Cutaneous vascular responses of the hands and feet to cooling, rewarming and hypoxia in humans

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ABSTRACT

Introduction. This study investigated skin vasomotor responses in the fingers and toes during cooling and rewarming with, and without, normobaric hypoxia.

Methods. Fourteen volunteers (8 males and 6 females) were exposed to gradual air cooling (mean ± SD - 0.4 ± 0.1°C·min^{-1}) followed by rewarming (+0.5 ± 0.1°C·min^{-1}) whilst breathing normoxic air (F_IO_2 0.21 at 761 ± 3 mmHg) or hypoxic gas (F_IO_2 0.12, at 761 ± 3 mmHg equivalent to ~5000 m above sea level). Throughout the gradual cooling and rewarming phases rectal temperature was measured, and skin temperatures and laser Doppler skin blood flow were measured on the thumb, little finger, great and little toe pads.

Results. During gradual cooling, skin but not deep body temperature decreased. No differences in cutaneous vascular conductance (CVC) were found for the toes or thumb (*P*=0.169 great toe; *P*=0.289 little toe; thumb *P*=0.422). CVC was reduced in the little finger to a greater extent at the same mean skin temperatures (34.5°C to 33.5°C) in the hypoxic compared to normoxic conditions (*P*=0.047). The onset of vasoconstriction and release of vasoconstriction in the thumb and little finger occurred at higher mean skin temperatures in hypoxia compared to normoxia (P<0.05). The onset of vasoconstriction and release of vasoconstriction in the toes occurred at similar skin temperatures (*P*=0.181 and *P*=0.132, respectively).

Conclusion. The earlier vasoconstrictor response and later release of vasoconstriction in the finger during hypoxic conditions may result in a greater dose of cold to that digit, taking longer to rewarm following the release of vasoconstriction.

INTRODUCTION

Cold injury (CI) is a frequent pathological consequence of exposure to altitude (>2800m) [1]. At altitude, hypoxia coexists with other stressors, in particular cold and dehydration. The central and peripheral responses to local and whole body cooling and, separately, the responses to natural or simulated altitude exposures have been investigated in detail. In contrast, the *combination* of these stressors has had less focus within integrated human research[2], despite their frequent combined occurrence in the natural world.

Cold exposure results in cutaneous vasoconstriction that lowers skin temperature, particularly in the extremities, and reduces the heat transfer from deep body tissues to the environment[3]. Thus, a

sustained period of vasoconstriction helps to preserve deep body temperature but increases the risk of frost bite and Non-Freezing Cold injury (NFCI, such as immersion foot or trench foot)[4]. The addition of a hypoxic stimulus (acute or chronic exposure) can prolong cold-induced cutaneous vasoconstriction by slowing rewarming[5,6]. Keramidas et al.[6] reported the acute effects of breathing a hypoxic gas mixture (F_IO_2 = 0.14) on hand skin temperature during rapid cooling in 8 °C water. They found no differences in the rate of hand skin cooling between normoxic and hypoxic conditions. This finding may be expected, as immersion in cold water would rapidly lower skin temperature, as well as provide a strong generalized sympathetic response, prompting a rapid and maximal vasoconstriction, which would likely be a stronger stimulus for peripheral vasoconstriction than hypoxia. Cooling of the digits whilst maintaining the rest of the body in a thermoneutral state may also allow cold induced vasodilatation (CIVD), a cyclical increase in local tissue temperature which accompanies a temporary return of blood flow to the digit[7]. No differences in CIVD response were found between normoxic and hypoxic conditions when the body remained warm and the hand cooled[6]. Gradual whole body cooling in a cold air environment during exposure may reduce mean body temperature and maintain sympathetic tone therefore CIVD is less likely to occur.[8] In the work of Keramidas *et al*, during the air rewarming, post-immersion thumb skin temperatures were significantly lower when breathing hypoxic gas compared to normoxic air[6]. They suggest that the lower skin temperatures during rewarming of the hand in hypoxia may be due to a reduced skin blood flow response, however blood flow was not directly measured in their study.

Any additional reduction in skin blood flow, caused by hypoxia in a cold environment increases the 'dose of cold' (a stimulus which results in physiological changes due to reductions in the environmental temperature, and is applied for a period of time) experienced by the extremities and has the potential to increase both the number and severity of cold injuries. In this way hypoxia may increase the risk of NFCI for a given air temperature. Whilst the exact dose required to increase the risk of NFCI is unclear, there are documented cases of NFCI which provide evidence of the conditions and duration of cold air exposure required,[9,10] however a range of factors may contribute to the mechanism of injury and its severity.[11]

The feet are more exposed to conditions likely to cause NFCI[12], have a lower blood flow and can maintain vasoconstrictor tone when deep body temperature is thermoneutral[13]. In addition, behavioral temperature regulation may be slower to respond to cooling of the toes as cortical models indicate that greater discomfort would be felt in the fingers during simultaneous cooling of both fingers and toes.

These factors suggest that vasomotor and behavioral responses to changes in temperature in the feet may be different to those of the hands on exposure to cold and hypoxic environments. However, this hypothesis has not been tested in a dynamic air environment, similar to that seen when at altitude, with participants warming and cooling with exercise and rest, shelter and exposure, and the vasomotor response switching from constriction to dilatation. It is the sensitivity of the cutaneous vasomotor response to such changes that determine 'dose' of cold experienced and thereby the risk of cold injury. Therefore, it was hypothesized (i) that during a standardized cooling and rewarming profile, vasoconstriction and release of vasoconstriction would occur at higher skin temperatures when breathing a hypoxic gas mixture (F_IO_2 = 0·12), compared to normoxic air; (ii) that vasoconstriction would occur earlier during cooling and be released later upon rewarming in the toes compared to the fingers.

METHODS

Volunteers

Fourteen healthy, non-smoking volunteers (8 males and 6 females) gave their written informed consent to participate in this study (mean [SD], age 23±2 years, height 1.72±0.11 m, mass 74.7±13.8 kg). Potential volunteers were excluded if they had sojourned at high altitude, flown in the month preceeding the experiment or reported peripheral vascular disease, Raynaud's or NFCI to any digits. This study was approved by the University of Portsmouth Science Faculty Research Ethics committee (SFEC 2014-018).

Procedures

Volunteers wearing shorts and a vest were instrumented with a rectal thermistor (Grant Instruments [Cambridge] UK, Ltd) which was self-inserted 15 cm past the anal sphincter, and skin thermistors (Grant Instruments [Cambridge] UK, Ltd) applied to seven sites (chest, arm, thigh, calf, forearm, distal pad of the right index finger and right great toe). Multi-channel Laser Doppler probes (VP1T/7 Moor Instruments, UK) were attached to the pads of the right thumb and little finger, great toe, and little toe and remained in position during both normoxic and hypoxic conditions. A pulse oximeter finger sensor (Nonin 7500, US) was positioned on the middle finger of the right hand and three lead electrocardiograph attached for calculation of heart rate (HR). Volunteers also wore a respiratory mask (Hans Rudolf, US) for the duration of the study for the measurement of end-tidal oxygen and carbon dioxide tensions ($P_{ET}O_2$ and $P_{ET}CO_2$) using a rapid responding oxygen and carbon dioxide analyzer (Rapidox, UK). Rectal and skin temperatures were recorded at minute intervals on a Squirrel 2020

electronic data logger (Grant Instruments [Cambridge] UK, Ltd). Laser Doppler (moorVMS-LDF, Moor Instruments, UK), pulse oximeter and oxygen and carbon dioxide gas analyzers were connected to an analogue to digital recorder (Power Lab, Australia), and were sampled at 400 Hz, 60 Hz and 400 Hz respectively and minute means calculated.

Participants cycled on a cycle ergometer (Monark, 874E, Sweden) at an external work rate of 60 W for 10-15 min in a temperature controlled chamber (ambient temperature 30 °C, relative humidity 50 %), to elevate deep body temperature by 0.3 °C and remove any central constrictor tone.[14] They were then positioned in a semi-recumbent position on a medical couch. After 10 minutes of rest air temperature was decreased at a rate of -0.4 $\pm$ 0.1 °C·min^{-1}. Once the volunteer had maximally vasoconstricted, as determined by Laser Doppler measurement and comparison of these values to a biological zero (manual inflation of a cuff around the limb to occlude blood flow during which laser Doppler measurements are recorded) taken prior to cooling, the room air was gradually rewarmed at a rate of +0.5 $\pm$ 0.1 °C·min^{-1}. When the ambient temperature had returned to 30 °C, with the volunteers' thumb and fingers maximally vasodilated, the same process was repeated a second time to apply the other condition. There was an interval of one hour between the completion of the first rewarm cycle and start of the second cooling cycle, during this period the volunteers remained in the temperature controlled chamber with their instrumentation attached.

Blood pressure (HEM-705C, Omron, UK) was recorded immediately prior to the start of cooling and the end of the passive rewarming phase of each condition. During the experiment volunteers breathed normoxic air for one ambient cooling–rewarming cycle and a humidified hypoxic gas mixture (F_IO_2 = 0·12), for the other ambient cooling-rewarm cycle. The order of presentation of the gas mixtures was balanced. During the hypoxic condition, volunteers were switched to breathing the normobaric hypoxic gas mixture five minutes prior to cooling the chamber air (Figure 1). The chamber wet bulb globe temperatures (WBGT) were measured (Edale instruments, UK) and recorded at minute intervals using a data logger (Squirrel 800 Logger, Grant Instruments [Cambridge] Ltd, UK) and the fraction of inspired oxygen and carbon dioxide concentrations (F_IO_2 and F_ICO_2) were measured at five minute intervals.

Thermal comfort and thermal sensation were assessed for the whole body using visual analogue scales which were modified from Zhang and Zhao[15] at five minute intervals during the exposures. The scales used consisted of a 20 cm continuous visual analogue scale ranging from "Very Hot" (0 cm); "Hot";

164 "Warm"; "Slightly Warm"; "Neutral"; "Slightly Cool"; "Cold"; to "Very Cold" (20 cm) for thermal
165 sensation and for thermal comfort very comfortable (20 cm), comfortable (16 cm), just comfortable (12
166 cm), just uncomfortable (10.5 cm), uncomfortable (4 cm), very uncomfortable (0 cm). On both thermal
167 perceptual scales the worded descriptions adjacent to the scales were used as a guide and volunteers
168 could position their vote at any point along the 20 cm scale.

169

170 *Data analysis*

171 Weighted mean skin temperature and weighted mean body temperatures were calculated[16,17].
172 Cutaneous vascular conductance (CVC) was calculated as the ratio of laser-Doppler blood flow to mean
173 arterial pressure (MAP).

174

175 Visual inspection of the minute by minute CVC was used to establish the onset of vasoconstriction,
176 maximal vasoconstriction and release of vasoconstriction. These points were determined independently
177 by two of the investigators, using *a priori* definitions (Table 1), following the same process previously
178 described by Maley *et al*.[18] Where there was disagreement, the investigators met to decide upon the
179 points collaboratively.

180

181 ********** insert Table 1 about here**********

182

183 *Statistical analysis*

184 *A-priori* power calculations using data from Keramidas *et al*.[6] , power at 0.8 and α = 0·05, determined
185 that 13 volunteers would provide appropriate power. However, to balance the design 14 volunteers
186 were recruited. Statistical analyses were undertaken using SPSS (IBM Version. 22, IBM, New York, NY,
187 USA). Following tests for normality, repeated measures ANOVA were used to establish if differences in
188 deep body temperature, cardiorespiratory, skin blood flow variables and subjective responses occurred
189 during normoxic and hypoxic exposures. A factorial ANOVA was used to compare the skin blood flow
190 responses between hypoxic and normoxic conditions. The skin blood flow responses at each 0.5 °C mean
191 skin temperature increment were compared between hypoxic and normoxic environments and
192 compared to other skin temperature increments (temperature [8] x condition[2]). Paired samples T-tests
193 were used to establish if the mean skin temperatures recorded at the onset of vasoconstriction, maximal
194 vasoconstriction and release of vasoconstriction were different when breathing hypoxic gas and

normoxic air. Significance was set at $P \leq 0.05$; data are presented as mean ± SD. Cohen's *D* effect sizes were also calculated, with values of 0.6, 1.2, 2.0 and 4.0 equalling a moderate, large, very large and extremely large effect size, respectively.[19]

RESULTS

Table 2 shows the cardiopulmonary responses to cooling and rewarming in hypoxic and normoxic environments. As expected, $P_{ET}O_2$, $P_{ET}CO_2$ and S_PO_2 were significantly reduced and HR and MAP increased during hypoxia compared to normoxia ($P_{ET}O_2$, $P < 0.001$; $P_{ET}CO_2$, $P = 0.003$; S_PO_2, $P = 0.002$; HR, $P = 0.013$; MAP, $P = 0.001$). MAP increased between baseline and normoxia ($P = 0.009$) and both MAP and HR increased in hypoxia compared to baseline (MAP $P = 0.001$; HR, $P = 0.003$), whilst $P_{ET}O_2$, $P_{ET}CO_2$ were reduced ($P_{ET}O_2$, $P < 0.001$; $P_{ET}CO_2$, $P < 0.001$; S_PO_2, $P = 0.004$).

**********Insert Table 2 about here**********

Mean rectal temperature was maintained at 37.3 °C ± 0.3 °C in normoxia and 37.3 °C ± 0.3 °C in hypoxia ($P = 0.363$, Figure 1). Mean body temperature reduced during cooling and increased during rewarming, but temperatures did not differ between normoxic and hypoxic conditions ($P = 0.464$, Figure 1). The cause of the reduction in mean body temperature was a reduction in mean skin temperature; hypoxic conditions resulted in higher mean skin temperatures during cooling and rewarming ($P<0.05$), but were not different at baseline prior to cooling. Accordingly, mean skin temperatures are compared rather than mean body temperatures. In subsequent figures, skin blood flow responses will be displayed against mean skin temperature, rather than a function of time, reflecting the stimulus for changes in blood flow.

**********Insert Figure 1 about here**********

Cutaneous vascular responses of the toes to changes in mean skin temperature

Figure 2 shows the CVC responses of the great toe and little toe to mean skin temperature change during whole body cooling and rewarming in air. As expected, when mean skin temperature fell there was a reduction in CVC. However, the CVC responses were not different between the normoxic and hypoxic cooling conditions ($P = 0.169$ great toe; $P = 0.289$ little toe). Additionally, during the rewarming process CVC responses did not differ between normoxia and hypoxia at any given mean skin temperature ($P = 0.693$ great toe, $P = 0.731$ little toe).

**********Insert Figure 2 and Figure 3 about here**********

Cutaneous vascular responses of the thumb and little finger to changes in mean skin temperature

Figure 3 shows the CVC responses to mean skin temperature during cooling and rewarming of the thumb and little finger. Responses were not different between normoxic and hypoxic exposures when the thumb was cooled (P = 0.422). During little finger cooling the CVC response to the hypoxic exposure was reduced to a greater extent at the same mean skin temperatures (34.5 °C to 33.5 °C) compared to the normoxic condition (P = 0.047, d = 0.63), but no differences were found at other mean skin temperatures. In comparison to normoxia, the CVC response to rewarming in hypoxia was reduced at a mean skin temperature of 33 °C (thumb P = 0.045, d = 0.43; little finger P = 0.040, d = 0.743); at all other skin temperatures CVC responses did not differ (thumb P > 0.165, d = 0.36).

**********Insert Table 3 **********

Mean skin temperature at the onset of, maximal and release of vasoconstriction

Table 3 shows the mean skin temperature at the onset of vasoconstriction, maximal vasoconstriction when cooling and release of vasoconstriction when rewarming. The thumb and little finger constricted and dilated at higher mean skin temperatures in hypoxia than normoxia (Table 3). This is in contrast to the responses of the great toe and little toe, where mean skin temperature did not differ at any point in normoxic and hypoxic conditions (Table 3).

**********Insert Table 4 about here**********

Table 4 shows the local skin temperatures at the onset of, maximal and release of vasoconstriction in hypoxic and normoxic conditions. Whilst there were no differences in the vasoconstriction and dilatation responses in hypoxic and normoxic conditions for local toe and thumb skin temperature, greater little finger skin temperatures were measured in hypoxia at maximal vasoconstriction (P = 0.036, d = 0.38) and the release of vasoconstriction (P = 0.039, d = 0.39), with a trend for greater skin temperatures at the onset of vasoconstriction in hypoxia (P = 0.059, d = 0.78).

The cooling and rewarming times were different for each individual, however mean cooling times did not differ between normoxia and hypoxia (normoxia 32.5 ±12.5 min and hypoxia 34.0 ±13.5 min, *P*=0.117). Similarly, rewarming time was also not significantly different between normoxia and hypoxia (normoxia 22±10.5 min, hypoxia 26.0±12.0, *P*=0.121), but there was a small effect *d*=0.4. In addition, the between condition cooling and rewarming durations showed strong positive correlations (cooling *r*=0.825, *P*=0.009, rewarming *r*=0.762, *P*=0.003). Further correlations revealed that body mass was positively related to the cooling duration (*r*=0.599, *P*=0.033), but not to rewarming (*r*=-0.333, *P*=0.266).

******* Include Figures 4 and 5 *******

Subjective responses to normoxic and hypoxic cooling

The subjective responses to cooling and rewarming are shown in Figures 4 and 5. As expected, there are main effects of temperature for both votes of thermal comfort and thermal sensation ($P < 0.001$) during cooling and rewarming. During cooling, there were no main effects of hypoxic exposure in either votes of thermal comfort ($P = 0.774$) or temperature sensation ($P = 0.350$). In contrast, during hypoxic rewarming volunteers were less thermally comfortable ($P = 0.039$) and had cooler temperature sensations ($P = 0.048$) than during normoxic rewarming.

Vasomotor differences between the thumb, little finger and toes

In normoxia, the onset of vasoconstriction occurred at higher mean skin temperatures in the toes than the fingers ($P = 0.044$, $d = 0.32$). In contrast, no differences in the mean skin temperature at the onset of vasoconstriction in the fingers and toes were found during the hypoxic exposure. Likewise, no differences between the mean skin temperatures at maximal vasoconstriction were found between the fingers and toes in either hypoxic or normoxic conditions. Finally, release of vasoconstriction in the thumb and little finger occurred at significantly lower mean skin temperatures than the toes during normoxic ($P = 0.001$, $d = 0.35$) but not hypoxic exposures (Table 3).

DISCUSSION

The present study identifies the skin blood flow responses of the thumb, fingers and toes to gradual cooling and rewarming in combination with an acute hypoxic exposure. This study found that acute

exposure to normobaric hypoxia in combination with skin cooling resulted in little finger and thumb vasoconstriction at higher mean skin temperatures compared to a normoxic condition, but this was not apparent in the toes. Similarly, the release of vasoconstriction occurred at higher skin temperatures in hypoxia than normoxia in the thumb and little finger, but again, not the toes. Therefore, the primary hypothesis, that vasoconstriction and the release of vasoconstriction would occur at higher skin temperatures in hypoxia, can be accepted for the fingers, but not the toes. Additionally, the onset of vasoconstriction and release of vasoconstriction in the toes was found to occur at higher mean skin temperatures than the little finger and thumb.

This study provides a clearer discrimination of changing vasomotor responses, particularly at the onset of vasoconstriction, during normoxic and hypoxic conditions. This was possible by gradually cooling the air in comparison to the rapid local or whole body cold water immersions used in previous studies[6,20,21]. The prolonged finger rewarming in hypoxia observed in the present study has also been reported elsewhere[5,6]. This may be the consequence of the greater sympathetic response to hypoxic environments or related to the hypoxic hyperventilation and hypocapnia that result in reduced perfusion of the extremities.[22]

In the current study, vasoconstrictor responses during hypoxic and normoxic exposures were complete by mean skin temperatures of 30 ^{o}C, local skin temperatures of 23 ^{o}C and air temperatures greater than 13 ^{o}C, whilst deep body temperature was maintained. Once vasoconstriction was confirmed at all test sites, gradual rewarming was performed. The ambient temperature range (30 °C to 13 °C) at first appears rather distinct from the ambient conditions likely be experienced at altitude, and those which may result in prolonged vasoconstriction and possibly NFCI. When clothing is factored in, the microclimate temperature range generated between garments and the skin is closer to the ambient temperature range used in the present study, where minimal clothing was worn. For instance, Cernych *et al*.[23] exposed volunteers dressed in long sleeved shirts and leggings to ambient conditions of 8°C, the microclimate next to the skin was in the region of 23-30 °C. Additionally, a-Ha *et al*.[24] exposed volunteers to ambient temperatures of 0°C and air flows of 0.26 $m \cdot s^{-1}$ air flows, dressed in ski suits and various undergarments (maximum clo of 2.5) they recorded mean skin temperatures of between 31 °C and 35 °C.

NFCI is considered a reperfusion injury that develops following a single or multiple periods of prolonged vasoconstriction.[25] Typically NFCIs have been found when ambient conditions fall between 15 °C and -0.5 °C for prolonged durations[4,26,27] this may be either exposure of naked skin to the environment, or in conditions where the microclimate next to the skin falls and is maintained below 15°C for some considerable time. Our results indicate that the ambient conditions were cold enough to induce maximal vasoconstriction. It seems that hypoxia may also increase the mean skin temperature at which maximal vasoconstriction and the release of vasoconstriction occurs in the thumb and little finger. If exposed for prolonged durations, in temperatures that maintain a vasoconstricted state, hypoxia may extend the period of poor perfusion of cooled tissues, and possibly increase the risk of NFCI.

The subjective perceptions of thermal comfort and sensation indicate that similar subjective responses were apparent during cooling, but upon rewarming, volunteers reported feeling cooler and less comfortable when hypoxic. These alterations in subjective responses may lead to behavioral changes in real world settings (such as an increased exercise intensity or adding of more clothing, where possible) in a hypoxic environment, which may reduce the extent and duration of vasoconstriction, possibly reducing the risk of NFCI.

Site-specific differences in vasomotor tone.

In this study, in normoxic conditions, the toes vasoconstricted at higher mean skin temperatures than the little finger and thumb. Likewise, upon rewarming, the finger and thumb skin blood flow returned more rapidly and the skin temperatures increased at a greater rate than that measured for the toes. It seems unlikely that a single factor is responsible, and the difference may be related to the morphological, neural and vasomotor characteristics of the feet and hands which culminate in a greater area-specific heat flow to the hands than the feet [13,28]. In this study, the onset and release of vasoconstriction in the toes occurred at higher mean and local temperatures than the thumb and fingers. This suggests that the toes receive a greater dose of cold. It is also possible that despite excluding those who had a history of cold injury, two participants may have had cold sensitive feet or a mild undiagnosed NFCI. Their toe data were excluded from these analyses as vasoconstriction persisted during passive rewarming and subsequent exercise. This is also why no determination of the release of vasoconstriction was made; only a small number of the volunteers' toes returned to baseline blood flow values during passive rewarming. This may be related to the sedentary nature of the experiment and maintenance of deep body temperature during passive rewarming, further exercise was required to fully

348 rewarm the toes of the remainder of the volunteers. Caldwell *et al*.[28] suggests maximal vasodilatation of
349 the toes occurs by inducing a level of hyperthermia. Indeed, the return of foot skin blood flow to enable
350 the continuation of the experiment was promoted during this study by a short bout of cycling exercise
351 which increased deep body temperature by no more than 0.3 ^{o}C as has been found previously by Eglin *et*
352 *al*.[14] Had the experiment focused solely on the fingers, the exercise bout would not be required as
353 maximal dilation was recorded with passive rewarming. Consequently, conclusions drawn from skin
354 blood flow or skin temperature research using one site cannot be universally applied to all sites of the
355 body, and separate site-specific investigations are necessary.[29]

356

357 Studies of non-glabrous skin suggest that cutaneous vasodilatation is augmented by exposure to hypoxic
358 conditions whilst applying cooling[30,31] and during passive heating.[32] These findings seem contradictory to
359 those presented for the fingers and hand in the present work, and to previous work,[33] in which skin
360 temperature was reduced in the fingers and toes during hypoxic exposure, compared to normoxia, in
361 thermoneutral environments. These differences are a likely consequence of the different structures and
362 autonomic innervation of the cutaneous circulation of the glabrous sites used in the present study (the
363 finger, thumb and toe pads) compared to non-glabrous skin[34]. However, in the hypoxic condition in this
364 study a small increase in weighted mean skin temperature occurred after five minutes of breathing
365 hypoxic gas, and the difference in weighted mean skin temperature is maintained throughout cooling;
366 interestingly, there were no differences in local finger and toe skin blood flow at this time. This may be a
367 consequence of the use of non-glabrous sites used to calculate weighted mean skin temperature.

368

369

370 *Limitations*

371 It should be noted that particularly during the later part of the cooling phase and early part of the
372 rewarming phase, the local skin temperature of the hands and feet may not be representative of
373 vasomotor responses, but more a reflection of the gradual warming due to ambient conditions.[35]
374 However, the ambient temperature change occurred at similar rates in both the normoxic and hypoxic
375 exposures: this is in fact more ecologically valid, as ambient conditions next to the skin may be
376 constantly changing, depending on the level of insulation worn and environmental temperature.

377

The volunteers were not blinded to the breathing gas mixture, as skin blood flow can be affected by emotional state[36], it may be that volunteers' perceptions influenced skin blood flow responses. However, there are a number of thermal and non thermal factors which stimulate change in skin blood flow.[37] Despite controlling posture and exercise during the study, it was not possible to control the volunteers' emotion in response to the exposure. Whilst heart rate and subjective votes between the exposures were similar at similar ambient temperatures, this factor cannot be excluded.

This study examined the vasomotor responses of both the fingers and toes to a combination of environmental stressors (hypoxia, gradual air cooling followed by rewarming). It is concluded that vasoconstriction and release of vasoconstriction of the thumb and little finger occur at higher skin temperatures during hypoxia than normoxia. Conversely, vasoconstriction and the release of vasoconstriction of the toes occurred at similar skin temperatures in hypoxic and normoxic environments. Consequently, site specific research is required for separate determination of vasomotor responses as application of the findings from the hands would lead to spurious conclusions about the feet. It is also concluded that the earlier vasoconstriction and later release of constriction in the finger and thumb during cold and hypoxic conditions could result in a prolonged dose of cold to those digits. In applied settings, this may mean that the fingers take longer to rewarm once vasoconstricted, which may increase the risk of NFCI.

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AUTHOR CONTRIBUTIONS

HM, JRH and MJT were involved in the study design, data collection, HM drafted the manuscript and JHR and MJT reviewed the manuscript.

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DISCLOSURE STATEMENT

None

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TABLES

Table 1. Definitions for *a priori* determination of the vasoconstriction and release of vasoconstriction

Table 2. Cardiopulmonary responses to cooling and rewarming in hypoxic and normoxic environments when at rest.

Table 3. Weighted mean skin temperatures (^{o}C ± SD) at the onset of vasoconstriction, maximal vasoconstriction and release of vasoconstriction in normoxic (N) and hypoxic (H) conditions.

Table 4. Local skin temperatures (mean, ^{o}C ± SD) at the onset of vasoconstriction, maximal vasoconstriction and release of vasoconstriction in normoxic (N) and hypoxic (H) conditions

FIGURES

Figure 1. Rectal temperature (Mean ± SD) (Triangles), body temperature (circles) and skin temperature (squares) in response to ambient gradual cooling (a) and rewarming (b) in normoxic (filled) and hypoxic (open) conditions. * P<0.05 difference between hypoxic and normoxic conditions

Figure 2. CVC (mean) response of the great toe and little toe to gradual whole body cooling and rewarming. A and B great toe cooling and rewarming respectively. C and D little toe cooling and rewarming respectively. *n*=12 normoxia, 20.9% O_2 (black circles), hypoxia, 11.5% O_2 (open circles)

Figure 3. CVC (mean) response of the thumb and little finger to gradual whole body cooling and rewarming. A and B thumb cooling and rewarming respectively. C and D little finger cooling and rewarming respectively. *n*=14. normoxia, 20.9% O_2 (black circles), hypoxia 11.5% O_2 (open circles). *P<0.05 for normoxia v hypoxia

Figure 4. Thermal comfort (mean ± SD) against mean skin temperature during (a) cooling and (b) rewarming. *n*=14. normoxia, 20.9% O_2 (black circles), hypoxia 11.5% O_2 (open circles). *P<0.05 main effect of condition

525 Figure 5. Temperature sensation (mean ± SD) against mean skin temperature during (a)
526 cooling and (b) rewarming *n*=14. normoxia, 20.9% O_2 (black circles), hypoxia 11.5% O_2
527 (open circles). *P<0.05 main effect of condition

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