静的膝伸展運動時における血管収縮作用に及ぼす局所冷却の影響

Effects of regional cooling on vasoconstriction in the non-exercised limb during sustained knee extension exercise

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Abstract

To test whether there are regional differences in the exercise pressor reflex within an exercising muscle, we evaluated the effect of regional cooling, which delays activation of the muscle metaboreflex, using vascular resistance as an index of sympathetic nerve activity in the non-exercised limb during exercise. Nine subjects performed 2 min of ischaemic isometric knee extension at 30% of maximal torque in three trials: without cooling (C-trial), with cooling of the proximal thigh portion (P-trial), and with cooling of the distal portion (D-trial). Heart rate, mean arterial pressure, calf blood flow in the non-exercised leg, and calf vascular resistance in the non-exercised leg were measured. In both cooling trials, regional cooling significantly decreased the skin temperature of the exposed portion, but had no effect on the opposite portion of the thigh. During exercise, heart rate and mean arterial pressure increased significantly, but were not affected by regional thigh cooling. In contrast, at 60 s of exercise, calf blood flow was significantly higher in the D-trial than in the C-trial (2.94 ± 0.23 vs. 1.97 ± 0.22 ml·100g^{-1}·min^{-1}, $P < 0.05$). Consequently, calf vascular resistance was significantly lower in the D-trial than in both the C- and P-trials at 60 and 90 s of exercise. These findings suggest that stimulating different regions within a given muscle causes a different magnitude of increase in calf vascular resistance evoked by the exercise pressor reflex during exercise.

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I. Introduction

Exercise elevates the cardiovascular response by two neural mechanisms: central command (Eldridge *et al*. 1985) and the exercise pressor reflex (Mitchell 1985; Kaufman and Rybichi 1987; Victor *et al*. 1989). The exercise pressor reflex acts via afferent nerves, which arise from the peripheral system and are capable of sensing thermal, chemical, and mechanical stimuli.

The region involved in sensing chemical and mechanical stimuli alters the magnitude of sympathetic outflow evoked by the exercise pressor reflex. For example, the exercise pressor reflex is affected by exercising limb; the pressor reflex arising from the forelimb is greater than that arising from the hindlimb both in animal (Hayashi *et al*. 2001) and human study (Saito1995). In addition, we recently provided the possibility that distal portion of the exercising muscle makes a greater contribution to the pressor response than does the proximal portion during incremental exercise (Mizuno *et al*. 2004). However, it remains unclear the mechanism(s) causing differential responses.

One chemical stimulus evoking exercise pressor reflex is a metabolic error signal resulting from the mismatch between metabolic demand and oxygen supply in exercising muscle (Hanna *et al*. 2002). Previously, we demonstrated that muscle blood flow (Mizuno *et al*. 2003a) and blood flow velocity (Mizuno *et al*. 2003b) decrease in the direction from the proximal to the distal portion in resting quadriceps muscle. Piiper *et al*. (1985) showed that oxygen supply to the exercising muscle is heterogeneous between proximal and distal portion associated with intramuscular pressure (Ameredes and Provenzano 1997). Since we applied a free perfusion exercise model to measure muscle oxygenation by near-infrared spectroscopy (Mizuno *et al*. 2004), we could not exclude the contribution of heterogeneous blood flow, which affect on the pressor reflex, between proximal and distal portion within a given muscle during exercise. Ischemic exercise model would allow us to counteract the heterogeneous blood flow.

The purpose of this study was to re-examine whether there are regional differences in the exercise pressor reflex within a given muscle using ischemic exercise model. Ray and colleagues (Ray and Gracey 1997; Ray *et al*. 1997) previously reported that muscle temperature affects the discharge of muscle sympathetic nerve activity (MSNA), and that muscle cooling delays the activation of the muscle metaboreflex, but not the muscle mechanoreflex, during exercise. We measured calf vascular resistance as an index of sympathetic nerve activity evoked by the exercise pressor reflex, and compared calf vascular resistance during static knee extension exercise with regional cooling of the proximal and distal portions of the femoral muscle groups. We presumed that a differential response in vascular resistance might occur depending on which region of the muscle was stimulated.

II. Methods

1. Subjects.

Nine men participated in this investigation; their mean (± SD) age was 24.6 ± 3.1 yr, height 169.9 ± 3.9 cm, and weight 64.7 ± 5.9 kg. The subjects were fully informed of the purpose, nature, and potential risks of the experiments, and gave their written

informed consent to participate in this study. This study was approved by our local ethics committee and all work conformed to the Declaration of Helsinki.

2. Procedures

1) Exercise

Static knee extension was used as the exercise in this study. Subjects were positioned supinely with the knee joint flexed to 45º. On a different day before the experimental sessions, maximal torque generated during static knee extension was determined in the right leg using the Cybex® dynamometer (Lumex Inc., Ronkonkoma, NY, USA). The average of three measurements was taken as the subject's maximal torque. For subsequent exercise tests, the load was set at 30% of maximal torque. Subjects monitored their exerted torque with an oscilloscope (DCS7020, Kenwood, Tokyo, Japan). Two minutes before exercise, a pneumatic cuff placed around the upper thigh was inflated to 250 mmHg to induce muscle ischemia, which was continued during 2 min of static knee extension exercise. The laboratory temperature was maintained at 22–25ºC.

2) Trials

The subjects performed three trials, each on a separate day, consisting of a control trial (C-trial, without cooling), a trial in which the proximal thigh was cooled (P-trial), and a trial in which the distal thigh was cooled (D-trial). The portions of the muscle to be cooled were determined by measuring 25% (proximal) and 75% (distal) of the length between the greater trochanter and the knee joint in the exercising leg. The specific region was cooled with an ice pack (10 cm × 15 cm) applied for 30 min before and during each P-trial and D-trial. Trial order was randomly assigned, with at least 24 h between sessions.

3) Measurements

Heart rate (HR) was determined using standard ECG leads (OEC-8108, Nihon Kohden, Tokyo, Japan). Blood pressure was measured with a finger cuff (2300 Finapres, Ohmeda Inc., Englewood, CO, USA). The monitoring finger cuff was placed around the middle finger of the left hand. Baseline values were obtained by averaging for 2 min before exercise. HR and mean arterial pressure (MAP) were calculated every 30 s during exercise.

Calf blood flow (CBF) in the nonexercising leg was measured by venous occlusion plethysmography using a mercury-in-silastic strain gauge (EC-5R, Hokanson Inc., Bellevue, WA, USA). The left leg was slightly elevated above heart level. The strain gauge was placed around the largest area of the left calf. A pneumatic cuff placed around the thigh was inflated to 60 mmHg to measure CBF at 30 s intervals throughout the experiment. Calf vascular resistance (CVR) in the nonexercising leg was calculated by dividing mean blood pressure by CBF.

Before exercise, local skin temperatures in the proximal and distal portions of thigh were measured every 10 min using 2-channel electronic thermometers having accuracy near 0.1°C (Model 6510, Mallinckrodt Inc., Hazelwood, MO, USA). The probes were attached to the skin at each portions of thigh. During exercise, skin temperatures were monitored at 1 min intervals. On a day other than the day of the exercise test, thigh fat thickness was measured in the portions that underwent cooling using an ultrasonic apparatus (SSD-1000, Aloka, Tokyo, Japan). There was no significant difference in thigh fat

thickness between proximal and distal portions (3.3 ± 0.5 and 3.7 ± 0.8 mm, respectively, $P > 0.05$).

4) Statistics

All data are represented as means ± SE. A two-way analysis of variance (ANOVA) for repeated measures, with time and trial as main effects, was employed to determine significant differences. If the *F*-test was significant, pairwise comparisons were performed using Bonferroni's post-hoc test. Values of $P < 0.05$ were considered statistically significant.

III. Results

1. Skin temperature

Changes in thigh skin temperature in each trial are summarized in **Figure 1**. No change was observed between proximal and distal portions in the C-trial (averaged 32.7 ± 0.1ºC vs. 32.0 ± 0.1ºC, respectively; Figure 1A). In contrast, in both the P- and D-trials, skin temperature in the cooled portion decreased significantly below that of the opposite portion. Immediately before exercise, skin temperature was 16.5 ± 0.6ºC in the P-trial and 17.4 ± 0.8ºC in the D-trial (Figures 1B and 1C). At the end of exercise, proximal skin temperature in the P-trial was significantly lower than distal skin temperature in the D-trial (13.7 ± 0.6ºC and 16.4 ± 0.8ºC, respectively; $P < 0.05$).

2. Cardiovascular response

Changes in HR and MAP are shown in **Figure 2**. Immediately before exercise, neither HR nor MAP differed significantly between trials. HR and MAP increased significantly during exercise ($P < 0.001$) in both trials, but there were no significant differences between trials.

Changes in CBF and CVR during exercise are shown in **Figure 3**. Immediately before exercise, neither CBF nor CVR differed significantly between trials (C-trial: 2.71 ± 0.27 ml·100g–1·min–1; P-trial: 3.01 ± 0.46 ml·100g–1·min–1, D-trial: 3.19 ± 0.34 ml·100g–1·min–1, F = 3.40, P = 0.58). CBF decreased significantly ($P < 0.001$) in both trials; as a result, CVR increased significantly ($P < 0.001$) during exercise in both trials. Neither CBF nor CVR differed significantly between C- and P-trials at any time. In contrast, at 60 s of exercise, CBF in the D-trial (2.94 ± 0.23 $ml \cdot 100g^{-1} \cdot min^{-1}$) was significantly higher than in the C-trial (1.97 ± 0.22 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P < 0.05$), but not in the P-trial (2.18 ± 0.34 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P = 0.058$). CBF in the D-trial at 90 s of exercise (2.68 ± 0.24 $ml \cdot 100g^{-1} \cdot min^{-1}$) showed a tendency to be higher than in the C-trial (1.98 ± 0.26 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P = 0.07$) and in the P-trial (1.81 ± 0.20 $ml \cdot 100g^{-1} \cdot min^{-1}$, $P = 0.066$). Consequently, at 60 and 90 s of exercise, CVR in the D-trial (40.7 ± 2.9 units at 60 s and 48.4 ± 4.4 units at 90 s) was significantly lower than in the C-trial (62.7 ± 4.5 units at 60 s, $P < 0.05$ and 71.5 ± 7.5 units at 90 s, $P < 0.05$) and P-trial (62.8 ± 8.3 units at 60 s, $P < 0.05$, and 73.0 ± 9.2 units at 90 s, $P < 0.05$). Statistical analysis for both absolute and percentage change from baseline value of CBF and CVR demonstrated the same results as those found for absolute value.

IV. Discussion

We provided the possibility that distal portion of the exercising muscle makes a greater contribution to the pressor response than does the proximal portion during incremental exercise (Mizuno *et al*. 2004). Although our suggestion lead by the relationship of inflection points between

cardiovascular responses and oxygenation during repetitive incremental exercise, it remains unclear whether during sustained constant load exercise there are regional differences in exercise pressor reflex within a given muscle. Furthermore, we could not address the mechanism(s) causing a differential response between portions: such as heterogeneous blood flow. To address these questions, we examined the effects of regional cooling on the pressor response during ischemic static exercise. The major finding was that cooling of the distal but not the proximal portion of the thigh attenuated an increase in vascular resistance during exercise.

A change in sympathetic outflow, which can be evaluated directly by recording MSNA, is closely matched to vascular resistance during exercise in humans (Seals 1989; Saito *et al*. 1990). Attenuation of the increase in CVR during exercise can be explained by data from a previous study showing that muscle cooling delays the burst frequency of MSNA (Ray *et al*. 1997). However, we observed regional cooling effects on CVR in the D-trial but not in the P-trial during exercise (Figure 3). This is an important observation that may explain the mechanisms involved in the cardiovascular response when different regions of skeletal muscle are cooled.

One possible explanation for the attenuation of the increase in CVR might relate to differences in the longitudinal distribution of skeletal muscle fibre type between proximal and distal portions. Animal studies indicate a non-uniform distribution of skeletal muscle fibre type; for example, the percentage of type I fibre decreases in the direction from proximal to distal in hindlimb muscles (Torrella *et al*. 2000; Wang and Kernell 2000). Wilson *et al*. (1995) demonstrated that the fibre type

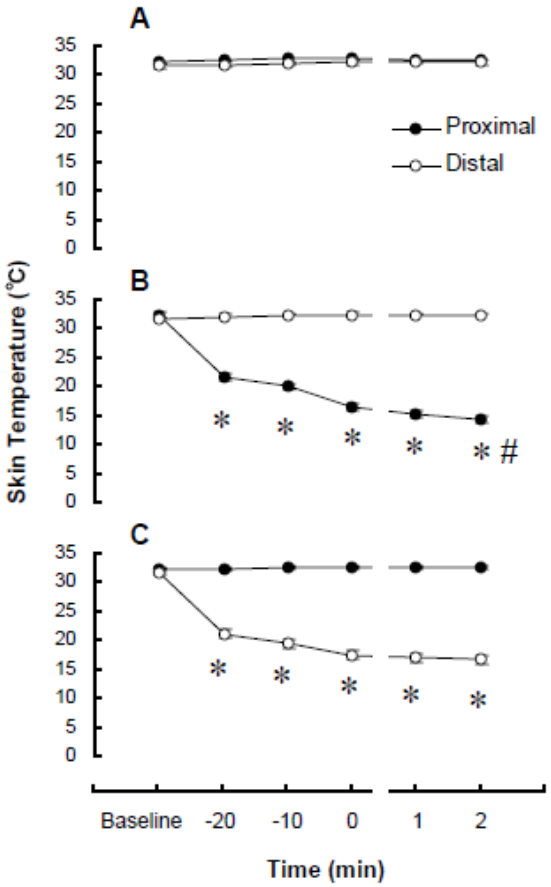

Figure 1. Changes in skin temperature during baseline, rest, and exercise in the C-trial (A), P-trial (B), and D-trial (C). Closed and open circles indicate skin temperature in the proximal and distal portions, respectively. *Significant difference vs. no cooling portion in each trial ($P < 0.05$). #Significant difference vs. skin temperature in the distal portion of the D-trial ($P < 0.05$).

of the contracting muscle influences the magnitude of the pressor response to a static contraction; that is, the pressor reflex to a static contraction is greater in a predominately glycolytic muscle than in a primarily oxidative muscle. Similarly, Sadamoto *et al*. (1992) reported that arterial blood pressure at the end of sustained handgrip exercise was positively correlated to the relative content of fast twitch fibres in the brachioradialis muscle.

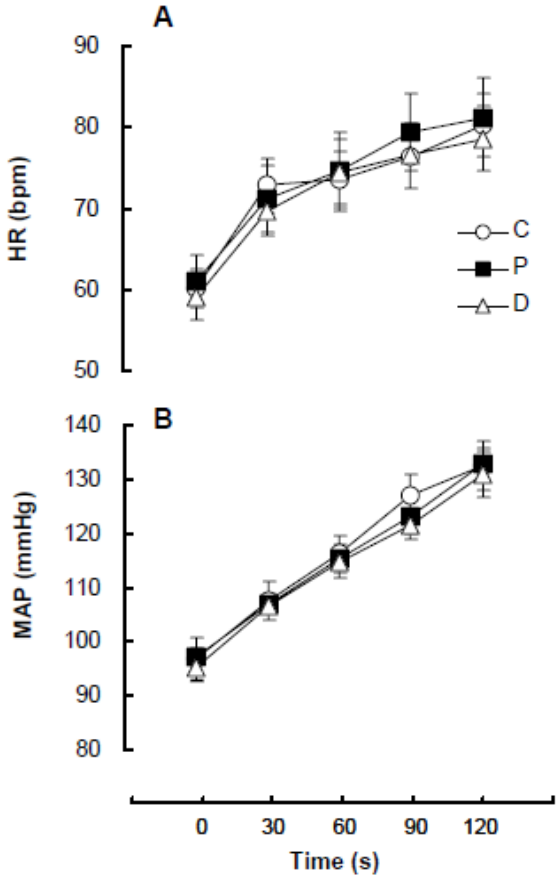

Figure 2. Changes in heart rate (A) and mean arterial pressure (B) during exercise in each trial. Open circles indicate the C-trial, closed squares indicate the P-trial and open triangles indicate the D-trial.

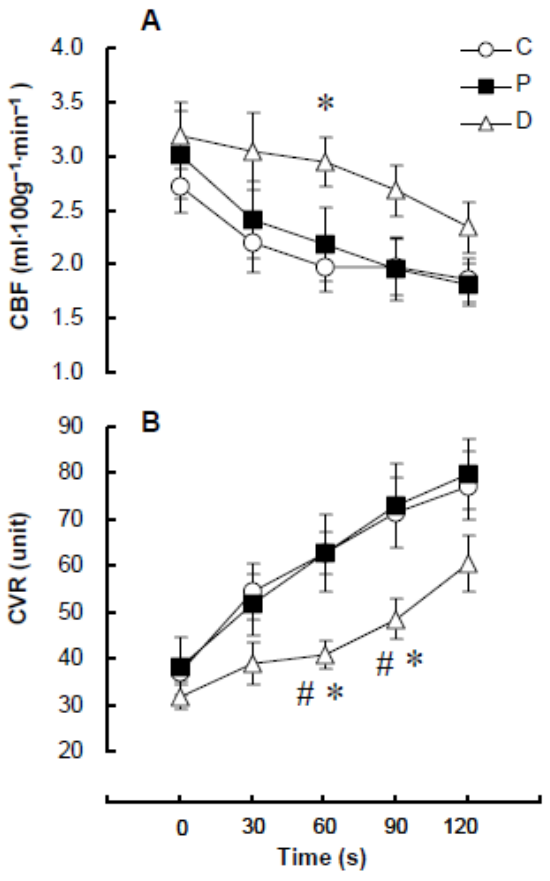

Figure 3. Changes in calf blood flow (A) and calf vascular resistance (B) during exercise in each trial. Open circles indicate the C-trial, closed squares indicate the P-trial and open triangles indicate the D-trial. *Significant difference vs. C-trial ($P < 0.05$); #Significant difference vs. P-trial ($P < 0.05$).

An alternative explanation is that the distribution or number of afferent nerves differs between proximal and distal portions within a muscle. Muscle temperature directly affects the discharge rate of muscle afferent nerves; decreasing muscle temperature attenuates the discharge frequency of chemically sensitive muscle afferents (Hertel *et al*. 1976; Kumazawa and Mizumura 1977). If our current data resulted from the effects of decreasing temperature on afferent nerves, it could be speculated that the distal portion has more afferent nerves than does the proximal portion. Kumazawa and Mizumura (1977) reported that muscular polymodal receptors were more frequently found in the head, the tail, and the edges of dog muscle. Further neurophysiological investigation is needed to identify the distribution of afferent nerves in human muscle. In contrast, decreasing muscle temperature alters muscle metabolism during isometric exercise in humans (Edwards *et al*. 1972), and it is unclear whether muscle cooling directly affects muscle afferent sensitivity or indirectly affects muscle metabolism.

Local skin cooling directly stimulates both cutaneous thermal receptors and nociceptors, which elicit an increase in sympathetic nerve activity (Kregel *et al*.

1992). We cannot completely exclude the possibility that the thermal stimulus could have operated in both cooling trials. However, we found no significant difference between trials in any pre-exercise variables despite decreased skin temperature (Figures 2 and 3). Moreover, we observed regional cooling effects on CVR during exercise in the D-trial but not in the P-trial, whereas skin temperature decreased significantly in both regions (**Figure 1**). These results argue against a role of cutaneous thermal receptors, and we believe it is unlikely that stimulation of cutaneous thermal receptors was responsible for the attenuation of CVR during exercise.

We observed no significant effect of regional cooling on heart rate and blood pressure measured at rest and during exercise (Figure 2), whereas cooling attenuated the increase in CVR during exercise. These findings are consistent with the study by Ray *et al*. (1997), which also found no effect on heart rate or arterial blood pressure with attenuation of MSNA by local cooling. It is possible that sympathetic outflow to other vascular beds may have increased to compensate for the decrease in CVR. Alternatively, the attenuation in CVR elicited by regional cooling may not have been of sufficient magnitude to change arterial pressure.

There are several methodological limitations of our study. We did not measure muscle temperature and thus could not determine the extent to which regional cooling might have decreased muscle temperature. However, the decrease in skin temperature caused by regional cooling in our study (average range 32.4 ± 0.2–15.5 ± º0.5C) was similar to that previously reported by Ray *et al*. (1997). Because there was no significant difference in thigh fat thickness in the cooled portions, we assume that heat conductivity was similar between these portions. A previous study by Gonzalez-Alonso *et al*. (2000) using magnetic resonance imaging showed similar knee extensor muscle mass between proximal and distal portions. Thus, we believe it unlikely that in our study the cooling effect was different in the two regions of the muscle exposed to cooling. In addition, muscle and skin temperatures change in parallel during cold-water immersion (Kregel *et al*. 1992). Further experiments are needed to measure cooling-induced changes in the muscle temperature to clarify this issue.

In conclusion, regional cooling of the distal portion of the thigh attenuated the increase in vascular resistance during exercise; however, no effect was observed with proximal cooling. These findings suggest that stimulating different regions within a given muscle causes different degrees of CVR evoked by the exercise pressor reflex during exercise, and that the differential responses between portions might not be caused by heterogeneous oxygen supply within an exercising muscle.

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