Research Square

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Medical history of freezing injury is associated with persistent decrease in local response to cold. A case control study

Melchior Arnal
Ecole Centrale d'Electronique (ECE)
Amelie Clave
Académie de santé des armées (ACASAN)
Nicolas Vincent
Académie de santé des armées (ACASAN)
Gustave Savourey
Institut de Recherche Biomédicale des Armées (IRBA)
Jean-Claude Launay
Institut de Recherche Biomédicale des Armées (IRBA)
Coralie Thieulin
Ecole Centrale d'Electronique (ECE)
Fabien Sauvet
fabien.sauvet@gmail.com

Institut de Recherche Biomédicale des Armées (IRBA)
Cyprien Bourrilhon
Institut de Recherche Biomédicale des Armées (IRBA)

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Abstract

Purpose: Tolerance to cold and cold injuries are characterized by a high inter-individual variability. We investigated the influence of frostbite history on physiological responses to local cold exposure during a hand cold water immersion test.

Methods. 18 subjects with a frostbite history (FS, >1 year, mean 5.0±6.5 years), and 18 control subjects (CS), immersed their right hand for 30min in a 5°C water bath (CWI), followed by 30min recovery (passive recovery) at ambient air temperature (Tdb=22°C), then 30min in a 43°C water bath (active recovery). Fingers and core temperatures, skin blood flow, heart rate and blood pressure were continuously recorded. Evaluation of pain and blood samples were made before, during immersion, and after recovery (active and passive).

Results. Fingers temperatures and cutaneous vascular conductance (CVC) of the FS ~~s~~ubjects were lower than CS during CWI (-0.8±0.5°C, $p<0.03$). Higher pain was reported during immersion in FS. After cold exposure, rewarming during passive recovery was slower for FS (-3.1±2.5°C, $p<0.01$). FS had higher endothelin levels since the beginning of the test until the active recovery and more elevated pCO_2 levels. No significant differences were observed in core temperature, heart rate, arterial blood pressure and arterial gas between the two groups throughout the experiment.

Conclusion. Frostbite history is associated with persistent changes in tolerance and physiological responses during cold exposure and recovery. Our results suggest that the slow rewarming kinetic could be an index of high vulnerability trait, with a reduced blood flow to the extremities caused by a stronger vasoconstrictor tone compared to uninjured subjects.

Introduction

Frostbite is a localized cold-induced lesion resulting from a prolonged exposure to a temperature below 0°C. Hands and feet are at greater risk of frostbite injury but ears, nose and cheeks can also be affected. This type of injury was first described during wars on militaries, but it also afflicts civil population, such as skiers and mountaineers during outdoor activities in cold environments. It results most of the time from inappropriate protection. Frostbites were classified depending on the seriousness of lesions from superficial, with no permanent tissue damage to various degrees of permanent tissue loss, leading at worst to an amputation. Currently, we often use a retrospective diagnosis and a 3 to 6 weeks waiting period is necessary to determine the severity of the lesions. Some researchers are focusing on new guidelines to predict the final outcomes of frostbites earlier (Cauchy et al. 2001), as well as on promising therapies (Zafren 2013). Physiological changes in the peripheries to local cold have been widely studied in the literature. Indeed, many authors have explained the action of sympathetic nervous system leading to vasoconstriction and reduced blood flow in the fingers when exposed to cold (Launay and Savourey 2009; Cheung 2015; Daanen and Van Marken Lichtenbelt 2016). Besides, since Lewis, who described the cold induced vasodilation (CIVD) phenomenon in 1930, many studies investigated this protective mechanism against local cold injuries (Sendowski et al. 1997, 2000; Daanen 2003; Daanen and Layden 2010; Flouris and Cheung 2010). A wide inter and intraindividual heterogeneity in CIVD response has been reported in various studies. In the same time, it has been shown that people with a reduced CIVD response have a higher risk of frostbite, leading researchers to work on CIVD enhancement (Daanen et al. 2012). However, a lack of consensus in the experimental protocols of studies that have assessed physiological response to a local cold exposure of the extremities was noticed by O'Brien in 2005 (O'Brien 2005), who suggested an homogenization of technics to ensure their reproducibility. Single-point Laser Doppler Flowmetry (LDF) is a reproducible non-invasive technique to study cutaneous vascular resistance (CVC) in the fingers, according to the studies of Roustit and Cracowsky (Roustit and Cracowski 2012; Cracowski and Roustit 2016). This tool is accurate at quantifying fast changes in skin blood flux and to study skin microcirculation. Alongside these methodological issues, few studies have been conducted to examine both the influence of frostbite in local response to cold and the predisposition of subjects with frostbite to future cold injury. However, it is well established that people who had suffered a frostbite episode keep years later a cold hypersensitivity and the apprehension of recurrence. This indicates that frostbite history modifies the local cold tolerance. As studies comparing local cold tolerance between frostbitten subjects with uninjured one's are rare, the purpose of this study was therefore to compare physiological vascular and temperature responses to cold water immersion between subjects with a frostbite history FS (> 1 year) with control subject CS. The interests of this study were i) to better elucidate the physiological responses during, and after (recovery), a cold hand test performed both in subjects with a medical history of freezing injuries and subjects without such a medical history and ii) following the results, to try to give advices on how to better prevent risks of recurrence.

Methods

Subjects

Forty healthy men, military members or mountain rescuers not acclimated or recently exposed to cold, volunteered participated in this study. The subjects were divided into two groups of twenty participants each, in order to compare an injured group (i.e. frostbite subjects FS) composed with subjects who had already suffered at least one frostbite episode at least one-year ago, to a control group (CS) with subjects who had never suffered cold injuries. Prior to the experiment, subjects from the injured group filled in a questionnaire on epidemiological data and information on their frostbites.

All subjects were medically screened for Raynaud's syndrome and cardiovascular diseases. This study was previously approved by the Ethics Committee of the University of Grenoble and follow the Helsinki's 2001 recommendations. All participants were clearly informed about the experimental protocol, potential risk and discomfort of the study, and signed an informed consent to participate. Subjects were free to withdraw from the experiment at any time and experiments were stopped in case of severe vagal faintness.

Biometric characteristics were measured and calculated. Body weight (W in kg) was assessed using an electronic balance (KCC300/ID 7, Mettler-Toledo GmbH, G), height (H in m) using a standard height gauge, and skin folds [tricipital (Pct), underscapular (Pcs)] using a Holtain skin fold calliper (Crymich, UK) (mean of two measurements of each by 2 physicians). Body surface area (AD in m2) was calculated using Dubois and Dubois Eq. (1916), the lean body mass

(LBM) was calculated with Lohman's formula (Lohman et al. 1975) and percentage of body fat mass was calculated as (1 - LBM/W) 9 100. Phototype classification was made using the Fitzpatrick Skin Phototype Classification, which ranges from very fair (skin type I) to very dark (skin type VI) (Roberts 2009).

Protocol

Subjects were dressed with shorts and a tee-shirt and, after both a medical examination and insertion of a venous blood catheter in the cubital vein, stayed at rest in a sitting position, in a temperature and humidity-controlled chamber, during all the experiment. Each subject was submitted to a right-hand cooling test in a random order fairly distributed determined with a chi2-test. For the first 30 min, the subject was monitored at a comfortable ambient temperature which was considered to be near a thermoneutral environment (dry bulb temperature Tdb = 22°C ± 1°C, relative humidity 35 ± 10%). Then, the subject was submitted to a 5°C water immersion of the right hand up to the styloid, for 30 min (cryostats HUBER 142w). Immediately after cold water immersion (CWI), the hand was taken out of the water and dried with a towel. The subject was again monitored for 30 min in a comfortable ambient air (22 ± 1°C), this period of time was called "passive recovery". After these 30 min, the subject plunged his right hand into a 43°C water bath for 30 min this period of time was called "Active recovery" (Sendowski et al. 1997; Johnson and Kellogg 2010; Daanen et al. 2012).

Materials

Temperatures and cutaneous vascular measurements

Right hand skin temperatures were continuously monitored using Cu-Ct thermocouples, previously calibrated in iced water, from eight sites (the five fingers, hypothenar eminence, thenar eminence and forearm) and finger skin temperatures were sampled every 10 seconds (Fig. 1).

The temperature sensor was isolated from the environment with neoprene, except at the contact surface with skin. CIVD reactions starts were defined as a continuous rise of at least 0.1°Cover the first minute combined with a continuous rise of at least 0.5°C in 3 minutes. In the same way, the end of CIVD was defined as a decrease of 0.5°C in 3 minutes from a temperature variation above 0.1°C in 1 minute. A temperature rise under these criteria was considered as "NO CIVD".

Multiple CIVD oscillations could be observed and counted for a given subject. The ONSET time is the period of time in seconds from the beginning of the immersion until the start of CIVD response. Each digit response to the cold stress stimulus was observed and monitored. However, we chose to use the index response of each subject as a reference to determine the onset time and the CIVD appearance or not.

Heart rate (Fc) was continuously monitored by Dynamap® Plus 8723 and Systolic (SAP) and Diastolic (DAP) arterial pressure were measured every minute. Mean arterial blood pressure (MAP) was calculated as: MAP = (2DAP + SAP)/3 (mmHg)

Skin blood flow was recorded continuously from the palmar side of the right index middle phalanx near the skin temperature measurement area, by single-point laser Doppler flowmetry (Perimed® PF405). This laser-based technique consists of one transmitting and one receiving optical fibers and is commonly used to assess human cutaneous blood flow. Superficial blood flow was measured by the laser (wavelength 780 nm) to a depth of approximately 0.6 mm. Cutaneous vascular conductance (CVC) was defined as the result of Skin Blood Flow (SkBf) divided by Mean Arterial Pressure (MAP).

Rectal temperature (*T*re) was measured by a (Cu-Ct) thermocouple probe inserted 12 cm into the rectum (accuracy ± 0.05°C). Skin temperatures were measured from ten sites (forehead, arm, forearm, hand, chest, abdomen, thigh, calf, ankle, back) with Cu-Ct thermocouples (accuracy ± 0.05°C). Weighted mean skin temperature (*T*sk) was calculated using the Colin and Houdas Eq. (1965).

For temperatures and CVC values analyses, the recorded Baseline period (Fig. 2) was the last 5-min of the 30 min baseline period in ambient air (22°C). CWI was the 30 min cold water immersion (5°C) period. Passive recovery was the last 5 min of the 30 min rewarming period in ambient air (22°C) and active recovery was the last 5 min of the 30 min rewarming period in hot water (43°C). Onset was defined as is the initial lower Tfi value. Tmin and Tmax were respectively the minimal and the maximal (after Onset) values during CWI immersion. Amplitude was the difference (Tmax-Tmin). Temperature of the 5 figers have been averaged and the variance between fingers calculated.

Blood samples and pain evaluation

Venous blood samples were obtained at 5 min before the end of the baseline period, two times during the immersion (at the end of the Onset and at the first peak of CIVD), at the end of passive recovery and at the end of the active recovery period (Fig. 2). For each blood sample, we filled two heparinized tubes (5 ml, S-Monovette, Sarstedt, G) one for assay of catecholamines and another for determination of nitrates and nitrites. We also used two EDTA tubes with aprotinine (5 ml, Vacutainer, Becton–Dickinson, Le Pont de Claix, France) for assay of renin and endothelin-1. Immediately after filling, veinous blood gas (pO_2, pCO_2) were analyses using Radiometer® ABL800 FLEX and the tubes were centrifuged, aliquoted and the aliquots frozen to -80°C.

Subjects were scored on pain sensation throughout the experiment immediately after each blood samples on a numeric scale ranging from "no pain at all"' (0) to "unbearable pain" (10).

Biochemical and hormone assays

The biochemical assays focused on vasoactive variables for which the half-life was compatible with the test duration. Free plasma catecholamines (CA), noradrenaline (NA), adrenaline (A) and dopamine (DA) were determined using a high-performance liquid chromatography assay with electrochemical detection (HPLC-ED) as previously described. The detection limit for plasma NA, A and DA were 32, 16 and 2 ng/L, respectively. Endothelin-1, the most important vasoconstrictive agent produced by endothelial cells, was measured with a commercial ELISA kit (R&D Systems, Minneapolis, MN, USA). The lower limit of detection was 0.1 pg/ml and inter- and intra-assay best coefficients of variation were 2.6 and 4.6%, respectively. In order to assess renin–angiotensin system activity, the most important hormonal vasoconstrictive pathway, renin was measured with a commercial RIA kit (Renin III generation, CisBio International, Gif sur Yvette, France). The lower limit of detection was 2.5 pg/ml and inter- and intra-assay coefficients of variation were 4.5 and 7.4%, respectively.

Statistical analysis

Comparison of biometrical parameters between FS and CS groups have been made using a Tukey t test. The primary endpoint was the average digital fingers temperature during immersion. Values observed during the different periods were compared using a 2 ways ANOVA: the group (2 levels, non-repeated measure) and the period (4 levels with repeated measure: Baseline, CWI, passive recovery and active recovery). We used the JAMOVI software for R language environment (v1.6.15, retrieved from http://www.jamovi.org). All data are expressed as mean ± SD with a significance level set at $P < 0.05$. The same method was applied for the secondary endpoint. When appropriate, a post Bonferroni analysis was made. For the primary endpoint and CVC, the effect size of the ANOVA analysis was assess using the η^2 (Eta squared). According to Cohen's (1988) guidelines, 0.01 is a small effect, 0.06 a medium effect and 0.14 a large effect.

Results

In 4 out of 40 subjects (2 in each group), the experiment was stopped during the immersion because the subjects' pain levels were higher than 7/10 and they decided to stop the protocol. So, we only used data of 36 subjects consisting of 18 frostbitten and 18 matched controls.

For the FS group, the delay between the test and the injury was at mean 5.0 ± 6.5 years. Questionnaire replies revealed that all the injured subjects had suffered only one frostbite episode except one subject who declared 2 episodes. Frostbites were mainly localized on feet (n=14, 75%), but they were associated on both feet and hand for 3 subjects (16.6%) and only localized only on hands for 2 of them. Hands' frostbites affected mostly the distal phalanx (n=12/14, 86%) and rarely the proximal phalanx (n=2). The average number of injuries was 3.0 ± 1.5. Severity of injuries was of the first-degree (n=13, 72%) and the most severe frostbites were of the second degree (n=5, 28%).

Frostbite happened mainly during the day (n=13, 72%), at an average altitude of 2000 meters but whose standard deviation was rather high, as the minimal altitude was 200 meters whereas the maximum one was 4600 meters. Average air temperature was -7.5°C, with 70% of cold injuries happening for sub-zero temperatures. Reported temperatures ranged from -30°C to 10°C. Wind was also an important factor, as 13 (72%) of frostbites happened in the presence of wind. 28% of frostbites (n=5) happened with a humidity lower than or equal to 20%, whereas 50% of these injuries (n=9) happened for values between 20% and 87%. The majority (n=13, 72%) of subjects wore wet clothes at the time of frostbite, 40% were dehydrated (n=7), 28% presented shivering (n=5), and 45% presented with sleep-deprivation (n=8).

No differences were present in biometrical characteristics between the two groups of participants excepting in the ring finger circumference which is greater for the frostbitten group than the control group (p=0.04) and phototype (p=0.01) (Table 1).

Table 1: Subjects biometrical characteristics

		Control (CS)	Frostbite (FS)	p
Age (years)		31.17 ± 6.62	30.25 ± 6.99	0.68
Weight (kg)		74.15 ± 7.95	78.40 ± 10.91	0.24
Height (cm)		176.47 ± 8.21	180.325 ± 5.56	0.08
Lean body mass (%)		61.37 ± 5.85	65.316 ± 7.74	0.10
Body Fat (%)		17.48 ± 3.88	16.755 ± 2.528	0.91
Thumb	Length (mm)	71.94 ± 6.45	72.51 ± 6.59	0.83
	Circumference (mm)	71.00 ± 5.053	74.21 ± 3.84	0.06
	Surface (mm²)	5117.78 ± 670.25	5375.24 ± 674.58	0.28
	Volume (mm3)	8034.91 ± 1052.29	8438.75 ± 1059.10	0.28
Index	Length (mm)	88.055 ± 12.73	86.35 ± 13.14	0.28
	Circumference (mm)	69.39 ± 4.95	70.91 ± 4.12	0.45
	Surface (mm²)	5899.11 ± 1068.77	6138.75 ± 1107.68	0.35
	Volume (mm3)	9261.60 ± 1677.97	9637.83 ± 1739.06	0.21
Middle Finger	Length (mm)	92.22 ± 10.74	94.75 ± 13.52	0.15
	Circumference (mm)	69.61 ± 4.578	71.81 ± 3.65	0.11
	Surface (mm²)	6443.33 ± 1039.2	6824.51 ± 117.55	0.07
	Volume (mm3)	10116.03 ± 1631.53	10714.46 ± 1851.89	0.07
Ring Finger	Length (mm)	86.89 ± 10.17	89.41 ± 13.31	0.35
	Circumference (mm)	63.83 ± 5.06	67.41 ± 4.01	0.04
	Surface (mm²)	5571.94 ± 971.94	6039.95 ± 1073.96	0.09
	Volume (mm3)	8747.95 ± 1525.94	9482.72 ± 1686.11	0.45
Little Finger	Length (mm)	68.56 ± 7.85	71.25 ± 11.46	0.06
	Circumference (mm)	59.11 ± 3.89	62.55 ± 6.04	0.10
	Surface (mm²)	4067.22 ± 812.23	4451.12 ± 788.66	0.56
	Volume (mm3)	6385.54 ± 1021.91	6988.07 ± 1238.19	0.10
Total finger area (mm²)		27099.39 ± 3954.50	28829.21 ± 4077.21	0.10
Body surface area (m²)		1.9022 ± 0.11	1.945 ± 0.18	0.12
Phototype		3.44 ± 0.61	4.25 ± 1.12	0.01

Values are mean ± SD

General response to cold water immersion test

The test period has a significant influence on subjects' general physiological response. During the baseline phase, heart rate and MAP remained constant in the two groups (HR= 68.7 ± 9.5 bpm; PAM = 85.5 ± 11.3 mmHg for all subjects). During CWI, we observed a significant increase in MAP (MAP = 92.7 ± 13.8 mmHg; p=0.03). During passive recovery, both heart rate and MAP decreased but while heart rate was significantly lower compared to baseline (HR= 63.5 ± 9.42, p=0.02), mean arterial pressure value was restored to its initial value (MAP = 81.8 ± 15.4, p=0.012). During active recovery, heart rate increased (HR= 73.5 ± 9.5, p=0.02) and reached baseline level while MAP remained stable.

Moreover, Tre and Tsk showed a significant decrease during both passive and active recoveries compared to baseline (Baseline: Tre=37.4°C and Tsk=33.0°C; passive recovery: Tre=37.1°C and Tsk=32.2°C; p=0.01 and p=0.004, respectively in comparison to baseline; active recovery: Tre=37.1°C, Tsk=32.3°C, p=0.02 and p=0.003, respectively). However, we didn't observe a group effect of interaction for heart rate, blood pressure, Tre and Tsk values.

Peripheries response to hand cooling test

Mean finger 2 skin temperatures (Baseline Tmean for Tfi= 31.3 ± 3.85°C), dropped immediately after hand immersion into the 5°C bath (Tmean = 9.8 ± 2.0°C), increased with time during passive recovery and reached a peak during the active recovery (Tmean = 40.0 ± 0.8°C).

We observed a significant group (Table 2, p=0.01) and Period (p=0.001) effect for the mean finger temperature, without interaction. Average finger digital temperature was lower in the FS group compared to CS during immersion (-0.8 ± 0.5°C, p<0.03) in cold water and Passive recovery ((-3.1±2.5°C, p<0.01)

(Figer 2). The amplitude of the difference between groups was higher during passive recovery than during CWI. We also observed lower values at onset and CIVD. Mean index skin temperature was minimal few minutes after immersion for both CS (t=6 min, Tmin =10.1°C) and FS (t=21 min, Tmin=8.4°C).

We did not observe a group effect on variance values. However, during the immersion and especially on the onset time, FS's fingertip temperatures were significantly lower than those of CS ($p<0.05$) and rewarming of the auricular on passive recovery is slower for the FS than the CS (Tauricular=19.1 ± 2.4°C; Tauricular=22.7 ± 4.9°C; p=0.02, respectively).

Figure 3 shows the evolution of the index temperature and CVC during the experiment for each group. A significant difference was observed during the immersion in the index CVC between the FS and the CS (p=0.02) with a lower one for subjects who had suffered frostbites (Table 3).

We show an interaction between the group and the period for CVC values. CVC values were lower for the FS only during passive recovery (-0.5±0.1 PU/mmHg, p=0.02). No difference has been observed during the other periods (table 3).

Table 2. ANOVA values.

Parameter	Factor	df	F	P	η²
Mean finger temperature	Group	2-18	6.65	0.01	0.18
	Period	3-17	71.69	0.001	0.22
	Group x period	6-17	0.94	0.45	0.02
Variance between fingers	Group	2-18	0.37	0.54	0.01
	Period	3-17	5.93	0.01	0.16
	Group x period	6-17	0.31	0.02	0.08
CVC	Group	2-18	0.35	0.64	0.01
	Period	3-17	4.94	0.01	0.13
	Group x period	6-17	0.31	0.03	0.09

df is the degree of freedom and η² is the effect size (Eta squared). According to Cohen's (1988) guidelines, 0.01 is a small effect, 0.06 a medium effect and 0.14 a large effect. CVC is cutaneous vascular conductance.

Tableau 3: Mean finger 2 temperature and CVC values between groups [frostbite (FS) and control (CS)] across periods.

	Baseline (22°C)						Immersion (5°C)						Passive Recovery (22°C)						A
	Control			Frostbite			Control			Frostbite			Control			Frostbite			C
T Mean (°C)	31.8	±	0.7	31.5	±	0.7	10.9	±	0.7	9.2	±	0.7	23.6	±	0.7	20.5	±	0.7**	3
T Min (°C)	30.3	±	0.7	30.3	±	0.7	7.5	±	0.7	6.8	±	0.7	10.9	±	0.7	9.1	±	0.8	2
T Max (°C)	32.8	±	0.9	32.6	±	0.9	31.3	±	0.9	29.8	±	0.9	28.0	±	0.9	25.0	±	0.9*	4
Amplitude (°C)	2.5	±	1.0	2.3	±	0.9	23.7	±	1.0	23.0	±	1.0	17.0	±	1.0	15.9	±	1.0	1
Delay Tmax (s)													1698.1	±	72.5	1706.4	±	74.6	5
Delay Tmin (s)							871.0	±	99.4	892.0	±	96.7							
Mean CVC (PU/mmHg)	0.9	±	0.2	0.7	±	0.2	0.5.0	±	0.2	0.5	±	0.2	0.9	±	0.2	0.4	±	0.2*	2

*significant difference between frostbite and control ($p<0.05$). CVC is cutaneous vascular conductance.

Pain

We observed an interaction between the group of the period ($F_{6\text{-}18}$= 4.6, p=0.01). The pain scores were maximal at the first peak of CIVD, few minutes after immersion (mean pain score = 5.0 +- 2.1) and decreased with time, during recovery phases. No significant differences occurred in pain sensation between subjects who had suffered frostbites (FS) and their matched controls (CS) throughout the experiment. However, mean pain score was maximal at the end of the ONSET time (mean pain score = 4.9 ± 1.9) for the CS, whereas maximal mean pain score was reached at the first peak of CIVD for FS (mean pain score = 5.3 ± 2.2).

Biological responses

The study of l biological response revealed a significant influence of the period on noradrenaline ($F_{3,18}$= 4.5, p =0.01) and adrenaline ($F_{3,18}$=3.6, = 0.01) concentrations whereas renin ($F_{3,18}$=1,4, p =0.12) and dopamine ($F_{3,18}$=1,7, p=0,10) concentrations remained constant throughout the experiment. Both adrenalin and noradrenalin concentrations followed an increasing trend past baseline to passive recovery and diminished on active recovery, reaching baseline levels.

A significant group effect ($F_{1,17}$=3,5, p=0.04) has been observed for Dopamine. Significant differences were observed between FS and CS in dopamine concentration on baseline (p=0.012), at the first minutes of immersion (p=0.001) and on the active recovery (p=0.024) whereas no differences were noticed in adrenaline, in noradrenalin neither in renin concentrations.

Endothelin levels (group effect $F_{1,17}$=4,5, p=0.02) were similar for all participants during the active recovery but were much more elevated for subjects with frostbite (FS) than CS on baseline (p<0.001) during the CWI p=0.009) and during passive recovery (p=0.04).

The study of local biological response showed for all subjects an evolution of pCO_2 and pO_2 in the opposite direction. Indeed, peripheral venous gas analyses revealed that pCO_2 and pO_2 curves were symmetric. While we observed a slight augmentation of pO_2 during CIVD, followed by an increase in pO_2 during the active recovery, pCO_2 decreased on the same way. Oxygen saturation followed exactly the evolution of pO2 throughout the experiment. Concerning the acidity of venous blood, HCO_{3-} stayed stable and decreased significantly during the active recovery. A significant change was observed during the active recovery on venous gases and acidity values compared to baseline levels for pCO_2 (p<0.001), pO_2 (p<0.001) sO_2 (p<0.001), pH (p<0.001) and HCO_{3-} (p<0.001), p<0.001, sO_2= 95.3 %, p<0.001; pH= 7,40, p<0.001 and HCO_{3-} = 24.1 mmol/L, p<0.001).

Venous gas analyses showed that the subject's status had a significant influence on venous blood gases and acidity. pCO_2 (group effect $F_{1,17}$ = 4.5, p=0.01, without interaction) is higher for FS than CS from beginning of the test (p=0.002) to the end of passive recovery (p=0.01), but no significant differences were observed during active recovery. However, pO_2 ($F_{1,17}$ = 3.6, p=0.01, without interaction) was significantly different between the two populations only at baseline, with a higher one for uninjured subject (p=0.027). Moreover, FS had a higher HCO_{3-} concentration from the beginning to the end of the experiment that CS. Subjects who had suffered frostbite (FS) had significantly lower pH levels (Interaction effect $F_{6,17}$ = 2.5, p=0.04) on baseline (p=0.02) and passive recovery (p=0.03) compared to CS but not during the immersion.

Table 4. Changes biologic values. * difference between frostbite and control, p<0.05

	Baseline		Onset		CIVD	
	Control	Frostbite	Control	Frostbite	Control	Frostbite
Rénine (pg/ml)	14.9 ± 1.7	14.1 ± 1.6	15.7 ± 2.0	14.8 ± 1.8	16.4 ± 2.0	18.3 ± 2.8
Noradrénaline (ng/l)	362.1 ± 22.3	376.4 ± 23.6	440.9 ± 24.4	448.3 ± 26.7	481.2 ± 30.4	485.5 ± 34.9
Adrénaline (ng/l)	45.6 ± 5.0	36.4 ± 4.0	67.2 ± 9.1	58.2 ± 7.2	83.5 ± 10.7	148.4 ± 32.2
Dopamine (ng/l)	17.8 ± 1.6	12.4 ± 1.1	16.6 ± 1.4	10.7 ± 0.8	15.2 ± 1.7	16.4 ± 3.2

Table 5. Changes in venous gas parameters. * difference between frostbite (FS) and control (CS), p<0.05

	Baseline		Onset		CIVD		Passive recovery
	Control	Frostbite	Control	Frostbite	Control	Frostbite	Control
pH	7.4 ± 0.1	7.4 ± 0.1	7.4 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1
pCO_2 (mmHg)	46.3 ± 0.9	50.8 ± 1.2*	47.2 ± 0.7	51.2 ± 0.9*	44.1 ± 0.6	46.8 ± 1.1	45.9 ± 1.1
pO_2 (mmHg)	43.0 ± 2.9	35.6 ± 2.1*	45.2 ± 2.2	39.2 ± 1.8	51.9 ± 2.5	45.6 ± 1.7	40.9 ± 3.2
sO_2 (%)	70.7 ± 4.1	59.4 ± 3.9*	75.8 ± 2.4	66.8 ± 2.5*	83.1 ± 1.9	77.2 ± 1.7	67.7 ± 3.7
HCO_{3-} (mmol/L)	26.4 ± 0.3	28.1 ± 0.4$	26.6 ± 0.2	28.1 ± 0.3*	25.9 ± 0.2	27.1 ± 0.4*	26.2 ± 0.3

Discussion

In this study we demonstrated the persistent differences in physiological responses to cold and subsequent recovery between subjects who had suffered frostbite and uninjured subjects. No differences were observed in general response to local cold exposure between the two populations, excepting the dopamine and endothelin concentration, but this study reveals many local differences in the extremity's response to cold. It showed finger skin temperatures of subjects with prior frostbite cold injuries were colder during immersion, compared to those of the control group. In contrast, rate of rewarming did not differ between injured and uninjured subjects, excepting for the auricular recovery, which was slower for injured subjects.

Vascular parameters were assessed on the index finger, due to physical constraints. Analysis of the thermal behavior of the hand showed a remarkable homogeneity, with the index finger following the trend and evolution of the rest of the hand. We can thus assume that the findings observed at the index finger can be expanded to the rest of the hand, within reason.

The physiological changes obtained during the 30 min CWI, are in line with previous results. Indeed, as described, by Cheung (Cheung 2015), skin temperature of the fingers rapidly and exponentially dropped to a minimal value closed to the bath temperature, due to a strong vasoconstriction directly following the immersion. The significant rises in heart rate and PAM at the time to immerse hand into cold water, seem to confirm the hypothesis of a vasoconstrictor tone driven, by sympathetic activity. Blood flow in the extremities rapidly adjusts to cold exposure, reducing the cutaneous microcirculation in favor of a central pooling of blood in deep body core. However, after few minutes of immersion, CIVD reactions occurred. As reported by Cheung and Mekjavic (Cheung and Daanen 2012), we observed asynchronous CIVD over different fingers of a same hand, CIVD responses in some fingers but not all and different magnitude of CIVD among fingers, within a given hand, during immersion. These observations confirm previous studies and report a wide heterogeneity of responses between fingers of a same hand to cold exposure. However, as we used the index skin temperature as a reference for the other finger's assessment, no significant differences were obtained between fingers, pooling all participants. Thus, it would have been interesting to assess each digit individually, to highlight the variability of finger responses, to a cold stress stimulus as suggested by Morrison (Morrison et al. 2015).

The index CVC was lower for subjects with frostbites than for uninjured subjects during immersion, which means that their blood flow was more reduced to the extremities than the control group.

The water temperature of the bath used on active recovery was determined to produce a maximal vasodilation of vessels, based on results obtained in a 2010 study (Johnson and Kellogg 2010). According to this study, forearm blood flow reaches maximal value near 42°C so a local warming of 43°C can be used to normalize blood flow and vascular conductance during the experiment, enabling comparisons between subjects and groups. Our results actually showed no differences between subjects with frostbite and uninjured ones (CS) during the 43°C immersion in PVBG analysis. Whereas from the beginning to the 43°C recovery, subjects with frostbites actually presented higher pCO_2 levels than uninjured ones.

The temperature disparity of the nail fold of the five fingers was calculated as the variance of SkBf (Pauling et al. 2012). The greater temporal and spatial resolution of Laser Speckle Contrast Imaging (LSCI) compared with thermic camera may facilitate the development of novel assessment tools of autonomic function and digital cutaneous perfusion.

A striking outcome of this study is the evolution of endothelin levels. We decided to present this result in the peripheral response part, first because this vasoconstrictor peptide site of action is located on the extremities, then because it induced local adaptations. Endothelin is suggested to be highly related to sympathetic hyperactivity, resulting in enhanced vasoconstrictor tone and reducing blood flow to the peripheries. Its concentration in venous blood was minimum during the active recovery (43°C immersion) and no differences were noted between the two groups of subjects, probably because the sudden and intensive rewarming led to maximal vasodilation and sympathetic activity attenuation. However, endothelin levels were much higher in injured subjects than in the control group from baseline to the end of passive recovery. Cold injured subjects therefore seem to permanently present a stronger vasoconstrictor tone in upper limb peripheries, than their matched controls.

Endothelin isn't directly linked to the central nervous system, unlike the other catecholamine hormones that were monitored in this experiment. Higher levels of endothelin, which appear to be innate due to said levels being increased even in the control phase, would point toward a tendency for vasoconstriction independent of any cold stimuli. [it could therefore be considered that endothelin might have an antagonistic role in the deployment of CIVD, which would explain the incidence of frostbite as the impediment of reflexive vasodilation would render them more prone to tissue injury. Endothelin's role in CIVD hasn't been explored in literature as of the writing of this article. A Lancet article regarding the role of endothelin on Raynaud's syndrome (Zamora et al. 1990), using the same experimental setup as the one described in the present article, also pointed towards a high shift of baseline Endothelin value compared to control tests. Knowing this, further analysis of endothelin's role in the CIVC/CIVD response could be of interest in elucidating the physiological ascendency of differences in cold sensitivity]

Moreover, results of venous blood gas analysis were never published in studies on physiological response to local cold exposure. However, venous blood gas sampling is more and more used as a substitute of arterial blood sampling in medicine because veins are easier to access than arteries. This method is particularly suitable in some cases in emergency departments (Lim and Kelly 2010; White et al. 2018). According to publications and a review (Byrne et al. 2014) investigating the comparability of venous blood gas and arterial blood gas analyses, venous blood gas seemed to well compare with arterial analysis for pH estimation but not for PCO_2 and PO_2. Thought, it seemed interesting to use venous as an alternative of arterial analysis to investigate the temporal evolution of venous blood gas and the acido-basic equilibrium when hand is exposed to local cold. It was more comfortable for subjects, easier to realize and with a fewer risk of vascular complications. Venous blood sampling is also useful for catecholamines, renin and endothelin determination. Catecholamines are hormones and neurotransmitters naturally produced by the body in the brain, in nerve tissues and in adrenal glands. The best known of these catecholamines are noradrenaline, adrenaline, and dopamine. They help the body to regulate heart rate and arterial blood pressure sending signal to nervous system. When confronted to a cold stress stimulus, they activate sympathetic nervous system in the aim to reduce blood flow to the extremities by vasoconstricting vessels. Endothelin is a peptide also enrolled in the regulation system with a strong vasoconstriction action on blood vessel. Endothelin is one of the most powerful vasoconstrictors and its secretion from the vascular endothelium affects vascular tone. Dosing of catecholamines was chosen in this study to observe general biology response to cold while venous blood end endothelin analyses reflect local response.

Moreover, the results of PVBG analyses showed a simultaneous rise in pO_2 and decrease in pCO_2 during CIVD which is in line with the description of the hunting response. The opening and closing of vessels and capillaries during CIVD reaction enable to increase blood flow to the extremities. As a consequence, venous blood is more oxygenated and contains less CO_2. This idea is reinforced by the PVBG values obtained during the 43°C recovery. This

immersion into hot water led to a sudden sharp increase in pO_2 and decrease in pCO_2. The values obtained during the 43°C immersion attest of an arterialization of venous blood. The rise in pH level during the 43°C recovery reflects the smaller concentration of CO_2, which is acidic, in venous blood.

We investigated the evolution of vasoconstrictors but it could also be interesting to study the levels of vasodilators to see if differences exist between subjects with frostbites and uninjured ones.

When the hand is immersed in cold water, local tissue temperature falls could also reduce perfusion by a physical change in the blood itself, associated by cold increases plasma and whole-blood viscosity. This response partly through greater red-cell aggregation and reduced red-cell deformability, and this effect is worsened locally by plasma leakage from cooled vessels, which further concentrates the cellular components of blood. (Regli et al. 2021; McIntosh et al. 2024). The combined effect of vasoconstriction and rising viscosity is a marked drop in tissue perfusion and oxygen delivery, so the extremity cools even faster and becomes progressively hypoxic. In our work, we observed in the frostbite history group, lower PO_2 a and higher PCO_2 values during recovery that could be explaine by this response. If exposure continues, tissue temperature can fall to the point where damaging cell membranes and initiating the tissue injury could be observed. In this way, cold-induced hyperviscosity is not simply an incidental laboratory finding but an active contributor to the ischemic cascade that makes fingers and hands especially vulnerable to freezing injury.

Another interesting finding was that the injured population had a higher phototype compared to the remaining group whereas the two groups had similar biometrical characteristics. Similar results were obtained in a study (Zagari et al. 2009), which suggested that humans with a higher phototype are more represented in the cold injured population. Besides, Cheung and Daanen (Cheung and Daanen 2012) reported that it is worthwhile to look at ethnic differences in local response to cold. Another study (Sullivan-Kwantes et al. 2017) showed that, ethnic background would influence the physiological reactions to cold and cold tolerance. Indeed, several studies identified ethnic differences in the ability to vasodilate the extremities when exposed to cold. According to them, Caucasians seem to better vasodilate the peripheries than people from Caribbean and African origin in very cold conditions thus, more blood and more heat is able to enter in their fingers. This observation reinforces the results of the study (DeGroot et al. 2003), which found that African-American men suffered cold injury hospitalization 3.7 times more than Caucasians and the highest incidence rate were in American Indian/Alaskan men. These results may confirm the idea of the existence of nonmodifiable risk factors for FCI including ethnic background that could influence cold tolerance and the vasodilatory response of the extremities to cold.

Cutaneous temperatures were monitored with ten separate sensors, each located at a different point according to anatomical features identified in the early stages of the experiment (Sendowksi (2000), Savourey (1996)). These sensors provide a punctual reading of the temperature, that is, the value at a given point without the possibility of moving its location. Further experiments should complement, if not outright replace, these contact measurements with ones based on the radiated infrared energy of the hand, giving a broad range of spatial values. Issues stemming from the use of thermocouples are the need for an acquisition channel for every single sensor, the necessity of mounting them to the subject's hand while thermally isolating them from the outside, and their inability to be relocated. We therefore are planning on utilizing thermal imagery to better monitor the temperature of the hand, while also shifting to an aerial means of cooling the hand allowing us to measure the cooling of the hand insofar as water's infrared absorption would prevent us from accurately gauging it with immersion methods. Another argument for this type of cooling is the potential behavioral difference regarding water-based cooling rather than aerial cooling, motivated by the difference in thermal conductivity and convective nature of heat transfer.

Another issue related to the use of water immersion to replicate cold exposure is the inability to detect blood perfusion using a speckle laser Doppler flowmeter. In this regard, as well as with the use of thermal imagery for hand temperature monitoring,

Conclusion

In conclusion, these results show that physiological response and cold tolerance during a local cold immersion are weakened for subjects with prior frostbite compared to uninjured subjects. The parameter that differed the most between the two populations was cutaneous temperature. Differences in blood circulation in the peripheries are significant, but we still don't know if these differences were inherited or acquired following a frostbite episode. However, higher endothelin levels and higher phototypes seem to characterize the injured population and may result in an altered ability to vasodilate the extremities when exposed to cold. The ability to visually map out the reheating of tissues, correlated with tissue perfusion, would be beneficial as a means of identifying these different behavioral profiles. Innate differences were identified, yet not to the extent of pathological phenomena like Raynaud's syndrome.

Another point of interest would be the reported feeling of cold in the case of subjects with a history of cold exposure, which begs the question of whether said heightened sensitivity of cold is the reason for their former cold injury, or if said sensitivity is itself caused by this previous event. Cold-injured subjects didn't present with a frostbitten right hand, which was the targeted area of our experiment, which would point toward an innate hypersensitivity to cold, both in nociception and potential wounding. Taken together, our findings are relevant to the prevention of frostbite in FS patients, who are at increased risk. Enhanced protective measures and, possibly, screening for the local response to cold and rewarming appear to be appropriate.

Cold exposure was done with a hydraulic system, of the Cold Pressor type, which doesn't reproduce the environmental conditions of actual on-field cold exposure. It would therefore be interesting to use an aerial cold exposure system.

Abbreviations

CIVD	Cold-induced vasodilation
CVC	Cutaneous vascular conductance
CS	Control subjects
DAP	Diastolic arterial pressure
FS	Frostbite subjects
MAP	Mean arterial pressure
SAP	Systolic arterial pressure
SkBf	Skin blood flow
Tfi max	Maximal finger skin temperature
Tfi mean	Mean finger skin temperature
Tfi min	Minimum finger skin temperature
Tre	Rectal temperature
Tsk	Skin temperature

Declarations

Statements and Declarations:

Authors disclosed no financial or non-financial interest in this study

AI Statement:

Any generative AI was used for data analysis or manuscript conception.

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Conflict of interest

Authors declared no conflict of interest

Author Contribution Statement

GS and JCL conceived and designed research. GS, JCL and CB conducted experiments. MA, AM, NV, FS and CB analyzed data. MA, AM, NV and FS wrote the manuscript. GS, JCL, CT and CP corrected the manuscript. All authors read and approved the manuscript.

Data Availability

The Ungdata surveys that support the findings of this study are available from IRBA, but restrictions apply to the availability of these data, which were used under licence for the current study and so are not publicly available. The data are, however, available upon request to the corresponding authors and with the permission of IRBA.

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Figures



Figure 1

A. Instrumentation of the immerged hand with thermocouples and laser doppler. B. Example of an instrumented hand (during baseline and passive recovery). C. Example of immersion of the right hand.

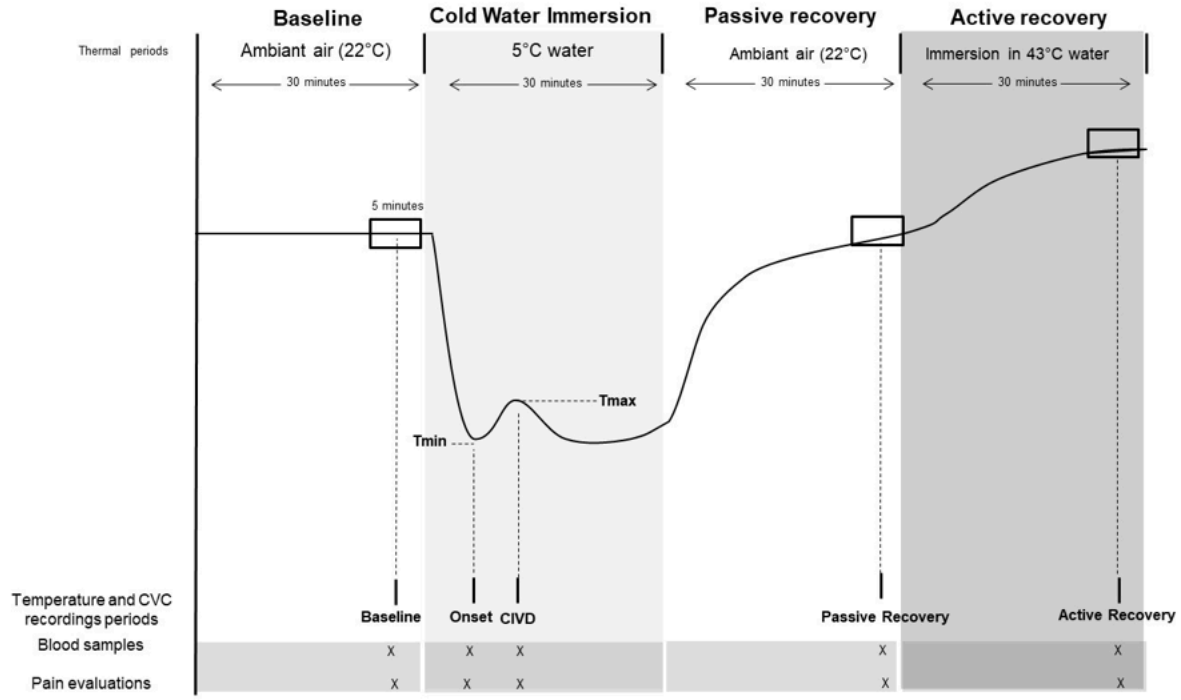


Figure 2

Experimental scheduling and calculated parameters. Baseline is the last 5 min of the 30 min baseline period in ambient air (22°C). Cold water immersion (CWI) is the 30 min immersion (5°C water) period. Passive recovery is the last 5 min of the 30 min rewarming period in ambient air (22°C) and active recovery the period of immersion in a 43°C water. Onset is the initial lower Tfi value during CWI immersion. Tmin is the minimal value during immersion. Tmax is the maximal after onset during CWI Amplitude is the difference Tmax - Tmin.

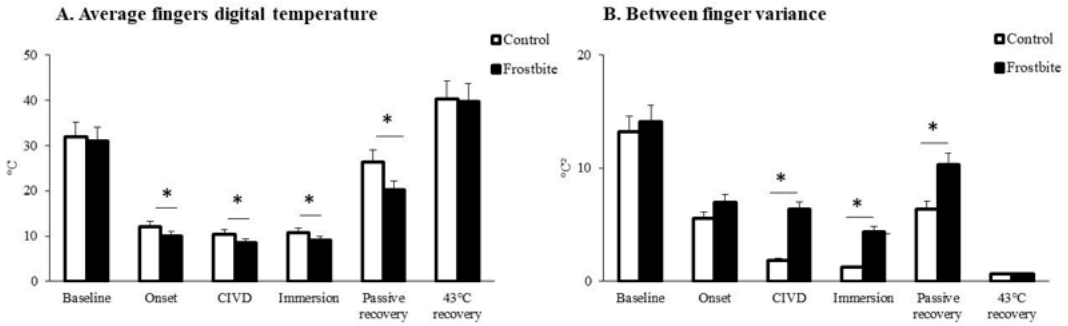


Figure 3

Average fingers temperature and between fingers variance

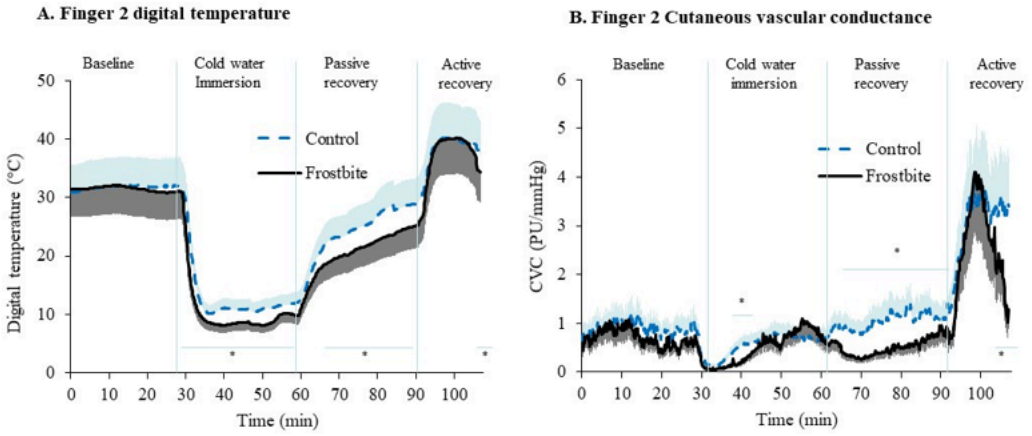


Figure 4

Index finger skin temperature and cutaneous vascular conductance CVC throughout the experiment. * is a difference between Control (CS) and Frostbite (FS) subjects ($p<0.05$).

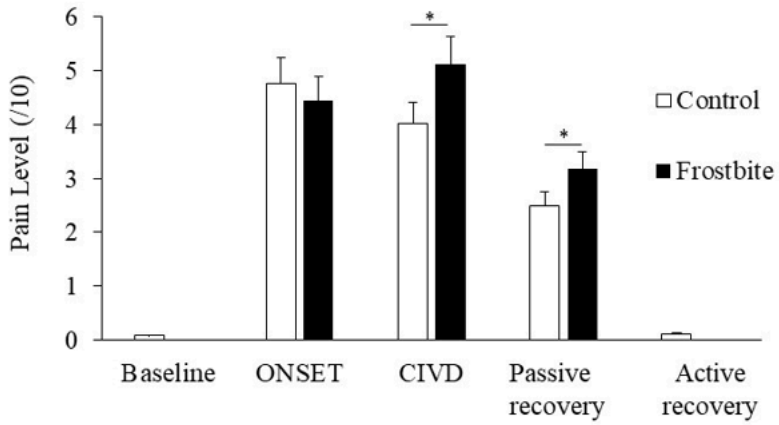


Figure 5

Pain level during test periods for frosbite (FS) and control (CS) subjects. *P<0.05. CIVD is cold induced vasodilation. * is a difference between FS and CS, $p<0.05$.

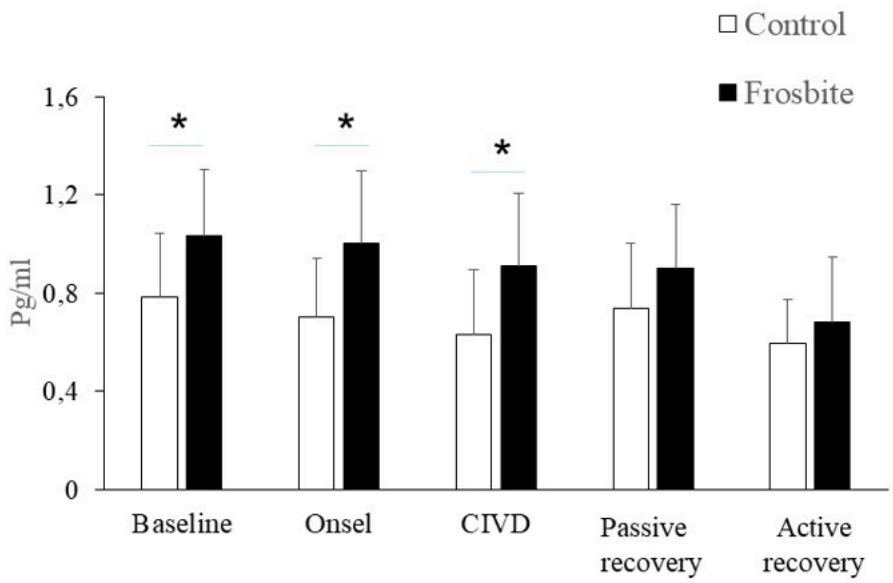


Figure 6

Endothelin values across periods in control and frostbite subjects. * is the difference between FS and CS ($p<0.05$). CIVD is cold induced vasolidation.