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Observations of cold-induced vasodilation in persons with spinal cord injuries

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STUDY DESIGN: Acute experimental study.

OBJECTIVES: Cold-induced vasodilation is a local mechanism of protection against frostbite in non-injured persons. We assessed whether an increase in skin blood flow (SkBF) during local cooling (LC) was observed in individuals with spinal cord injuries (SCIs) and if the response patterns differed between region levels or sites.

SETTING: Laboratory of Wakayama Medical University and the affiliated clinics, Japan.

METHODS: A local cooler device (diameter 4 cm) was placed on the chest (sensate) and right thigh (non-sensate) in persons with cervical (SCI_C; $n = 9$) and thoracolumbar SCIs (SCI_{TL}; $n = 9$). After the surface temperature under the device was controlled at 33 °C for 10 min (baseline), LC (-0.045 °C/s) was applied and the skin temperature was maintained at 15 and 8 °C for 15 min of each stage. SkBF (laser Doppler flowmetry) was monitored using a 1-mm needle-type probe inserted into its center.

RESULTS: The percent change in SkBF (% Δ SkBF) on the chest remained unchanged until the end of 15 °C stage; thereafter, it increased to a level at least 70% greater than the baseline during the 8 °C stage in both groups. The % Δ SkBF on the thigh in both SCI_C and SCI_{TL} notably increased from 8 and 6 min respectively, during the 8 °C stage, compared to 1 min before the stage; however, it did not exceed the baseline level.

CONCLUSIONS: An increase in SkBF during LC was observed both in the sensate and non-sensate areas in SCIs, although the magnitude was larger in the sensate area.

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INTRODUCTION

Cold-induced vasodilation (CIVD) was first reported by Lewis [1]. CIVD is the physiological phenomenon in which skin temperature on the fingers and toes decreases initially when immersed in cold water <10 °C while the skin temperature increases for 5–10 min after the onset of cooling [2, 3]. This phenomenon prevents frostbite at the acral site [4, 5].

CIVD has been reportedly observed in the fingers, palms, toes, soles, nose, ears, and other areas where arteriovenous anastomoses are abundant [4–7], and its vasomotor control is driven only by the vasoconstrictor components of the sympathetic nervous system of the skin [8]. CIVD has been observed occasionally in the trunk and extremities that are in contact areas subjected to pressure, such as the gluteus maximus, patella, and elbow [9]. Bergersen et al. [10] and Geurts et al. [11] indicated that CIVD was a local reaction not mediated by the central nervous system. Daanen et al. [12] indicated that CIVD was not likely to be induced by the axon reflection of the sensory nerves and local areas but by the vascular endothelial function of the skin [4, 5]. The release of nitric oxide (NO) might be related to this phenomenon. However, it remained unclear whether CIVD would appear in individuals with spinal cord injuries (SCIs).

The sympathetic nervous system is completely segregated from the central nervous system in persons with complete cervical SCIs (SCI_C), although the disruption to sympathetic control is dependent on the level of the lesion in individuals with thoracolumbar SCIs (SCI_{TL}). However, sensory function depends on the level of the lesion in patients with complete SCIs. The sensory nerve is intact on the subclavian area of the chest in persons with SCI_C when the level of lesion is lower than C5. As a withdrawal of the sympathetic nervous system directly contributes to modify the CIVD response in the finger [13], if CIVD occurs on the chest and thigh in both SCIs, the response patterns would be different between SCI_C and SCI_{TL}. If intact sensory nerves influence the skin blood flow (SkBF) responses, these patterns would also differ between the chest and thigh.

We assessed whether response patterns of an increased SkBF would be different on the chest of patients with SCI_C and SCI_{TL} and between the chest and thigh in both groups. Local cooling (LC) was performed at 15 °C and 8 °C using a local cooler with a Peltier device. The reasons why the temperature during LC was set at approximately 10 °C were that an increase in SkBF was observed in the acral skin at approximately 10 °C in previous studies [2, 3, 7, 10–13] and our preliminary experiment confirmed that an

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Table 1. Characteristics of participants (SCI_C and SCI_{TL}).

	Participants	Sex	Age (y)	Height (cm)	Weight (kg)	Lesion level	Time since injury (y)
SCI _C	1	Male	40	173	52.0	C5	19.0
	2	Male	46	178	55.0	C6	24.0
	3	Male	28	168	58.0	C5	12.0
	4	Male	47	160	35.0	C6	30.0
	5	Male	28	181	61.2	C5	6.8
	6	Male	53	181	80.8	C5	35.0
	7	Male	47	168	57.9	C7	15.5
	8	Male	38	175	41.6	C5	10.3
	9	Male	28	165	51.7	C5	1.1
		Mean ± SD		39 ± 9	172.1 ± 6.9	54.8 ± 12.1	
SCI _{TL}	1	Male	56	172	69.0	L1	8.0
	2	Male	44	170	67.0	Th9	4.0
	3	Female	44	167	57.0	L5	1.0
	4	Male	37	181	64.3	Th7	1.5
	5	Male	34	165	59.9	Th9	15.2
	6	Male	48	170	57.8	Th7	30.8
	7	Male	51	163	68.6	Th10	10.4
	8	Male	28	168	71.9	Th3	1.0
	9	Male	38	163	40.6	Th12	1.0
		Mean ± SD		42 ± 8	168.8 ± 5.2	61.8 ± 9.0	
	<i>p</i> value		0.53	0.29	0.21		0.09

The characteristics of participants with cervical cord injury (SCI_C) and thoracolumbar spinal cord injury (SCI_{TL}) are presented. Lesion level: cervical cord (C), thoracic cord (Th), and lumbar cord (L). Values are presented as the mean ± standard deviation (SD).

increase in SkBF was observed even in the non-acral areas of non-injured persons at approximately 10 °C.

METHODS

This acute experimental study was performed in the laboratory of Wakayama Medical University and in clinics that were convenient for participants to visit.

Participants

Participants were aged ≥20 years and were able to communicate. The inclusion criteria were: (1) persons with SCI_C with lesions between levels C5–8 and persons with SCI_{TL} with lesions between levels Th1–L5; (2) the time since injury was > 6 months; and (3) a SCI_C classified as A on the American Spinal Injury Association Impairment Scale (AIS); Grade “A” indicates clinically complete injury, suggesting that both motor and sensory functions are compromised at the lesions level [14, 15]. Experienced physiatrists examined the AIS score in each participant. The exclusion criteria included: (1) inflammatory skin diseases including decubitus ulcers, infectious diseases, diabetes or hypertension, dyslipidemia, arteriosclerosis obliterans, collagen disease, liver disease, skin atrophy; (2) those aged ≥65 years, and (3) those receiving steroid therapy. This study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained voluntarily from all patients before participating in the study. The right to withdraw consent at any time without stating the reason was guaranteed without any individual disadvantage for subsequent medical care. The experimental protocol was approved by the Institutional Review Board for Human Research at the University of Wakayama Medical, Japan (# 2837). The experiment was conducted under the supervision of a physiatrist, with due consideration for safety. The characteristics of the study participants are listed in Table 1.

Protocol

The participants were laid on a bed in a room temperature of 25–27 °C and relative humidity of 30–40% for at least 30 min. LC was performed on the right sides of the thigh (L2–5 region) and chest (C4 region) separately in a

random order, with intermittent 30 min breaks, except for the first three participants of each group (see Table 1) for whom LC was performed simultaneously on both areas using two Peltier systems. Circular plates of Peltier thermoelectric element, each with 4 cm in diameter, were placed on the skin surfaces, avoiding areas with large veins. The LC area was checked beforehand to determine if participants felt cold. All participants in the SCI_C and SCI_{TL} groups felt cold with LC on their chest but not on their thigh. The surface temperature of the Peltier was controlled using a thermoception analyzer (Intercross-210, Intercross Co, Tokyo). The Peltier thermoelectric element had a 1-mm diameter for inserting a needle probe to measure SkBF. As shown in Fig. 1A, the surface temperature of the Peltier was set at 33 °C. After 10 min of rest, the temperature was decreased to 15 °C at a rate of 0.045 °C/s and maintained for 15 min, then decreased to 8 °C at the same rate and was maintained for 15 min.

Measurements

SkBF was recorded as the blood flow (mV) using a laser Doppler flowmeter (Omega Flow, OMEGAWAVE, Tokyo). A needle-type probe for measuring SkBF was inserted into the 1-mm hole at the center of the Peltier. The blood flow on the skin surface at the LC site was recorded via a 16-bit analog-to-digital converter (AI-1664 LAX-USB, CONTEC, Osaka, Japan) at a sampling frequency of 1000 Hz, with the mean value calculated every 1 s.

A thermocouple (copper-constantan, OMEGA Engineering, Norwalk, CT, USA) was used to measure the sublingual (T_{SL}) and skin temperatures at the three sites: the left side of the chest, forearm, and thigh, which were recorded continuously via another analog-to-digital converter (LT-6 Series, Nikkiso Thermo, Tokyo) with a sampling period of 1 s. Mean skin temperatures were calculated as follows with weighted average values: T_S = 0.43 × chest + 0.25 × forearm + 0.32 × thigh [16], taking into account the body surface area ratio of each site. A thermocouple sensor was placed between the Peltier device and skin surface.

Heart rate (HR) was calculated from the R–R interval of the electrocardiogram (BSM2401, NIHON KOHDEN, Tokyo). Continuous blood pressure response was measured from the index finger using a beat-by-beat blood-pressure monitoring device (MUB101, Medisence, Tokyo) in the first three participants of each group (see Table 1). The systolic (SBP) and diastolic BPs (DBP) were measured by the auscultatory method using a

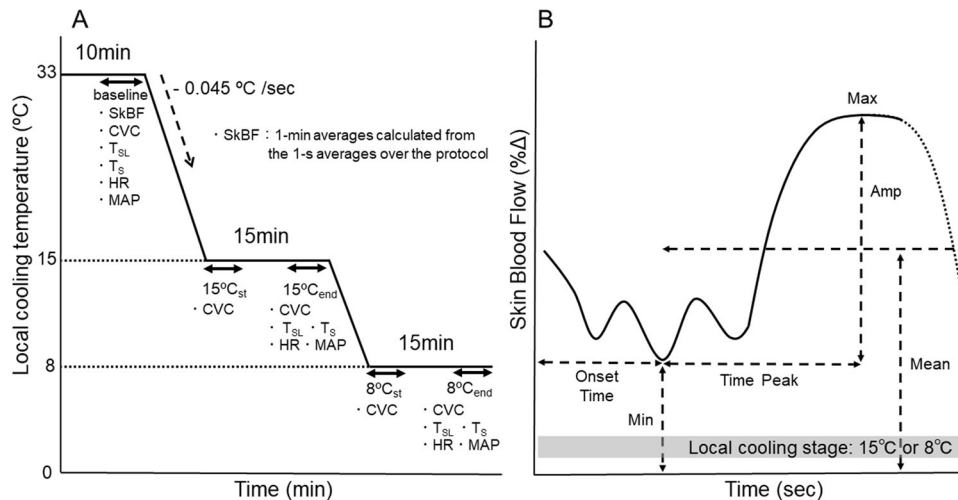


Fig. 1 Explanations for a protocol of local cooling and analyses of vasodilation. **A** Protocol of local cooling (LC). The surface temperature of the Peltier device was maintained at 33 °C (baseline) for 10 min, then reduced to 15 °C for 15 min, and then to 8 °C for 15 min. The temperature was decreased from 33 °C to 15 °C and 15 °C to 8 °C at a rate of 0.045 °C/s. Analysis time intervals of measurement items are shown. SkBF (Skin blood flow), CVC (Cutaneous vascular conductance), HR (Heart rate), MAP (Mean arterial pressure), T_{SL} (Sublingual temperature), T_S (Mean skin temperature). The baseline value was averaged for the last 5 min of 33 °C. LC of each stage is the first 5 min (15 or 8 °C_{st}) and last 5 min (15 or 8 °C_{end}). **B**: Analysis of vasodilator response at the 15 and 8 °C stage. Min (Minimum), Time_{onset} (Time until the Min), Max (Maximum), Time_{peak} (Time to peak), Amp (Amplitude, calculated as Max–Min), Mean (Averaged vasodilation).

sphygmomanometer (STBP780; COLIN; Komaki, Japan) every minute in the remaining six participants of each group.

Analysis

The 1-s mean value of SkBF [mV] was calculated using a commercially available software (The Kissei Comtec; BIMUTUS II; Matsumoto, Japan). The 1-min averages were calculated from the 1-s averages. The decrease in LC temperature from 33 to 15 °C was calculated as an average for the end value 40 s, while the 15 to 8 °C decrease was also calculated as an average for the final value 36 s because the rate of decrease in temperature was 0.045 °C/s, which means that it took 400 s and 156 s for the temperature to decrease from 33 to 15 °C and 15 to 8 °C, respectively. The mean value for the last 5 min of rest at 33 °C was averaged as the baseline, and the percent (%) change (Δ) in SkBF from the baseline was calculated (% Δ SkBF). A comparison between SCI_C and SCI_{TL} was made using data obtained from 41 points (1 point as the baseline, 7 points from 33 °C of the local temperature to 15 °C, 15 points from 15 °C LC, 3 points from 15 to 8 °C, and 15 points from 8 °C LC). Characteristics of vasodilation responses during 15 °C and 8 °C LC were assessed as discussed here. The 1-s mean value of SkBF was smoothed by 11-point moving average. The minimum (Min), onset time (Time_{onset}), maximum (Max), amplitude (Amp), mean, and time to peak (Time_{peak}) of each response were calculated in accordance with the methods reported by Daanen et al. [5] (Fig. 1B): (1) the Min was determined as the lowest value of the % Δ SkBF during each LC stage before the onset of the increase in the % Δ SkBF, (2) the Time_{onset} was the time since the onset of each LC stage until the onset of the increase in the % Δ SkBF [s], (3) the Max was the highest value of the % Δ SkBF, (4) the Amp was calculated as Max–Min, (5) the mean was the averaged value of the % Δ SkBF, and (6) the Time_{peak} was calculated from the onset of each increase to Max. An increase in SkBF was noted in participants where the % Δ SkBF exceeded over 50% of Min.

The T_{SL} and T_S were calculated using the 1-min averages. The SBP and DBP were detected from the continuous blood pressure wave; then, the MAP was calculated as (SBP - DBP)/3 + DBP. The HR and MAP were averaged every minute only during the baseline and 15 and 8 °C stages (31 points; 1 point as the baseline, 15 points from 15 °C LC, and 15 points from 8 °C LC). The mean values of the HR and MAP at rest were the mean values during the last 5 min of rest. Cutaneous vascular conductance (CVC = SkBF/MAP) was calculated every minute and averaged for 5 min at baseline and for the first and last 5 min of each LC stage (Fig. 1A; baseline, 15 °C_{st}, 15 °C_{end}, 8 °C_{st}, 8 °C_{end}); moreover, the % Δ CVC from the baseline was calculated. The MAP using a sphygmomanometer in six of each group and that using continuous blood pressure wave in three of each group were used to calculate CVC.

Statistical analyses

Participant characteristics in Table 1 were analyzed using an unpaired t-test. Analyzed using two-way analysis of variance (ANOVA), the % Δ SkBF, T_{SL} , and T_S were repeatable factors for time (41 levels; baseline, decreasing from 33 to 15 °C, 15 °C stable, decreasing from 15 to 8 °C, 8 °C stable) and factors between groups (2 levels; SCI_C and SCI_{TL}). The HR and MAP were analyzed as repeatable factors for time (31 levels; baseline, 15 °C stable, 8 °C stable) and in groups (2 levels) from the baseline. CVC was a repeatable factors for time (5 levels; baseline, 15 °C_{st}, 15 °C_{end}, 8 °C_{st}, 8 °C_{end}) and the factors between groups were 2 levels. The characteristics of % Δ SkBF response were also tested by two-way ANOVA for repeated measurements; the repeatable factors were temperature (2 levels; 15 °C and 8 °C LC) and the factor between groups (2 levels). The Bonferroni method was applied as a post-hoc test to assess significance in changes during LC, both from the baseline value and from the level measured 1 min before the onset of each stage. The number of participants in whom increased % Δ SkBF was observed were tested by chi-square at 15 °C and 8 °C LC. Statistical significance was set at $p < 0.05$. A statistical power of the F test (ANOVA: repeated measure within factors) was calculated using G*Power 3.1.9.2 (Heinrich-Heine University, Düsseldorf, Germany) assuming an α error of 0.05. Effect sizes were calculated using the variances explained by the effect and within-group variances. All analyses were performed using SPSS ver. 25 (IBM, Armonk, NY, USA). All values were expressed as means \pm standard deviations (means \pm SDs).

RESULTS

There were no significant differences in age, height, weight, and time since injury between the groups (Table 1).

SkBF [mV] and CVC [mV/mmHg] on the chest and thigh

SkBF [mV] on the chest in both SCI_C and SCI_{TL} significantly increased from 1 min before the onset of 8 °C LC during 10 to 15 min and 12 to 15 min at 8 °C LC, respectively (Fig. 2A). SkBF on the thigh in SCI_C significantly decreased from the baseline for the first minute of 8 °C LC (Fig. 2B), then returned to the baseline level at 8 °C LC. The statistical powers (1 - β) of the F test of SkBF on the chest in SCI_C and SCI_{TL} were 0.98 (effect size 0.26) and 0.99 (0.28), that on the thigh were 0.22 (0.11) and 0.46 (0.15), respectively. CVC levels on the chest and thigh did not significantly deviate from the baseline in both SCI_C and SCI_{TL} (Fig. 3A, B). The 8 °C_{end} levels on

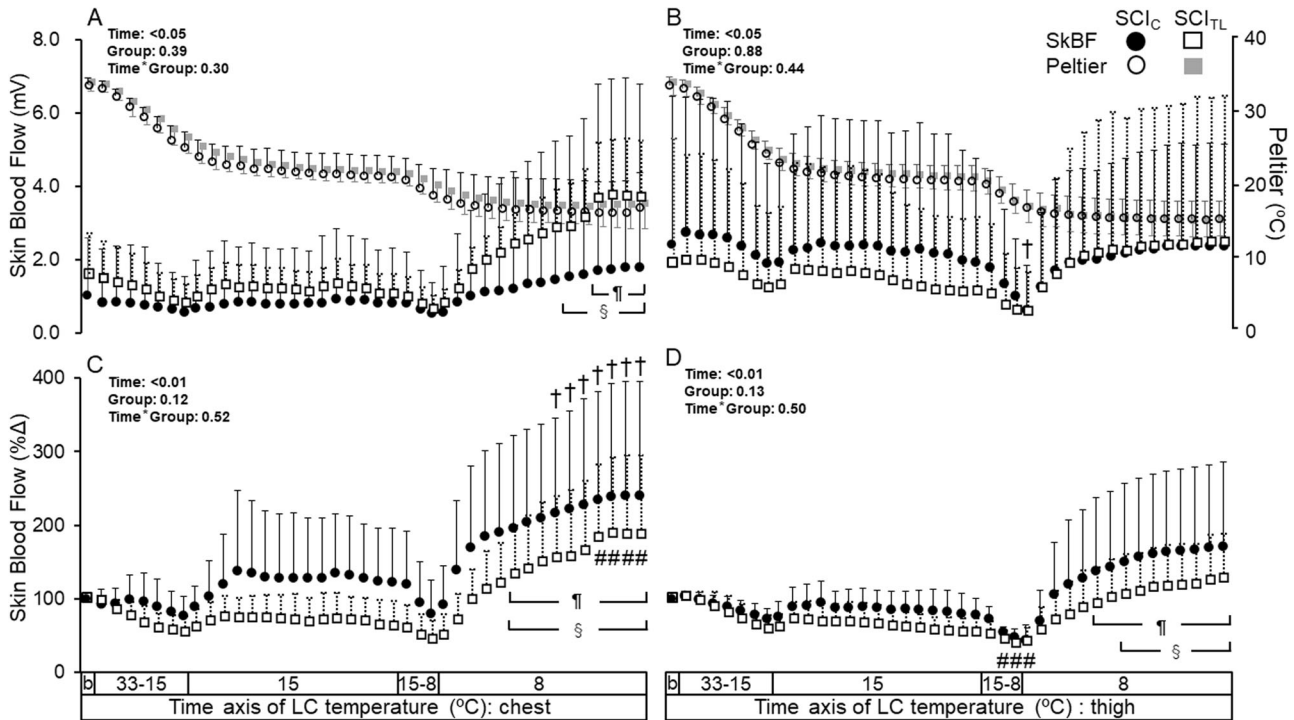


Fig. 2 Skin blood flow during local cooling (LC) is shown as the mean \pm SD in cervical (SCI_C; $n = 9$; ●) and thoracolumbar spinal cord injuries (SCI_{TL}; $n = 9$; ○). The lateral axis shows the time, with baseline, 33 °C to 15 °C, 15 °C stage, 15 °C to 8 °C, and 8 °C stage. **A:** mV value on the chest, **B:** mV value on the thigh, **A and B:** temperature change between the Peltier device and skin during LC is shown as the mean \pm SD in SCI_C; $n = 9$; ● SCI_{TL}; $n = 9$; ○. **C:** % change from the baseline value (% Δ) on the chest, **D:** % Δ on the thigh. The averaged values for the last 5 min of 33 °C was set as the baseline (0 min). Symbols represent statistical significance: †, vs baseline; ‡, vs 1 min before the onset of each stage (15 and 8 °C LC); SCI_C #, vs baseline; §, vs 1 min before the onset of each stage (15 and 8 °C LC); SCI_{TL} #, vs baseline; ¶, vs 1 min before the onset of each stage (15 and 8 °C LC); SCI_{TL} at the level of p value < 0.05. The results of two-way ANOVA repeated measurements of SkBF are shown in the upper left of the figure. [mV] on the chest and thigh were as follows: effects within participants (Time), $F(40, 640) = 6.039$ and 3.576 , $p = 0.02$ and 0.04 ; between participants (Group), $F(1, 16) = 0.79$ and 0.02 , $p = 0.38$ and 0.88 ; interaction effects of Time \times Group, $F(40, 640) = 1.20$ and 0.84 , $p = 0.29$ and 0.44 , respectively. The results of two-way ANOVA repeated measurements of SkBF [% Δ] on the chest and thigh were as follows: effects within participants (Time), $F(40, 640) = 15.29$ and 9.08 , $p < 0.001$ and 0.004 between participants (Group), $F(1, 16) = 2.64$ and 2.57 , $p = 0.12$ and 0.13 ; and interaction effects of Time \times Group, $F(40, 640) = 0.63$ and 0.58 , $p = 0.52$ and 0.50 , respectively.

the chest were significantly higher than the 15 °C_{st} in SCI_C and the 15 °C_{st} and 15 °C_{end} in SCI_{TL} (Fig. 3A). The CVC remained constant on the thigh (Fig. 3B).

% Δ SkBFs and % Δ CVC on the chest and thigh

The % Δ SkBFs on the chest in SCI_C significantly increased over the baseline from 9 to 15 min of 8 °C LC, while that in SCI_{TL} increased only in the last 4 min of 8 °C LC compared with the baseline (Fig. 2C). The % Δ SkBF on the thigh in SCI_{TL} significantly decreased from the baseline value of the last 2 min during the decreasing phase from 15 to 8 °C and the first minute at 8 °C LC (Fig. 2D). The % Δ SkBF in both SCI_C and SCI_{TL} significantly increased from 8 and 6 min, respectively, to the end of the 8 °C LC stage, compared to the level 1 min before the onset of 8 °C LC (Fig. 2D). The statistical powers (1 - β) of the F test of the % Δ SkBF on the chest in SCI_C and SCI_{TL} were 0.99 (effect size 0.28) and 0.99 (0.36), that on the thigh were 0.99 (0.28) and 0.99 (0.28), respectively. The % Δ CVC levels on the chest in SCI_C and SCI_{TL} significantly increased from the baseline at the 8 °C_{end} (Fig. 3C). The 8 °C_{end} levels on the chest and thigh were significantly higher than the 15 °C_{st} and 15 °C_{end} in both groups (Fig. 3C, D). The % Δ CVC on the thigh in SCI_{TL} significantly decreased from the baseline value at the 15 °C_{end} (Fig. 3D).

Characteristics of an increased % Δ SkBF response

There were no significant differences in the Time_{onset} between 15 °C and 8 °C LC and between SCI_C and SCI_{TL} on both sites

(Table 2). The Min in both groups were significantly lower in 8 °C LC than in 15 °C LC on the thigh, while that on the chest remained unchanged in SCI_C. The Amp in SCI_C and SCI_{TL} were significantly higher in 8 °C LC than those in 15 °C LC on both sites ($p < 0.05$ and 0.01 , respectively) with no difference between groups and sites. The mean in both groups were higher in 8 °C LC than in 15 °C LC only on the chest (all $p < 0.05$). The Time_{peak} in SCI_C and SCI_{TL} were longer in 8 °C LC than in 15 °C LC on both sites (all $p < 0.05$). The % Δ SkBF increased over the 50% value of the Min in all participants and both sites at 8 °C LC but not in all at 15 °C LC (Table 2).

Body temperature and cardiovascular responses

The T_{SL} and T_S were comparable between the groups throughout the experiment in the chest and thigh (Table 3). The HR on the chest and thigh in participants with SCI_C remained unchanged throughout the experiment and SCI_{TL}, respectively (Table 3). The MAP also showed no significant differences between SCI_C and SCI_{TL} and no changes among stages in both sites.

DISCUSSION

The main findings of this study were as follows: (1) increased % Δ SkBF was observed at 8 °C LC in all participants and sites; thus, both intact sensory neurons and sympathetic nerve activity from the central nervous system were not necessary to initiate it, (2)

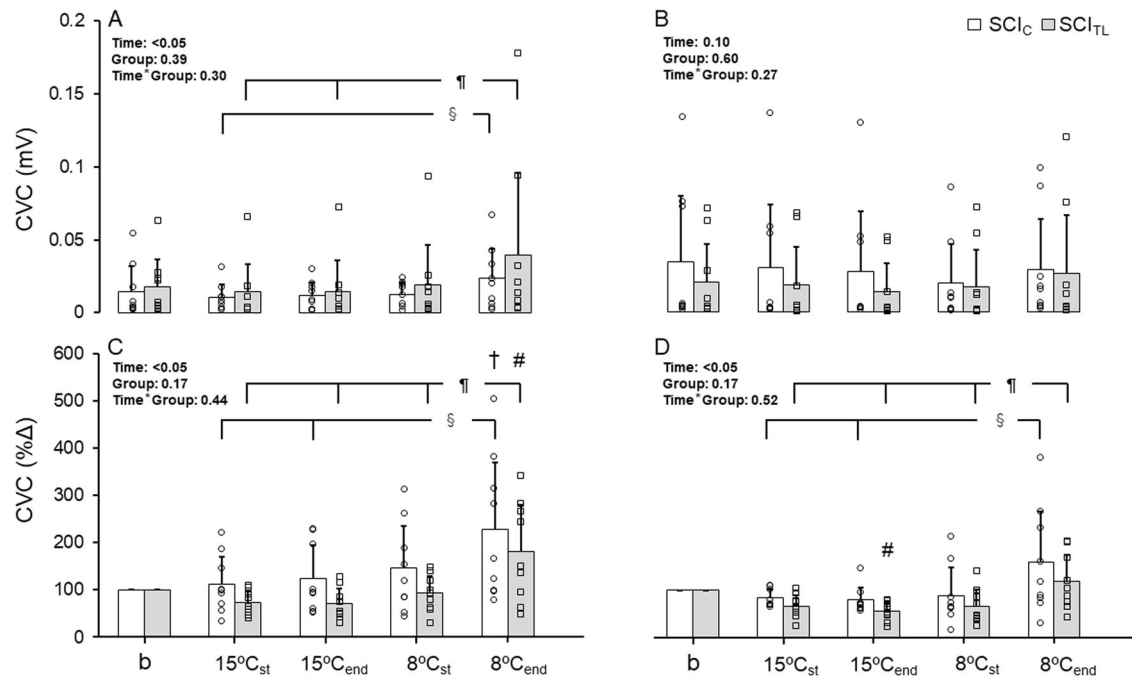


Fig. 3 Cutaneous vascular conductance (CVC) during local cooling (LC) is shown as the mean \pm SD in cervical (SCI_C; $n = 9$; □) and thoracolumbar spinal cord injuries (SCI_{TL}; $n = 9$; ■). **A:** mV/mmHg on the chest, **B:** mV/mmHg on the thigh, **C:** % change from the baseline value (% Δ) on the chest, **D:** % Δ on the thigh. The averaged values for the last 5 min of 33 °C was set as the baseline, LC of each stage is the first 5 min (15 or 8 °C_{st}) and last 5 min (15 or 8 °C_{end}). Symbols represent statistical significance: †, vs baseline; §, vs 8 °C_{end} stage (SCI_C); #, vs baseline; ¶, vs 8 °C_{end} stage (SCI_{TL}) at the level of p value < 0.05. The results of the two-way ANOVA for repeated measurements of CVC [mV/mmHg] on the chest and thigh were as follows: effects within participants (Time), $F(4, 64) = 5.78$ and 2.56 , $p = 0.02$ and 0.10 ; between participants (Group), $F(1, 16) = 0.35$ and 0.29 , $p = 0.56$ and 0.60 ; and interaction effects of Time \times Group, $F(1, 64) = 0.79$ and 1.37 , $p = 0.41$ and 0.27 , respectively. The results of the two-way ANOVA of % Δ CVC on both sites were as follows: effects within participants (Time), $F(4, 64) = 13.55$ and 8.52 , $p = 0.001$ and 0.005 ; between participants (Group), $F(1, 16) = 2.11$ and 2.10 , $p = 0.16$ and 0.17 ; and interaction effects of Time \times Group, $F(4, 64) = 0.77$ and 0.53 , $p = 0.44$ and 0.52 , respectively.

the % Δ SkBF on the chest showed a significant increase from the baseline, but not on the thigh, indicating that this could be explained with the state of the sensory nerve, which was disrupted from the central nervous system in the thigh but not in the chest. This is the first study to observe increased % Δ SkBF in the non-acral skin area by LC in individuals with SCIs.

Initiation of the increased SkBF

SkBF is determined by the balance between vasodilation and vasoconstriction activities [17, 18]. As the MAP remained unchanged throughout the present protocol, the increase or decrease in SkBF suggested cutaneous vasodilation or vasoconstriction, respectively. The observed increase in % Δ SkBF on both the chest and thigh at 8 °C LC in both groups (Figs. 2, 3, and Tables 2, 3) suggests that the trigger for this response involves local mechanisms such as dilators released from endothelial cells of cutaneous blood vessels or from sensory nerves. Since all participants had complete SCIs, it appears that the increased % Δ SkBF is not governed by central nervous drive. Yamazaki et al. [19] showed that LC stimulus of 24 °C produced a cutaneous vasodilator response in the early stage of cooling on the skin surface area where bretylium was infiltrated by an iontophoresis technique. Hodges et al. [20] reported that nitric oxide synthase (NOS) inhibitors suppressed the increase in SkBF during early cooling. These results indicate that NO is released within the vascular endothelium during LC of the skin. Therefore, vascular endothelium function could have triggered the increased % Δ SkBF in this study, although this has not been confirmed yet in SCIs.

Potential for modifying increased SkBF through intact sensory neurons

SkBF and CVC responses on the chest in both groups (sensitive) were greater than those on the thigh (non-sensitive) (Figs. 2, 3) because SkBF on the chest exceeded the baseline but that on the thigh did not. Transient receptor potential channels can detect a low temperature of <17 °C [21–23], can act as a primary vascular cold sensor, and can first induce vasoconstriction due to an increased release of noradrenaline from the sympathetic nerve ending. It might then mediate the vasodilation response by involving a release of calcitonin gene-related peptide, substance P, and NOS-induced NO from the nerve endings of C-fibers [24]. Therefore, intact sensory neurons might strengthen the response of the increased SkBF, although no studies have examined whether cutaneous vasodilation activity is related to CVD in humans. If vasodilation activity is greater than vasoconstriction activity [8, 18], CVD is observed.

Modification of the increased SkBF by the sympathetic nervous system

An increase in the concentration of noradrenaline has suppressed the CVD response during cold immersion of fingers at 5 °C [25]. Hodges et al. [13] also suggested that neural activity was consistently reduced before CVD, and sympathetic nervous system withdrawal directly contributes to CVD onset. However, SkBF and CVC levels on the thigh did not exceed baseline values during 8 °C LC whereas they increased on the chest in both groups (Figs. 2, 3C, D). Although the sympathetic nervous system on the chest is intact in SCI_{TL}, while not in SCI_C, the responses between groups did not differ. The integrity of the sympathetic nervous

Table 2. Characteristics of increased % Δ SkBF at 15 °C and 8 °C in local cooling.

	Site	15 °C LC		8 °C LC		p value within, between, interaction
		SCI _C	SCI _{TL}	SCI _C	SCI _{TL}	
Time _{onset} (s)	Chest	125 ± 174	81 ± 151	23 ± 28	26 ± 28	$p = 0.06, p = 0.61, p = 0.55$
	Thigh	54 ± 95	32 ± 35	30 ± 24	32 ± 17	$p = 0.46, p = 0.60, p = 0.47$
Time _{peak} (s)	Chest	270 ± 284	274 ± 208	630 ± 220 [†]	730 ± 151 [#]	$p < 0.01, p = 0.42, p = 0.58$
	Thigh	372 ± 251	131 ± 117	660 ± 235 [†]	750 ± 196 [#]	$p < 0.01, p = 0.37, p = 0.01$
Min (%baseline)	Chest	70 ± 26	50 ± 22	74 ± 44	43 ± 23 [#]	$p = 0.70, p = 0.07, p = 0.25$
	Thigh	62 ± 17	52 ± 17	36 ± 18 [†]	34 ± 9 [#]	$p < 0.01, p = 0.29, p = 0.34$
Max (%baseline)	Chest	171 ± 112	94 ± 31	260 ± 155 [†]	206 ± 111 [#]	$p < 0.01, p = 0.19, p = 0.63$
	Thigh	106 ± 34	81 ± 26	182 ± 126	138 ± 63 [#]	$p = 0.01, p = 0.22, p = 0.67$
Amp (%baseline)	Chest	101 ± 101	45 ± 24	186 ± 125 [†]	163 ± 117 [#]	$p < 0.01, p = 0.35, p = 0.47$
	Thigh	44 ± 31	29 ± 19	146 ± 117 [†]	104 ± 58 [#]	$p < 0.01, p = 0.28, p = 0.51$
Mean (%baseline)	Chest	125 ± 78	71 ± 30	196 ± 123 [†]	140 ± 72 [#]	$p < 0.01, p = 0.14, p = 0.93$
	Thigh	84 ± 23	62 ± 22	136 ± 95	98 ± 49	$p = 0.02, p = 0.15, p = 0.64$
Increase in SkBF (N = 9)	Chest	7/9	6/9	9/9	9/9	
	Thigh	4/9	5/9	9/9 [†]	9/9 [#]	

The characteristics of increased %change (Δ) in skin blood flow (SkBF) during local cooling (LC; 15 °C and 8 °C stage) in participants with cervical cord injury (SCI_C) and thoracolumbar spinal cord injury (SCI_{TL}) are shown as the mean \pm SD. Time_{onset}, a time to the onset of the increase in % Δ SkBF during each LC stage; Time_{peak}, time to peak from the Time_{onset}; Min, the lowest values before the onset of increased % Δ SkBF during each LC stage; Max, the maximum value during each LC stage; Amp, the amplitude calculated as Max–Min; Mean, the averaged value of each increased response after the onset; Increase in SkBF, number of participants in whom % Δ SkBF increased over 50% of Min. *P* values of ANOVA are shown in order of within factor (Temperature), factor of between participants (Group), and interaction of Temperature and Groups in SCI_C and SCI_{TL}. [†], [#], compared between 15 °C vs 8 °C LC in SCI_C and SCI_{TL}, respectively. Significance level is $p < 0.05$.

Table 3. Body temperature and cardiovascular responses.

	Site	SCI _C			SCI _{TL}			p value within, between, interaction
		baseline	15 °C	8 °C	baseline	15 °C	8 °C	
T _{SL} [°C]	Chest	36.3 ± 0.6	36.3 ± 0.6	36.2 ± 0.6	36.7 ± 0.4	36.4 ± 0.4	36.4 ± 0.6	$p = 0.60, p = 0.18, p = 0.26$
	Thigh	36.3 ± 0.5	36.4 ± 0.5	36.3 ± 0.5	36.6 ± 0.6	36.6 ± 0.4	36.5 ± 0.5	$p = 0.44, p = 0.24, p = 0.41$
T _S [°C]	Chest	32.7 ± 0.5	32.5 ± 0.6	32.6 ± 0.6	32.0 ± 1.2	32.0 ± 1.3	32.0 ± 1.3	$p = 0.65, p = 0.23, p = 0.42$
	Thigh	32.6 ± 0.5	32.3 ± 0.4	32.5 ± 0.5	32.0 ± 1.2	31.8 ± 1.3	31.8 ± 1.3	$p = 0.09, p = 0.20, p = 0.60$
HR [beats/min]	Chest	66 ± 16	66 ± 16	66 ± 15	76 ± 20	75 ± 20	77 ± 21	$p = 0.37, p = 0.31, p = 0.75$
	Thigh	68 ± 15	67 ± 15	66 ± 15	79 ± 17	77 ± 17	78 ± 19	$p = 0.01, p = 0.25, p = 0.23$
MAP [mmHg]	Chest	80 ± 13	81 ± 13	80 ± 12	91 ± 12	90 ± 12	90 ± 11	$p = 0.66, p = 0.19, p = 0.40$
	Thigh	73 ± 8	74 ± 10	74 ± 9	89 ± 12	89 ± 12	90 ± 11	$p = 0.67, p = 0.01, p = 0.71$

Sublingual temperature (T_{SL}, °C), mean skin temperatures (T_S, °C), Heart rate (HR, beats/min) and mean arterial pressure (MAP, mmHg) are shown as representative values at 33 °C (baseline), 15 °C, and 8 °C as the mean of the last 5 min for each stage. The values are presented as the mean \pm standard deviation (SD) in participants with cervical spinal cord injury (SCI_C) and those with thoracolumbar spinal cord injury (SCI_{TL}). *P* values of ANOVA are shown in order of within factor (Time), factor of between participants (Group), and interaction of Time and Group in SCI_C and SCI_{TL}.

system appears to play a minor role in the modification of the increased SkBF.

Vasoconstriction during LC on the thigh

Herein, the % Δ SkBF decreased before CIVD was observed on the thigh, where the sensory nerve was not intact and activities of sympathetic nervous control were disrupted. As previously reported, after administration of bretylium, which inhibits catecholamine release from the nerve endings, by iontophoresis, LC stimulation at 24 °C or 29 °C increased SkBF and then decreased it. Hence, the vasoconstrictive effects of noradrenaline would be necessary only during early phase of LC [19, 26, 27]. Thus, the main mechanism of cutaneous vasoconstriction induced by LC on the thigh could involve the increased action of noradrenaline secreted from sympathetic nerve terminals by local mechanisms such as the mediation of Rho kinase [28, 29], non-

catecholamine constriction by reduced NO, or by some actions from sensory nerve terminals [19, 20, 26, 29].

Limitations

Criticisms of the small sample size are inevitable. However, this study found a significant difference in the patterns of SkBF between the sites. It is possible that responses in the paralyzed region could vary based on the time elapsed since injury. The variability in the time since injury remained unsolved. We did not measure any electrical activities related to indexes of skin sympathetic nervous activity in the present participants.

CONCLUSION

An increase in SkBF was observed in the non-acral area (chest and thigh) by a LC stimulus (8 °C) in all participants with SCIs. The

central neural mechanisms do not trigger the increase, and local mechanisms may be responsible for the changes, although the response is modified by the intact/disrupted state of the sensory nerves. The increased SkBF during LC suggests that CIVD is expressed even in SCI_C and SCI_{TL} and that the defense mechanism against frostbite exists in paralyzed areas.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

- Lewis T. Observations upon the reaction of the vessels of the human skin to cold. *Heart*. 1930;15:177–208.
- Cheung SS, Mekjavic IB. Cold-induced vasodilatation is not homogenous or generalizable across the hand and feet. *Eur J Appl Physiol*. 2007;99:701–5.
- Daanen HA, Van de Linde FJ, Romet TT, Ducharme MB. The effect of body temperature on the hunting response of the middle finger skin temperature. *Eur J Appl Physiol Occup Physiol*. 1997;76:538–43.
- Cheung SS, Daanen HA. Dynamic adaptation of the peripheral circulation to cold exposure. *Microcirculation*. 2012;19:65–77.
- Daanen HA. Finger cold-induced vasodilation: a review. *Eur J Appl Physiol*. 2003;89:411–26.
- Brajkovic D, Ducharme MB. Facial cold-induced vasodilation and skin temperature during exposure to cold wind. *Eur J Appl Physiol*. 2006;96:711–21.
- Van der Struijs NR, Van Es EM, Raymann RJ, Daanen HA. Finger and toe temperatures on exposure to cold water and cold air. *Aviat Space Environ Med*. 2008;79:941–6.
- Kellogg DL Jr. In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *J Appl Physiol*. 2006;100:1709–18.
- Fox RH, Wyatt HT. Cold-induced vasodilatation in various areas of the body surface of man. *J Physiol*. 1962;162:289–97.
- Bergersen TK, Hisdal J, Walløe L. Perfusion of the human finger during cold-induced vasodilatation. *Am J Physiol*. 1999;276:R731–R737.
- Geurts CL, Sleivert GG, Cheung SS. Effect of cold-induced vasodilatation in the index finger on temperature and contractile characteristics of the first dorsal interosseus muscle during cold-water immersion. *Eur J Appl Physiol*. 2005;93:524–9.
- Daanen HA, Ducharme MB. Axon reflexes in human cold exposed fingers. *Eur J Appl Physiol*. 2000;81:240–4.
- Hodges GJ, Mallette MM, Cheung SS. Cutaneous neural activity and endothelial involvement in cold-induced vasodilatation. *Eur J Appl Physiol*. 2018;118:971–8.
- ASIA and ISCoS International Standards Committee. The 2019 revision of the international standards for neurological classification of spinal cord injury (ISNCSCI)-what's new? *Spinal Cord*. 2019;57:815–7.
- Roberts TT, Leonard GR, Cepela DJ. Classifications in brief: American Spinal Injury Association (ASIA) impairment scale. *Clin Orthop Relat Res*. 2017;475:1499–504.
- Roberts MF, Wenger CB, Stolwijk JA, Nadel ER. Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol Respir Environ Exerc Physiol*. 1977;43:133–7.
- Folkow B. Role of the nervous system in the control of vascular tone. *Circulation*. 1960;21:760–8.
- Kamijo Y, Lee K, Mack GW. Active cutaneous vasodilation in resting humans during mild heat stress. *J Appl Physiol*. 2005;98:829–37.
- Yamazaki F, Sone R, Zhao K, Alvarez GE, Kosiba WA, Johnson JM. Rate dependency and role of nitric oxide in the vascular response to direct cooling in human skin. *J Appl Physiol*. 2006;100:42–50.
- Hodges GJ, Zhao K, Kosiba WA, Johnson JM. The involvement of nitric oxide in the cutaneous vasoconstrictor response to local cooling in humans. *J Physiol*. 2006;574:849–57.
- Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, et al. A TRP channel that senses cold stimuli and menthol. *Cell*. 2002;108:705–15.
- McKemy DD. The molecular and cellular basis of cold sensation. *ACS Chem Neurosci*. 2013;4:238–47.
- Aubdool AA, Graepel R, Kodji X, Alawi KM, Bodkin JV, Srivastava S, et al. TRPA1 is essential for the vascular response to environmental cold exposure. *Nat Commun*. 2014;5:5732.
- Schlereth T, Schukraft J, Krämer-Best HH, Geber C, Ackermann T, Birklein F. Interaction of calcitonin gene related peptide (CGRP) and substance P (SP) in human skin. *Neuropeptides*. 2016;59:57–62.
- Sendowski I, Savourey G, Launay JC, Besnard Y, Cottet-Emard JM, Pequignot JM, et al. Sympathetic stimulation induced by hand cooling alters cold-induced vasodilatation in humans. *Eur J Appl Physiol*. 2000;81:303–9.
- Hodges GJ, Traeger JA 3rd, Tang T, Kosiba WA, Zhao K, Johnson JM. Role of sensory nerves in the cutaneous vasoconstrictor response to local cooling in humans. *Am J Physiol Heart Circ Physiol*. 2007;293:H784–H789.
- Pérgola PE, Kellogg DL Jr, Johnson JM, Kosiba WA, Solomon DE. Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *Am J Physiol*. 1993;265:H785–92.
- Bailey SR, Eid AH, Mitra S, Flavahan S, Flavahan NA. Rho kinase mediates cold-induced constriction of cutaneous arteries: role of alpha2C-adrenoceptor translocation. *Circ Res*. 2004;94:1367–74.
- Johnson JM, Kellogg DL Jr. Local thermal control of the human cutaneous circulation. *J Appl Physiol*. 2010;109:1229–38.

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AUTHOR CONTRIBUTIONS

This study was conducted at the Wakayama Medical University of Rehabilitation Medicine. The study was designed by Yoshi-ichiro Kamijo, Manabu Shibasaki, and Yukihide Nishimura. It was supervised by Chikako Kaminaka, Tatsuya Yoshikawa, and Fumihiro Tajima. The measurements were performed by Yasuhisa Fujita, Yoshi-ichiro Kamijo, Tokio Kinoshita, Takamasa Hashizaki, Kouta Murai, and Yasunori Umemoto. The data collection and analysis were performed by Yasuhisa Fujita, Manabu Shibasaki, and Yoshi-ichiro Kamijo. This manuscript was drafted by Yasuhisa Fujita and Yoshi-ichiro Kamijo. All authors have approved the final manuscript submitted for publication and agree to be accountable for all work and interpretations. All authors revised the manuscript, especially Yasuhisa Fujita and Yoshi-ichiro Kamijo, and have contributed to revisions in preparation for resubmission.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The procedures in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki as revised in 2013. This study was approved by the Ethics Committee of Wakayama Medical University (# 2837). Informed consent was obtained from all participants included in the study before participation. Additional informed consent was obtained from all participants whose identifying information is included in the study.

HUMAN AND ANIMAL ETHICS

Human ethics has been approved.

ADDITIONAL INFORMATION

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