

# Suppression of activation of muscle sympathetic nerve during non-noxious local cooling after the end of local cooling in normal adults

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## Abstract

**Purpose** While non-noxious local cooling is widely used in physical medicine, its effect on muscle sympathetic nerve activity (MSNA) and cardiovascular regulation are not clear. The purpose of the present study was to assess the responses of MSNA, blood pressure (BP), heart rate (HR) and local blood flow during non-noxious local cooling.

**Methods** The study included two protocols. Both protocols consisted of 10-min rest in supine position, followed by 15-min local cooling (15 °C) of the shin and anterior foot, and 20-min recovery. MSNA of the right common peroneal nerve, BP, HR, and shin skin temperature (TSK) were recorded in eight men in the first protocol, while leg blood flow (LBF) was measured in the same subjects by strain-gauge plethysmography in the second protocol.

**Results** TSK gradually decreased from  $31.5 \pm 0.02$  to  $16.0 \pm 1.01$  °C (mean  $\pm$  SEM) during local cooling, and gradually increased after the end of local cooling. No subject complained of pain, and BP and HR remained constant. The MSNA burst rate increased significantly ( $p < 0.05$ ) to  $141.1 \pm 12.5$  % during local cooling, but decreased significantly ( $p < 0.05$ ) to  $73.6 \pm 5.9$  % during the recovery period. Total MSNA also increased to  $148.0 \pm 14.2$  % ( $p < 0.05$ ) during local cooling, and decreased to

$74.0 \pm 13.9$  % ( $p < 0.05$ ) at recovery. LBF remained constant through the experiment.

**Conclusions** The results suggest that MSNA is activated by non-noxious local cooling, and attenuated after the end of local cooling without any changes in HR and BP.

**Keywords** Regional blood flow · Local cooling · Muscle blood flow · Microneurography · Plethysmography

## Abbreviations

BP	Blood pressure
DBP	Diastolic blood pressure
ECG	Electrocardiogram
HR	Heart rate
LBF	Leg blood flow
MBP	Mean blood pressure
MSNA	Muscle sympathetic nerve activity
SBP	Systolic blood pressure
TSK	Skin temperature

## Introduction

Muscle sympathetic nerve activity (MSNA) can be recorded from the muscle nerve fascicles in the limbs by the technique of microneurography (Delius et al. 1972a, b, c; Fagius et al. 1989; Hagbarth et al. 1972; Hagbarth and Vallbo 1968; Mark et al. 1985; Mitchell and Victor 1996; Seals and Victor 1991; Vallbo et al. 1979, 2004; Victor et al. 1987). MSNA has been studied during various types of physiological stimuli and induced various changes by these stimuli (Cui et al. 2007; Mano 1998; Mizushima et al. 1998; Nakamura et al. 2005; Niimi et al. 1997; Tanaka et al. 1991). There is general agreement that MSNA primarily represents the activity of the vasoconstrictor fibers

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throughout the body, which innervate skeletal blood vessels and thus play an important role in the regulation of systemic blood pressure (BP) (Charkoudian et al. 2005; Mitchell and Victor 1996; Saito et al. 1990). Therefore, activation of MSNA generally induces a rise in BP, while reduction of MSNA induces a fall in BP.

We reported previously that non-noxious local heat application to the leg reduced MSNA but neither changed BP nor heart rate (HR) in healthy men (Takahashi et al. 2011). We concluded that the response of MSNA during non-noxious local heat was induced by a local reflex mechanism based on the lack of change in BP, and thus the control of BP and HR was not affected by reduction in MSNA, suggesting that the effect of MSNA is limited to the local area. On the other hand, in the cold pressor test, which is often used to evaluate the sympathetic nervous system, BP, HR and MSNA increase after immersion of the hand in ice cold water, together with induction of intense pain (Kregel et al. 1992; Victor et al. 1987; Mizushima et al. 1998). To our knowledge, however, the response of BP, HR and MSNA to non-noxious local cooling of the skin has not been examined in detail.

Non-noxious local cooling, also known as cryotherapy, is one of the most generally used physical therapy in rehabilitation. Cryotherapy decreases local blood flow by causing vasoconstriction, and reduces tissue metabolism, oxygen utilization, inflammation, and muscle spasm (Nadler et al. 2004). The decrease in local blood flow is produced by the direct effect of the applied cold and by reflex mechanisms ranging from a simple monosynaptic reflex to a complex polysynaptic reflex (Lehmann and Lateur 1990). Previous studies demonstrated that application of mild non-noxious cold ice pack on the leg induced a decrease in leg blood flow (LBF) (Taber et al. 1992; Weston et al. 1994). However, Fiscus et al. (2005) reported that cold-water immersion of the lower part of the leg did not change LBF.

The present study was designed to determine the effects of non-noxious local cooling of the leg [i.e., cooling of skin temperature (TSK)] on BP, HR, MSNA and regional blood flow in normal subjects. The study was performed by measuring BP, HR, TSK, LBF and MSNA in the leg before, during, and after 15 min of non-noxious local cooling of the leg.

## Methods

### Subjects

Eight healthy adult men aged 21–35 years participated in this study after signing a written informed consent. All subjects were normotensive, taking no medications, and free of cardiovascular or neuromuscular diseases, based on medical history and physical examination. The study was

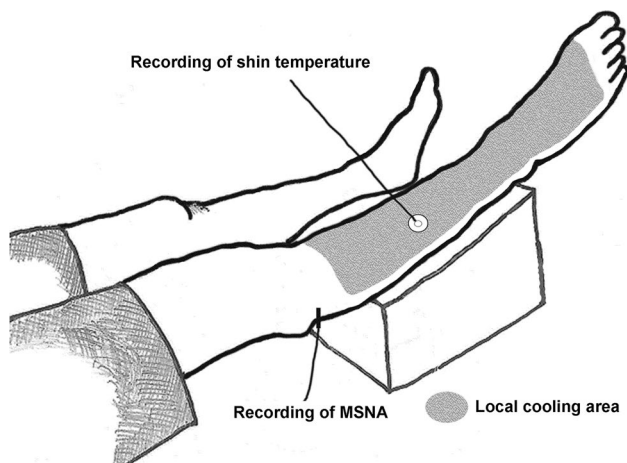
approved by the Human Investigation Committee of the Wakayama Medical University, Japan.

### Recording of sympathetic nerve activity

Multiunit recording of postganglionic MSNA was obtained from the right common peroneal nerve fascicles at the level of the fibular head in the lower extremity, according to the method described previously (Mizushima et al. 1998; Nakamura et al. 2005; Takahashi et al. 2011). For this purpose, a tungsten microelectrode with an uninsulated tip (diameter 1–5  $\mu\text{m}$ ) expanding to an insulated shaft (0.2 mm) was inserted into the nerve fascicles. A surface reference electrode (diameter 3 cm) was placed medially 2–3 cm from the recording electrode. The neural activity was collected through a microclipper using a shielded cord, amplified and filtered (bandwidth = 500–2000 Hz) using an electromyogram recording system (Nicolet, Viking SELECT VIASIS). For quantification of the electric signal, the amplified signal was fully rectified and integrated using a time constant of 100 ms. The mean voltage of the MSNA neurogram was continuously displayed together with the electrocardiogram (ECG) and raw MSNA neurogram on a chart recorder (NEC, Omnicore II RA1300) at a paper speed of 5 mm/s. The nerve signal was also continuously routed to a loudspeaker for audio recognition of MSNA bursts. The MSNA recording was accepted when the following three criteria were met (Vallbo et al. 1979; Wallin 1983), first, when electrical stimulation (0.1 mA; 0.2 ms; 1 Hz) through the electrode inserted into the common peroneal nerve elicited an involuntary muscle contraction but not paresthesia; second, when tapping or stretching the muscle or tendon supplied by the impaled fascicle elicited afferent mechanoreceptor discharges; and third, when the neurogram displayed spontaneous, intermittent, pulse-synchronous bursts that increased during a sustained expiration and phases 2 and 3 of the Valsalva maneuver but not during an acoustic stimulus (hand clap).

### Recording of leg blood flow, cardiovascular variables and skin temperature

Heart rate was obtained from the R–R interval of the ECG and arterial pressure was measured manually with a sphygmomanometer (model ES-H55D, Terumo). The mean blood pressure (MBP) was calculated from the diastolic blood pressure (DBP) and systolic blood pressure (SBP) as  $\text{MBP} = \text{DBP} + (\text{SBP} - \text{DBP})/3$ . LBF was determined on the right side using the plethysmographic technique (plethysmography: model ECR5, Hokanson) as described previously (Whitney 1953; Gamble et al. 1993). A silicon tube filled with mercury was placed around the right calf at maximum circumference and positioned 20 cm above the heart



**Fig. 1** The recording positions of muscle sympathetic nerve activity (MSNA) and shin temperature. The local cooling area is *marked*

level. The maximum circumference of the calf region was located 3 cm above a midpoint between the medical condyle of the femur and upper edge of the medical malleolus. This setup ensured that the silicone tube was not directly cooled by the application of ice packs. Changes in the calf circumference were measured by a bridge amplifier during proximal venous occlusion. In a series of preliminary experiments, we assessed the effect of local pressure on LBF by applying a weight identical to that of the ice pack to the area where the ice pack would be applied. The results showed that while the weight placement changed the baseline, it did not affect the impedance during venous occlusion. TSK of the right shin temperature was measured using a thermocouple thermometer (model LT8A, Gram Corporation).

### Local cooling

Local cooling was applied to the ipsilateral anterior area from 4 cm below the midcalf region down to the toes using two ice packs (Fig. 1). The ice pack was covered with double-folded paper towels to protect the skin. The temperature on the surface part of the towels in contact with the skin was 15 °C immediately before application of the ice pack.

### Experimental protocols

In the first protocol, MSNA was measured in eight subjects in a temperature-controlled room ( $26.0 \pm 0.5$  °C). Each subject reported to the laboratory at 16:00 h. After voiding, the subject rested comfortably in a supine position on the examination table wearing short pants and a T-shirt. After recording all variables for 10 min at rest (control period), local cooling was applied for 15 min (LC period) while monitoring continued. After removal of the ice packs, recovery measurements were made for 20 min (recovery

period). Thus, MSNA was measured continuously for 45 min. TSK, HR and MBP were recorded every 1 min, and averaged every 2 min except during the first minute of local cooling. Furthermore, in a preliminary study, we confirmed that TSK of the right shin was stabilized after 15 min of local cooling (the selection of this time period was based on the fact that cryotherapy is usually applied for 15 min in the clinic). For this reason, we used the 15-min duration for local cooling in present study. On the other hand, the recovery spanned over 20 min (i.e., longer than local cooling).

In the second protocol, LBF was measured in the same group of eight normal subjects without recording the MSNA, and all other parameters were recorded in the same manner as in the first protocol. The second protocol was conducted on a separate day within 1 week of the first protocol, and under conditions (room temperature and time of experiment) identical to the first protocol.

### Data analysis

Records were divided into 1-min periods for analysis. Acceptable bursts of MSNA were identified by inspection of the mean voltage neurogram on the chart recorder, synchronized with the audio signal from loudspeaker and the pulse wave of the ECG. All microneurographic recordings were analyzed by the same investigator. MSNA was expressed as both burst rate (bursts/min) and total activity (total MSNA) in arbitrary units (bursts/min  $\times$  average burst amplitude/min), according to the method described previously (Mark et al. 1985; Wallin 1983). MSNA was averaged each minute of the control, local cooling and recovery periods. Data from the 10-min control period were averaged to obtain single baseline values. To analyze the MSNA response, the mean values during local cooling and recovery periods were expressed as percentages of those recorded during the control period.

All data were reported as mean  $\pm$  standard error of the mean. The data of each parameter showed skewed distribution. Changes in each variable due to treatment (control, LC period, and recovery) were examined for statistical significance using the Mann–Whitney *U* test. A *p* value less than 0.05 was considered significant in all statistical analyses.

## Results

### Protocol 1

#### *Skin temperature*

Shin TSK gradually and significantly decreased ( $p < 0.01$ ) during local cooling from  $31.5 \pm 0.02$  °C to the lowest

value of  $16.0 \pm 1.01$  °C. All subjects were questioned verbally about pain and hot sensation, and none complained of pain or soreness during local cooling. The shin TSK gradually increased after removal of the cold pack, but remained significantly lower ( $p < 0.01$ ) compared with the control period until the end of the recovery period (Fig. 2).

#### Heart rate and mean blood pressure

HR and MBP remained constant throughout LC period and recovery (Fig. 3a, b).

#### Muscle sympathetic nerve activity

The mean MSNA burst rate during the control period was  $14.5 \pm 2.04$  bursts/min, increased significantly during LC period to  $141.1 \pm 12.5$  % ( $p < 0.05$ ) and decreased significantly ( $p < 0.05$ ) to become lower ( $73.6 \pm 5.9$  %) than the control value after the end of local cooling (Fig. 4a). The total MSNA also increased significantly ( $p < 0.05$ ) to  $148.0 \pm 14.2$  % during local cooling and decreased significantly ( $p < 0.05$ ) to  $74.0 \pm 13.9$  % relative to the baseline after removal of the ice pack (Fig. 4b). The percent change in MSNA burst rate during local cooling negatively correlated significantly with the shin temperature ( $R = -0.34$ ,  $p = 0.0105$ , Fig. 5).

#### Protocol 2

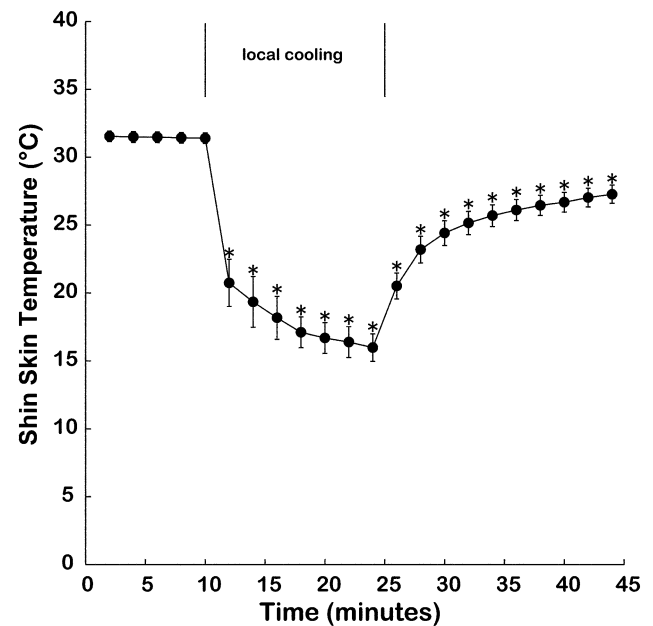
##### Leg blood flow

LBF in the right leg remained constant throughout LC period and recovery (Fig. 6).

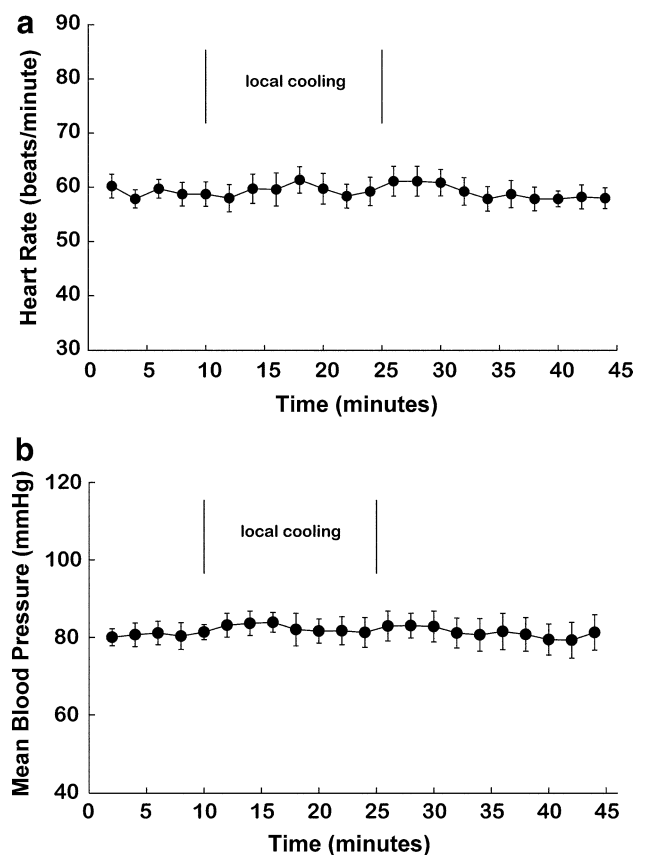
#### Discussion

The major findings of the present study were the following; (1) Local cooling (15 °C) of the skin over the shin and anterior foot increased MSNA of the ipsilateral common peroneal nerve, and removal of local cooling induced reduction of MSNA, and (2) HR, MBP and LBF remained constant throughout the study. These findings suggest that the fall in shin TSK induced activation of MSNA while increase in shin TSK induced attenuation of MSNA, and that the change in MSNA was induced by a local reflex mechanism in the absence of any response in HR and MBP.

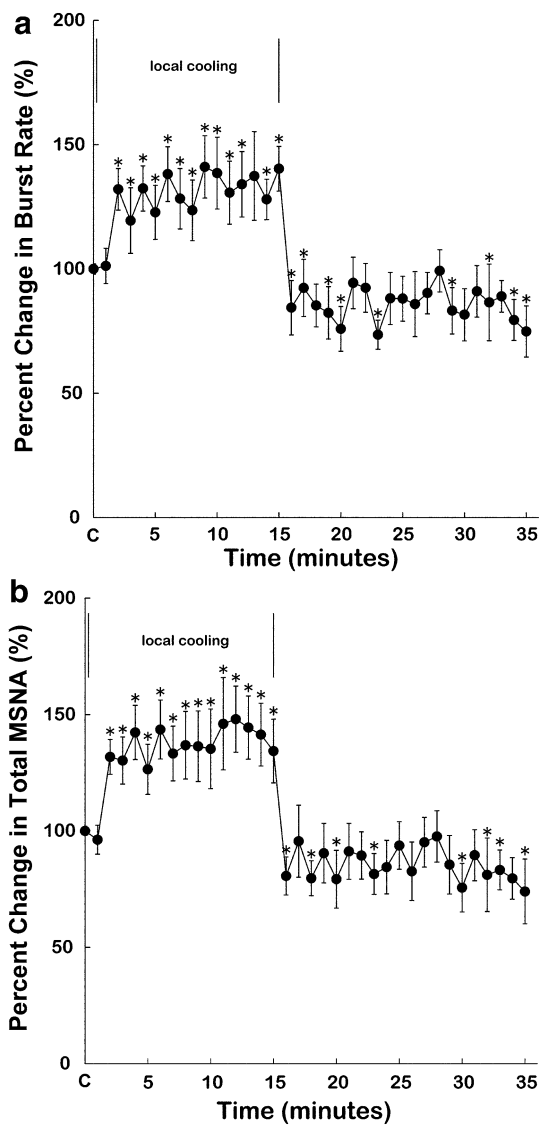
The cold pressor test is known to induce a rapid increase in HR and MBP, as well as increase in MSNA (over 150 %) (Mizushima et al. 1998). The test usually involves the use of low-temperature water (around 0 °C) to cool the hand. On the other hand, the local cooling applied in the present



**Fig. 2** Serial changes in shin skin temperature during local cooling. Values are mean  $\pm$  SEM. \* $p < 0.01$  vs. control period

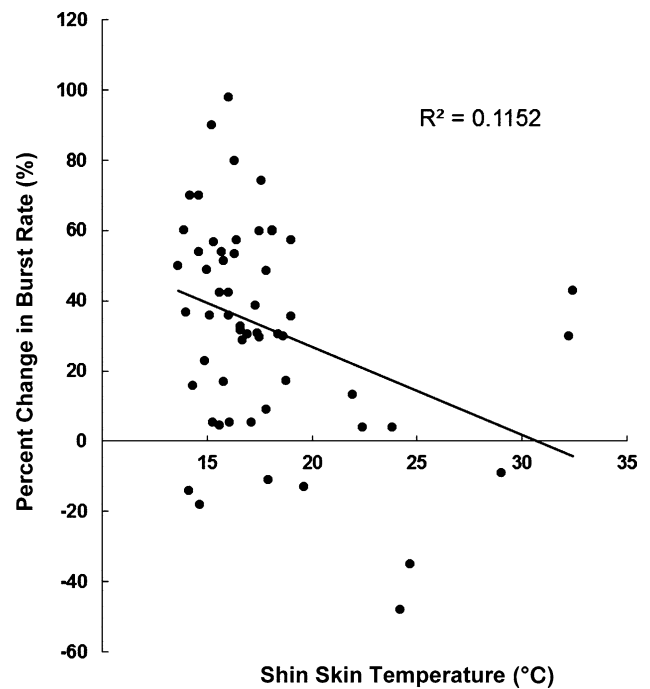


**Fig. 3** Serial changes in heart rate (a) and mean blood pressure (b) during local cooling. Values are mean  $\pm$  SEM

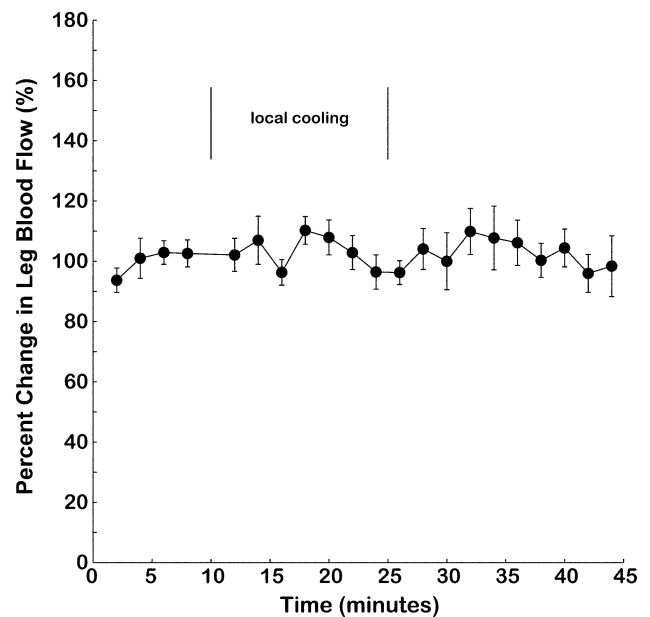


**Fig. 4** Percent changes in burst rate of MSNA (a) and total MSNA (b) during local cooling. Values are mean ± SEM. \**p* < 0.05 vs. control period. C Average of the control period

study involved cooling the skin to mild temperature (15 °C) using ice packs. Such mild cooling induced a mild increase in MSNA (<50 %), relative to the cold pressor test. The present findings of changes in MSNA during and after mild local cooling without changes in HR suggest that the threshold of the MSNA response to changes in shin TSK differed from that affecting the response of cardiac sympathetic and parasympathetic nerve activities. The stable MBP and changes in MSNA during and after mild local cooling suggest that the response in MSNA may represent a local reflex response elicited in the local cooling area. Ray et al. (1997) also reported that the MBP, HR and MSNA in the lower leg did not change by 30 min mild local cooling of the forearm. Thus, the threshold change in shin TSK



**Fig. 5** Relationship between percent change in burst rate of MSNA and shin skin temperature during local cooling. Each data point represents the average of every 2 min of data from all subjects



**Fig. 6** Percent changes in leg blood flow during local cooling. Values are mean ± SEM

necessary to elicit a local MSNA response seems lower than that required to change HR and BP. The stability of HR and MBP during and after mild local cooling implies a stable cardiovascular control during and after local cooling.



In the present study, none of the participating subjects complained of pain during local cooling and HR did not increase during local cooling. Victor et al. (1987) reported that tachycardia during the cold pressor test is induced by pain sensation. In contrast, several studies that have previously addressed the effects of somatic afferent stimulation on MSNA reported increased MSNA response (Kregel et al. 1992; Mizushima et al. 1998; Nakamura et al. 2008). Somatic afferent stimulation activates the ascending tracts in the spinal cord. In particular, the spinoreticular tract projecting to the medullary reticular formation activates the vasomotor center in the medulla oblongata. Activation of the projection from the vasomotor center to the intermediolateral cell column in the thoracolumbar cord results in excitation of the sympathetic preganglionic neurons causing increased MSNA (Nakamura et al. 2008). In this regard, Kregel et al. (1992) reported that immersion of the hand in 7 °C water induced intense pain and increased MSNA in the lower leg, whereas immersion of the hand in 14 °C water that did not induce intense pain did not increase MSNA in the lower leg. Furthermore, Mizushima et al. (1998) showed that immersion of the hand in cold water increased MSNA, BP and HR and was associated with a sensation of intense pain. The significant relationship between somatic afferent stimulation and MSNA suggests that somatic stimulation during local cooling in the present study did not activate the ascending tract in the spinal cord and vasomotor center in the medulla, and that the change in MSNA in the absence of pain during local cooling was induced by a local reflex mechanism.

In the present study, MSNA of the common peroneal nerve increased rapidly during 15 min local cooling (15 °C) on the ipsilateral lower leg, and then decreased to a level lower than the control value after removal of the cold pack. Moreover, MSNA activity during local cooling correlated significantly with shin temperature. In comparison, Mizushima et al. (1998) reported that MSNA of the common peroneal nerve increased during 3 min of the cold pressor test (hand immersion in 0 °C water) and also similar to our protocol it decreased after the cold pressor test, but it remained during the 3-min recovery period at a level higher than the control value. Thus, the increase in MSNA induced by local cooling observed in the present study was similar to that seen in the cold pressor test, but a different pattern was noted after the end of local cooling. The cold pressor test induces stimulation of the MSNA throughout the entire body, where our test stimulated MSNA in only the local area of application through a local reflex mechanism. To the best of our knowledge, the present study is the first report to evaluate the response of MSNA to non-noxious local cooling.

We reported previously that non-noxious local heat application (42 °C) to the shin and anterior foot induced a significant increase in LBF (Takahashi et al. 2011). In the

present study, non-noxious local cooling (15 °C) to the same area did not change LBF. Fiscus et al. (2005) reported that immersion of the lower leg in warm-water (40 °C) increased LBF whereas cold-water (13 °C) immersion did not. The LBF response observed in our study is similar to the latter. As discussed by Fiscus et al. (2005), the lack of change in LBF during cold-water immersion may represent a body reaction to preserve core body temperature or a vasomotor response. In the present and above studies, LBF was measured using the plethysmographic method. This method measures total regional blood flow of the leg, which includes mainly flow to muscles and skin. It is well known that any increase in MSNA is associated with vasoconstriction of skeletal muscle blood vessels. In the present study, the significant increase in MSNA from the common peroneal nerve recorded during local cooling of the shin and anterior foot was not associated with a decrease in LBF. We assume that the area that controls MSNA of the common peroneal nerve is located in part of the leg, and that MSNA and vasomotor response of skeletal blood vessels in other areas might show a response different to the area of the common peroneal nerve. Moreover, it is well known that the vasoconstrictor skin sympathetic nerve activity (SSNA) controls the tone of cutaneous vessels. In this regard, mild local skin cooling induced an increase in vasoconstrictor SSNA and a decrease in local skin blood flow (Sawasaki et al. 2001; Yamazaki et al. 2006). Therefore, the local skin blood flow during local cooling could have decreased in the present study, and the change in skin blood flow could also be different in the locally cooled area compared with other areas of the leg. On the other hand, several other studies (Taber et al. 1992; Weston et al. 1994) reported a decrease in LBF during application of mild cooling (10–20 °C) to parts of the leg. In these studies, the ice packs were applied for 20 min, whereas local cooling in the present study lasted 15 min. It is possible that LBF would have decreased had the local cooling been applied for longer.

Cryotherapy is widely used clinically and our finding showed that cryotherapy is a harmless treatment in improving patient care based on lack of both hypo-perfusion and changes in cardiovascular responses. Such lack of change in LBF suggests that the reduction in local blood flow observed in cryotherapy is localized in a limited area and that the sympathetic nerve response during cryotherapy seems to be limited to the local area.

### Technical considerations

Our study was performed by simultaneously measuring LBF and MSNA in the lower leg before, during, and after 15 min of local cool application. In the first protocol, MSNA was measured in eight subjects while in the second protocol; LBF was measured in the same group

of eight normal subjects without recording MSNA. Such design (two separate protocols) was necessary because the calf of the plethysmograph made contact with the tungsten microelectrode inserted into the common peroneal nerve. We acknowledged that such design was not ideal (i.e., no simultaneous measurements), but nevertheless necessary.

## Conclusions

The present study demonstrated suppression of activation of change in MSNA during non-noxious local cooling of the skin after the end of local cooling, without changes in BP and HR. The findings indicate different MSNA control mechanisms between non-noxious local cooling and noxious local cooling, and suggest that the MSNA response during non-noxious local cooling was induced by a local reflex mechanism.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare no conflict of interest.

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