = Glabrous toe.

4
5

1 Table 4 Median (IQR) and mean (SD) maximum percentage change in skin
 2 blood flow in response to iontophoresis of noradrenaline

Ethnicity		
Site	CAU	AFD
Forearm ^	-39[15] % <i>n</i> = 11	-29[55] % <i>n</i> = 11
Non-glabrous finger	-65[23] % <i>n</i> = 12	-54[25] % <i>n</i> = 11
Glabrous finger	-64[21] % <i>n</i> = 11	-65[25] % <i>n</i> = 11
Non-glabrous toe	-44[32] % <i>n</i> = 12	-46[27] % <i>n</i> = 11
Glabrous toe	-58[27] % <i>n</i> = 11	-46[33] % <i>n</i> = 9

3 ^ Median (IQR).

4

5

6

1 Table 5 Summary of skin blood flow results

Group	Response to Acetylcholine	Response to Sodium nitroprusside	Response to Noradrenaline
CAU	↑ forearm, NGF, NGT	↑ forearm, NGF, NGT	↓ forearm, NGF, GT
AFD	↑ forearm, NGF, NGT	↑ forearm	↓ forearm, NGF, GF, NGT, GT
CAU vs. AFD	NGF and NGT CAU > AFD	Forearm CAU > AFD	No differences

2 NGF = Non-glabrous finger, GF = Glabrous finger, NGT = Non-glabrous toe, GT = Glabrous toe.

3

4

1 REFERENCES

- 2 Anderson CR, Morris RL, Boeh SD, Panus PC, Sembrowich WL (2003) Effects
3 of iontophoresis current magnitude and duration on dexamethasone
4 deposition and localized drug retention. *Phys Ther* 83:161–170
- 5 Army Medical Surveillance Activity (2013) Update: cold weather injuries, active
6 and reserve components, U.S. armed forces, july 2008-june 2013. *Med*
7 *Surveill Mon Rep* 20:12–17
- 8 Bailey SR, Eid AH, Mitra S, Flavahan S, Flavahan NA (2004) Rho kinase
9 mediates cold-induced constriction of cutaneous arteries: role of alpha2c-
10 adrenoceptor translocation. *Circ Res* 94:1367–1374. doi:
11 10.1161/01.RES.0000128407.45014.58
- 12 Burgess JE, Macfarlane F (2009) Retrospective analysis of the ethnic origins of
13 male British army soldiers with peripheral cold weather injury. *J R Army*
14 *Med Corps* 155:11–15
- 15 Bylund D, Eikenberg D, Hieble J, Langer S, Lefkowitz R, Minneman K, Molinoff
16 P, Ruffolo RJ, Trendelenburg U (1994) International union of pharmacology
17 nomenclature of adrenoceptors. *Pharmacol Rev* 46:121–136
- 18 Candler WH, Ivey H (1997) Cold weather injuries among U.S. soldiers in
19 Alaska: a five-year review. *Mil Med* 162:788–791
- 20 Cardillo C, Kilcoyne CM, Cannon RO, Panza JA (1999) Attenuation of cyclic
21 nucleotide mediated smooth muscle relaxation in blacks as a cause of
22 racial differences in vasodilator function. *Circulation* 99:90–95. doi:
23 10.1161/01.CIR.99.1.90
- 24 Cardillo C, Kilcoyne CM, Cannon RO, Panza JA (1998) Racial differences in
25 nitric oxide mediated vasodilator response to mental stress in the forearm
26 circulation. *Hypertension* 31:1235–1239. doi: 10.1161/01.HYP.31.6.1235
- 27 Chotani MA, Flavahan S, Mitra S, Daunt D, Flavahan N a (2000) Silent
28 alpha(2c)-adrenergic receptors enable cold-induced vasoconstriction in
29 cutaneous arteries. *Am J Physiol Heart Circ Physiol* 278:H1075–H1083
- 30 Chotani MA, Mitra S, Su BY, Flavahan S, Eid AH, Clark KR, Montague CR,
31 Paris H, Handy DE, Flavahan N a (2004) Regulation of alpha(2)-
32 adrenoceptors in human vascular smooth muscle cells. *Am J Physiol Heart*
33 *Circ Physiol* 286:H59–H67. doi: 10.1152/ajpheart.00268.2003
- 34 Clark E. (1938) Arterio-venous anastomoses. *Physiol Rev* 18:229–247

- 1 Cleophas TJ, Fennis JF, van't Laar A (1982) Finger temperature after a finger-
2 cooling test: influence of air temperature and smoking. *J Appl Physiol*
3 52:1167–71
- 4 Conway GA, Husberg BJ (1999) Cold-related non-fatal injuries in Alaska. *Am J*
5 *Ind Med* 36:39–41
- 6 Daanen HA, van der Struijs NR (2005) Resistance index of frostbite as a
7 predictor of cold injury in arctic operations. *Aviat Space Environ Med*
8 76:1119–1122
- 9 DeGroot DW, Castellani JW, Williams JO, Amoroso PJ (2003) Epidemiology of
10 U.S. army cold weather injuries, 1980-1999. *Aviat Space Environ Med*
11 74:564–570
- 12 Duffy SJ, Tran BT, New G, Tudball RN, Esler MD, Harper RW, Meredith IT
13 (1998) Continuous release of vasodilator prostanoids contributes to
14 regulation of resting forearm blood flow in humans. *Am J Physiol*
15 274:H1174–1183
- 16 Easter MJ, Marshall JM (2005) Contribution of prostanoids to endothelium-
17 dependent vasodilatation in the digital circulation of women with primary
18 Raynaud's disease. *Clin Sci* 109:45–54
- 19 Ekenvall L, Lindblad LE, Norbeck O, Ezzell BM (1988) Alpha-adrenoceptors and
20 cold-induced vasoconstriction in human finger skin. *Am J Physiol*
21 255:1000–1003
- 22 Endrich B, Laprell-Moschner C, Brendel W, Messmer K (1982) Effects of
23 prolonged cold injury on the subcutaneous microcirculation of the hamster.
24 I. Technique, morphology and tissue oxygenation. *Res Exp Med* 181:49–61
- 25 Fearheller DL, Park J-Y, Sturgeon KM, Williamson ST, Diaz KM,
26 Veerabhadrapa P, Brown MD (2011) Racial differences in oxidative stress
27 and inflammation: in vitro and in vivo. *Clin Transl Sci* 4:32–37. doi:
28 10.1111/j.1752-8062.2011.00264.x
- 29 Félétou M (2011) The endothelium: Part 1: Multiple functions of the endothelial
30 cells—focus on endothelium-derived vasoactive mediators. 306
- 31 Furchgott RF (1983) Role of endothelium in responses of vascular smooth
32 muscle. *Circ Res* 53:557–573. doi: 10.1161/01.RES.53.5.557
- 33 Gainer J, Stein CM, Neal T, Vaughan DE, Brown NJ (2001) Interactive effect of
34 ethnicity and ACE insertion/deletion polymorphism on vascular reactivity.
35 *Hypertension* 37:46–51. doi: 10.1161/01.HYP.37.1.46

- 1 Golden FSC, Francis TJR, Gallimore D, Pethybridge R (2013) Lessons from
2 history: morbidity of cold injury in the Royal Marines during the Falklands
3 conflict of 1982. *Extrem Physiol Med* 2:1–12. doi: 10.1186/2046-7648-2-24
- 4 Grant RT (1930) Observations on direct communications between arteries and
5 veins in the rabbit's ear. *Heart* 15:281–303
- 6 Grant RT, Bland EF (1931) Observations on arteriovenous anastomoses in
7 human skin and in the bird's foot with special reference to the reaction to
8 cold. *Heart* 15:385–411
- 9 Griendling KK, FitzGerald GA (2003) Oxidative stress and cardiovascular injury
10 part I: basic mechanisms and in vivo monitoring of ROS. *Circulation*
11 108:1912–1916. doi: 10.1161/01.CIR.0000093660.86242.BB
- 12 Hashmi MA, Rashid M, Haleem A, Bokhari SA, Hussain T (1998) Frostbite:
13 epidemiology at high altitude in the Karakoram mountains. *Ann R Coll Surg*
14 *Engl* 80:91–95
- 15 Hendry RG, Marshall JM (2004) Vasoconstrictor products of cyclo-oxygenase
16 activity limit acetylcholine-induced cutaneous vasodilatation in young men.
17 *Clin Sci* 107:323–330. doi: 10.1042/CS20040077
- 18 Holowatz LA, Kenney WL (2010) Peripheral mechanisms of thermoregulatory
19 control of skin blood flow in aged humans. *J Appl Physiol* 109:1538–1544.
20 doi: 10.1152/jappphysiol.00338.2010
- 21 Holowatz LA, Thompson CS, Minson CT, Kenney WL (2005) Mechanisms of
22 acetylcholine-mediated vasodilatation in young and aged human skin. *J*
23 *Physiol* 563:965–973. doi: 10.1113/jphysiol.2004.080952
- 24 Honda M, Suzuki M, Nakayama K, Ishikawa T (2007) Role of alpha2c-
25 adrenoceptors in the reduction of skin blood flow induced by local cooling in
26 mice. *Br J Pharmacol* 152:91–100. doi: 10.1038/sj.bjp.0707380
- 27 Hope K, Eglin C, Golden F, Tipton M (2014) Sublingual glyceryl trinitrate and
28 the peripheral thermal responses in normal and cold-sensitive individuals.
29 *Microvasc Res* 91:84–9. doi: 10.1016/j.mvr.2013.11.002
- 30 Jia J, Pollock M (1997) The pathogenesis of non-freezing cold nerve injury.
31 Observations in the rat. *Brain* 120:631–646
- 32 Jia J, Pollock M (1998) Cold injury to nerves is not due to ischaemia alone.
33 *Brain* 121:989–1001
- 34 Johnson JM, Pergola PE, Liao FK, Kellogg DL, Crandall CG (1995) Skin of the
35 dorsal aspect of human hands and fingers possesses an active vasodilator
36 system. *J Appl Physiol* 78:948–954

- 1 Jones DS, Andrawis NS, Abernethy DR (1999) Impaired endothelial-dependent
2 forearm vascular relaxation in black americans. *Clin Pharmacol Ther*
3 65:408–412
- 4 Kahn DF, Duffy SJ, Tomasian D, Holbrook M, Rescorl L, Russell J, Gokce N,
5 Loscalzo J, Vita JA (2002) Effects of black race on forearm resistance
6 vessel function. *Hypertension* 40:195–201. doi:
7 10.1161/01.HYP.0000024571.69634.ED
- 8 Kalinowski L, Dobrucki IT, Malinski T (2004) Race-specific differences in
9 endothelial function: predisposition of african americans to vascular
10 diseases. *Circulation* 109:2511–2517. doi:
11 10.1161/01.CIR.0000129087.81352.7A
- 12 Kim BJ, Seo Y, Kim J-H, Lee DT (2013) Effect of caffeine intake on finger cold-
13 induced vasodilation. *Wilderness Environ Med* 24:328–336. doi:
14 10.1016/j.wem.2013.06.007
- 15 Komori K, Suzuki H (1987) Heterogeneous distribution of muscarinic receptors
16 in the rabbit saphenous artery. *Br J Pharmacol* 92:657–664
- 17 Koutsavlis AT, Kosatsky T (2003) Environmental-temperature injury in a
18 Canadian metropolis. *J Environ Health* 66:40–45
- 19 Lewis T (1930) Observations upon the reactions of the vessels of the human
20 skin to cold. *Heart* 15:177–208
- 21 Lundberg J, Norgren L, Ribbe E, Rosen I, Steen S, Thorne J, Wallin BG (1989)
22 Direct evidence of active sympathetic vasodilatation in the skin of the
23 human foot. *J Physiol* 417:437–446
- 24 Mäkinen TM, Jokelainen J, Näyhä S, Laatikainen T, Jousilahti P, Hassi J (2009)
25 Occurrence of frostbite in the general population – work-related and
26 individual factors. *Scand J Work Environ Health* 35:384–393. doi:
27 10.5271/sjweh.1349
- 28 Maley MJ, Eglin CM, House JR, Tipton MJ (2014) The effect of ethnicity on the
29 vascular responses to cold exposure of the extremities. *Eur J Appl Physiol*
30 114:2369–2379. doi: 10.1007/s00421-014-2962-2
- 31 Mata-Greenwood E, Chen D-B (2008) Racial differences in nitric oxide-
32 dependent vasorelaxation. *Reprod Sci* 15:9–25. doi:
33 10.1177/1933719107312160
- 34 Melikian N, Wheatcroft SB, Ogah OS, Murphy C, Chowienczyk PJ, Wierzbicki
35 AS, Sanders TAB, Jiang B, Duncan ER, Shah AM, Kearney MT (2007)
36 Asymmetric dimethylarginine and reduced nitric oxide bioavailability in

- 1 young black African men. *Hypertension* 49:873–877. doi:
2 10.1161/01.HYP.0000258405.25330.80
- 3 Miller D, Bjornson DR (1962) An investigation of cold injured soldiers in Alaska.
4 *Mil Med* 127:247–252
- 5 Morris SJ, Shore AC (1996) Skin blood flow responses to the iontophoresis of
6 acetylcholine and sodium nitroprusside in man: possible mechanisms. *J*
7 *Physiol* 496 (Pt 2):531–542
- 8 Noon JP, Walker BR, Hand MF, Webb DJ (1998) Studies with iontophoretic
9 administration of drugs to human dermal vessels in vivo: cholinergic
10 vasodilatation is mediated by dilator prostanoids rather than nitric oxide. *Br*
11 *J Clin Pharmacol* 45:545–550
- 12 Oakley H (2009) The hidden enemy: non-freezing cold injury since the
13 Falklands conflict. Proceedings of the NATO, TRO, HFM-168 Symposium
14 on “Soldiers in Cold Environment.” pp KN2, 1–8
- 15 Parkington HC, Coleman HA, Tare M (2004) Prostacyclin and endothelium-
16 dependent hyperpolarization. *Pharmacol Res* 49:509–514. doi:
17 10.1016/j.phrs.2003.11.012
- 18 Pueyo ME, Michel J (1997) Angiotensin II receptors in endothelial cells. *Gen*
19 *Pharmacol Vasc Syst* 29:691–696
- 20 Ramsay JE, Ferrell WR, Greer IA, Sattar N (2002) Factors critical to
21 iontophoretic assessment of vascular reactivity: implications for clinical
22 studies of endothelial dysfunction. *J Cardiovasc Pharmacol* 39:9–17
- 23 Rang HP, Dale MM, Ritter JM, Fowler RJ, Henderson G (2012) Rang and
24 Dale’s Pharmacology, 7th ed. Elsevier Churchill Livingstone, Edinburgh
- 25 Rosenbaum DA, Pretorius M, Gainer J, Byrne D, Murphey LJ, Painter CA,
26 Vaughan DE, Brown NJ (2002) Ethnicity affects vasodilation, but not
27 endothelial tissue plasminogen activator release, in response to bradykinin.
28 *Arterioscler Thromb Vasc Biol* 22:1023–1028. doi:
29 10.1161/01.ATV.0000017704.45007.1D
- 30 Roustit M, Blaise S, Cracowski J-L (2014) Trials and tribulations of skin
31 iontophoresis in therapeutics. *Br J Clin Pharmacol* 77:63–71. doi:
32 10.1111/bcp.12128
- 33 Russell KW, Imray CH, McIntosh SE, Anderson R, Galbraith D, Hudson ST,
34 Cochran AL (2013) Kite skier’s toe: an unusual case of frostbite.
35 *Wilderness Environ Med* 24:136–140. doi: 10.1016/j.wem.2012.11.013

- 1 Sarnoff SJ, Simeone FA (1947) Vasodilator fibers in the human skin. *J Clin*
2 *Invest* 26:453–459
- 3 Sauerstein K, Klede M, Hilliges M, Schmelz M (2000) Electrically evoked
4 neuropeptide release and neurogenic inflammation differ between rat and
5 human skin. *J Physiol* 529:803–810. doi: 10.1111/j.1469-
6 7793.2000.00803.x
- 7 Sawada S (2005) Assessment of local cold tolerance of individuals by using
8 conventional and unconventional methods based on observation of CIVD
9 reactivity. *Prevention of Cold Injuries. Meeting Proceedings RTO-MP-HFM-*
10 *126, Paper 12., Neuilly-sur-Seine, France: RTO, pp 12–1 – 12–6*
- 11 Schmelz M, Michael K, Weidner C, Schmidt R, Torebjork HE, Handwerker HO
12 (2000) Which nerve fibers mediate the axon reflex flare in human skin?
13 *Neuroreport* 11:645–648
- 14 Shibasaki M, Wilson TE, Cui J, Crandall CG (2002) Acetylcholine released from
15 cholinergic nerves contributes to cutaneous vasodilation during heat stress.
16 *J Appl Physiol* 93:1947–1951. doi: 10.1152/jappphysiol.00036.2002
- 17 Shore AC (1996) Vascular biology and physiology. In: Tooke JE, Lowe GDO
18 (eds) *A Textbook of Vascular Medicine*. Arnold, London, pp 8–42
- 19 Stein CM, Lang CC, Nelson R, Brown M, Wood AJJ (1997) Vasodilation in
20 black americans: attenuated nitric oxide-mediated responses. *Clin*
21 *Pharmacol Ther* 62:436–443
- 22 Stein CM, Lang CC, Singh I, He HB, Wood AJJ (2000) Increased vascular
23 adrenergic vasoconstriction and decreased vasodilation in blacks. Additive
24 mechanisms leading to enhanced vascular reactivity. *Hypertension*
25 36:945–951. doi: 10.1161/01.HYP.36.6.945
- 26 Taylor MS (1992) Cold weather injuries during peacetime military training. *Mil*
27 *Med* 157:602–604
- 28 Thompson-Torgerson CS, Holowatz LA, Flavahan NA, Kenney WL (2007) Cold-
29 induced cutaneous vasoconstriction is mediated by Rho kinase in vivo in
30 human skin. *Am J Physiol Heart Circ Physiol* 292:1700–1705. doi:
31 10.1152/ajpheart.01078.2006
- 32 Ungley CC, Blackwood W (1942) Peripheral vasoneuropathy after chilling
33 “immersion foot and immersion hand.” *Lancet* 2:447–451
- 34 Ungley CC, Channell GD, Richards RL (1945) The immersion foot syndrome.
35 *Wilderness Environ Med* 33:17–31

- 1 Waeber B, Schaller MD, Nussberger J, Bussien JP, Hofbauer KG, Brunner HR
- 2 (1984) Skin blood flow reduction induced by cigarette smoking: role of
- 3 vasopressin. *Am J Physiol* 247:H895–901

- 4 Wilson O, Goldman RF (1970) Role of air temperature and wind in the time
- 5 necessary for a finger to freeze. *J Appl Physiol* 29:658–664

- 6