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Original Article

Effects of static and dynamic stretching of lower limb muscles on oxygen uptake, heart rate variability, oxidized hemoglobin of muscular blood vessels and muscular discharges during incremental exercise

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ABSTRACT
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Objective: This study was conducted to clarify the effects of three types of warming up (WU), namely static stretching (SS), dynamic stretching (DS) and cycle ergometer riding at 20 watts (ergo), on cardiopulmonary function and muscle activity during incremental exercise.

Methods: Ten healthy adult males (aged 20.6 ± 0.5 years) participated in this study. Oxygen uptake, heart rate variability, oxidized hemoglobin and discharges of lower limb muscle were continuously recorded during WU and the subsequently performed incremental exercise.

Results: The sympathetic nervous activity index analyzed from heart rate variability, oxygen uptake and cardiac output significantly increased, and the parasympathetic nervous activity index decreased after DS and ergo compared to those after SS. However, no significant differences were found between those values while exercise was maintained at the anaerobic threshold level.

Conclusion: DS and ergo were suggested to be effective for adapting to exercise, but these effects seemed to disappear at the anaerobic threshold level.

Key words: static stretching, dynamic stretching, incremental exercise, heart rate variability, electromyogram

Introduction

Warming up (WU) is often performed to prevent injury and achieve high kinetic performance. The purpose of WU is to facilitate blood flow, increase muscle temperature, and prevent muscular injury by increasing the extensibility of connective tissue and muscle elasticity [1]. Static stretching (SS) and dynamic stretching (DS) are often used as WU, which is performed for the purpose of improving muscular flexibility. The procedure of SS is as follows: the subject slowly stretches the target muscle to the limit of the range of motion of the joint by themselves, and keeps at the limit position for 20 to 30 s [2]. Because muscles are stretched without recoil in SS, signals from the Golgi tendon organ suppress alpha motor neurons in the anterior horn of the spinal cord via inhibitory interneurons [3]. The stretch reflex seems to hardly occur since the muscle is slowly stretched, and muscular tension is reported to decrease [2, 3]. In DS, stretching effects can be obtained in the antagonistic muscle by repeated flexion and extension. The target muscles are repeatedly contracted without using recoil to the final range of motion of the joint. In this way, the stretching effect of an antagonistic muscle is reported to be obtained by reciprocal suppression [4]. Several researches have been reported on the instantial effect of stretching. Noguchi et al. [5] reported that 2 min of SS performed on the soleus muscle significantly increased the dorsiflexion angle of the ankle joint and that no significant difference in soleus muscle blood flow was found. Fujibayashi and Nara [6] reported that

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DS, in which a hip joint flexion exercise was repeated in the knee joint extension position, increased the flexibility of the hamstrings, and that muscular torque and integrated value of the electromyogram were maintained.

The anaerobic threshold (AT) calculated by the gradual increase load exercise is the largest oxygen uptake ($\dot{V}O_2$) at which the balance between $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$) is maintained, and is known as an index of exercise tolerance [7]. Exercise at the AT level is used as a prescription for exercise therapy because it does not cause a significant rise in blood catecholamine or acidosis [8, 9]. It has been reported that peak oxygen uptake (peak) obtained by incremental exercise is an indicator of prognosis in patients with heart disease [7]. We examined the effects of SS or DS of lower limb muscles on exercise tolerance and the autonomic nervous system. This study is considered to be significant in verifying the effects of WU on incremental exercise at the AT level or peak and circulatory risk management.

This study examined the effects of three types of WU, namely SS or DS of lower limb muscles, and cycle ergometer riding at 20 watts, on oxygen uptake, ventilatory equivalent, heart rate variability, oxidized hemoglobin of muscular blood vessels and muscular discharges during incremental exercise in healthy adult males.

Methods

1. Subjects

The subjects of this study were 10 healthy adult males (age 20.6 ± 0.5 years, height 168.3 ± 6.1 cm, weight 62.8 ± 9.5 kg) without present illness or medical history of respiratory, circulatory, or lower limb locomotor disorders. Before the start of this study, the subjects were explained the purpose and method of the study in writing and verbally, and agreed by signature. This study was conducted with the approval of the ethics committee of Kawasaki University of Medical Welfare (approval number: 18-063).

2. Methods

The subjects were asked to avoid taking alcohol and strenuous exercise for 24 h and to fast for 2 h before the experiment. They were interviewed about their physical condition before the experiment, and they participated in the experiment when problems were not found.

2.1 Measurement items

Button-type disposable electrodes were attached to the anterior chest to a lead electrocardiogram using a wireless electrocardiograph (Trigno Wireless System, Trigno). Sensors with three-axis accelerometers were attached to the vastus lateralis and the lateral head of the gastrocnemius muscle using a wireless transfer type electromyograph (Trigno Wireless System, Trigno). The attachment site was located at two-thirds of the line connecting the anterior superior iliac spine to the superior border of the patella in the vastus lateralis muscle, and one-third of the line connecting the caput fibulae to the heel in the lateral head of the gastrocnemius muscle [10]. A computer connected to the Trigno Wireless System was used for recording, and the electrocardiogram and electromyogram were analyzed using LabChart (ver. 7 for Windows, AD Instruments). Two probes were attached to the vastus lateralis and the lateral head of the gastrocnemius muscle, and the skeletal muscle oxygenation was measured by spatially resolved spectroscopy using near-infrared spectroscopy (NIRS, multichannel tissue oximeter Hb14-2, ASTEM). The probes for NIRS were attached near the sensor of the electromyograph, and the data were obtained using software (ver. 1.04 for Windows, Hb14). Data of the NIRS were adjusted according to subcutaneous fat thickness measured using an ultrasound diagnostic equipment (Prosound SSD-3500SX, Aloka). The breath-by-breath method of the respiratory gas analyzer (AT-1100A, ANIMA) was used to measure expired gas concentration and ventilatory equivalent, and the measurement data were recorded by the AT-1100A software.

2.2 Study protocol

A cycle ergometer (Strength ergo 8, Mitsubishi Electric Engineering) was used for the incremental exercise. The height of the saddle was adjusted so that the heel was centered on the pedal when the pedal was at the lowest point, and the knee joint was in the extended position. The position of the foot was set so that the second metatarsal head was on the central axis of the pedal. The subjects performed WU for 4 min and incremental exercise of 20 watts/min at the pedal rotation rate of 60 rpm after resting for 3 min. Incremental exercise was terminated when the pedal rotation rate fell below 50 rpm over 3 s, or when the subject informed that it was difficult to continue the exercise.

2.3 Types of warming up (WU)

Three types of WU for the incremental exercise were employed in this study, namely (1) cycle ergometer riding at 20 watts (“ergo”), (2) static self-stretching of the lateral vastus and lateral head of the gastrocnemius muscle (“SS”), and (3) dynamic stretching of the lateral vastus and lateral head of the gastrocnemius muscle (“DS”). All subjects performed the three types of WU in the experiment on different days, and the order of the WU was randomly determined. Each experiment was performed at intervals of at least 48 h to exclude the influence of fatigue.

In “ergo”, the saddle height and foot position were the same as for the incremental exercise, and the subject was asked to maintain riding at 20 watts at 60 rpm for 4 min. The "ergo" was considered as the control, since cycle ergometer riding is frequently employed as WU before incremental exercise. In "SS", the subject performed two sets of slow self-stretching for 20 s in order of the left quadriceps femoris, the right quadriceps femoris, the left triceps surae, and the right triceps surae muscle [11, 12]. The stretching strength was controlled at the level to feel moderate muscle tension without pain [13]. In "DS" for the quadriceps group, the knee joint flexion and extension exercise was performed in an upright posture while holding the hip joint in the neutral position. In "DS" for the triceps surae muscle group, the ankle plantarflexion and dorsiflexion exercise was performed in the standing posture. One set of "DS" was slowly performed five times, and then rapidly 10 times [2], and two sets were performed in order of the quadriceps femoris muscle group and the triceps surae muscle group.

2.4 Measurement items and data analysis

One cycle of pedaling motion was determined from the value of the three-axis accelerometer, and the mean power frequency (MPF) of the electromyogram during three cycles was calculated, and it was considered as an index of muscle fatigue [14]. The heart rate (HR), very low frequency (VLF) from 0 to 0.04 Hz, low frequency (LF) from 0.04 to 0.15 Hz, high frequency (HF) from 0.15 to 0.40 Hz, and total power (TP) from 0 to 0.40 Hz were calculated by frequency analysis of heart rate variability. The HF normalized unit (HF nu) = HF × 100 / (TP−VLF) was obtained by correcting HF with TP and VLF, and was used as an index of parasympathetic nervous activity, and LF/HF was used as an index of sympathetic nervous activity. The information about autonomic nervous activity decreases significantly because the R-R interval fluctuation is close to white noise during severe exercise [15]. In this study, LF/HF and HF nu during exercise above the AT level were excluded from the analysis. The average value of oxygen hemoglobin (Oxy-Hb) recalibrated by subcutaneous fat thickness, was used as an index of skeletal muscle oxygenation. AT was determined by the V-slope method using the calculated minute ventilation ($\dot{V}E$) and $\dot{V}O_2$/kg. The stroke volume (SV) was calculated from $\dot{V}O_2$ and HR [16], and cardiac output (CO) was calculated from the product of SV and HR.

2.5 Statistical analysis

Statistical analysis was performed using software (SPSS ver. 22.0, IBM) and the significance level was set at 5%. Friedman's test was performed, followed by the Wilcoxon signed rank test, and the results were corrected by the Holm method.

Results

The experimental environment was a temperature of 24.5 ± 1.2°C and humidity of 44.8 ± 6.1%. In this study, all subjects performed the three types of WU and rode the cycle ergometer until oxygen uptake reached peak value ("peak"). The HR, LF/HF, $\dot{V}E$, $\dot{V}O_2$/kg, and CO gradually increased as the incremental exercise proceeded. Conversely, the HF nu gradually decreased (Table 1, 2).

1. Parameter values during resting

We confirmed that no significant differences were found in HR, HF nu, LF/HF, $\dot{V}E$, $\dot{V}O_2$/kg, CO and Oxy-Hb before the three types of WU (Table 1−3).

Table 1. Comparison of heart rate variability between the three types of warming up (WU).

		ergo	SS	DS
HR (bpm)	rest	75.1 ± 6.4	75.1 ± 6.9	73.2 ± 9.0
	WU	92.9 ± 6.5	81.1 ± 7.1*	90.8 ± 8.8†
	AT	115.6 ± 6.9	108.2 ± 9.7	113.1 ± 8.9
	peak	185.2 ± 13.4	180.0 ± 16.1	187.6 ± 12.0
HF nu (nu)	rest	52.1 ± 7.0	55.2 ± 10.6	54.6 ± 3.9
	WU	21.5 ± 7.4	44.5 ± 10.1*	19.3 ± 7.2†
	AT	6.3 ± 3.0	8.4 ± 2.2	7.2 ± 2.4
LF/HF	rest	1.4 ± 0.5	1.3 ± 0.2	1.4 ± 0.3
	WU	3.2 ± 1.2	1.7 ± 0.4*	3.0 ± 0.2†
	AT	7.5 ± 3.1	8.0 ± 1.3	7.5 ± 1.6

Mean ± standard deviation.
*$p < 0.05$ ergo vs. SS, DS; †$p < 0.05$ SS vs. DS.
AT, anaerobic threshold; peak, peak oxygen uptake; SS, static stretching; DS, dynamic stretching; HR, heart rate; HF, high frequency; LF, low frequency.

Table 2. Comparison of expiratory gas and ventilatory equivalent between the three types of WU.

		ergo	SS	DS
$\dot{V}E$ (L/min)	rest	7.0 ± 1.8	6.9 ± 1.4	6.6 ± 1.7
	WU	13.7 ± 1.9	9.9 ± 1.6*	12.2 ± 2.6†
	AT	24.3 ± 6.8	24.8 ± 6.3	27.0 ± 7.0
	peak	91.0 ± 24.7	92.9 ± 26.5	97.9 ± 23.8
$\dot{V}O_2$/kg (mL/min/kg)	rest	4.0 ± 0.7	4.4 ± 1.1	4.1 ± 0.8
	WU	8.9 ± 1.4	6.3 ± 1.1*	7.6 ± 1.3*†
	AT	17.9 ± 4.9	18.1 ± 3.5	20.5 ± 6.4
	Peak	40.7 ± 6.8	39.5 ± 3.5	41.9 ± 6.2
CO (L/min)	rest	3.7 ± 0.7	4.0 ± 0.6	3.8 ± 0.9
	WU	7.0 ± 1.0	5.3 ± 0.9*	6.3 ± 1.2†
	AT	10.9 ± 2.0	10.9 ± 1.5	11.6 ± 2.1
	peak	16.1 ± 2.6	15.7 ± 2.2	16.7 ± 2.5

Mean ± standard deviation.
*$p < 0.05$ ergo vs. SS, DS; †$p < 0.05$ SS vs. DS.
$\dot{V}E$, minute ventilation; $\dot{V}O_2$, oxygen uptake; CO, cardiac output.

Table 3. Comparison of skeletal muscle oxygenation between the three types of WU.

		ergo	SS	DS
Oxy-Hb (μM) Vastus lateralis muscle	rest	39.0 ± 16.6	39.7 ± 16.1	41.2 ± 12.3
	WU	32.3 ± 10.0	40.2 ± 14.4*	32.8 ± 9.4†
	AT	36.2 ± 13.6	38.7 ± 16.1	40.7 ± 8.4
	peak	38.9 ± 13.9	37.5 ± 12.8	38.6 ± 8.9
Oxy-Hb (μM) Lateral head of gastrocnemius muscle	rest	50.1 ± 12.8	42.5 ± 12.9	51.8 ± 15.4
	WU	35.6 ± 9.9	42.7 ± 12.4*	34.6 ± 9.5†
	AT	43.6 ± 10.2	37.7 ± 13.7	43.7 ± 9.8
	peak	40.4 ± 11.6	34.4 ± 10.0	39.8 ± 12.5

Mean ± standard deviation.
*$p < 0.05$ ergo vs. SS, DS; †$p < 0.05$ SS vs. DS.
Oxy-Hb, oxygen hemoglobin.

2. Comparison of parameter values just after the three types of WU

The HR, LF/HF, $\dot{V}E$, $\dot{V}O_2$/kg and CO showed significantly higher values just after ergo and DS compared to those just after SS ($p < 0.05$, Table 1−3). Contrarily, the HF nu and Oxy-Hb of the vastus lateralis and lateral head of the gastrocnemius muscle showed significantly lower values just after ergo and DS compared to those just after SS ($p < 0.05$, Table 1−3).

3. Comparison of parameter values after the three types of WU at the exercise level of AT and peak oxygen uptake

To examine the effect of WU on exercise tolerance, exercise loads at the AT level and peak oxygen uptake were compared between the three types of WU. There were no significant differences in both loads between the three types of WU (Table 4). In addition, no significant differences were found in any of the parameters (HR, HF nu, LF/HF, $\dot{V}E$, $\dot{V}O_2$/kg, CO, Oxy-Hb and MPF) at both loads between the three types of WU (Table 1−3, 5).

Discussion

Stretching is generally employed as WU for exercise. Although SS has been reported to have the effect of improving muscle flexibility, it has been reported that muscle weakness may occur temporarily [2, 3, 17]. DS has been reported to have the effect of improving muscular flexibility, maximum muscular strength, knee extension power and agility [6, 18, 19]. It is important for circulatory risk management to examine the effects of SS or DS of lower limb muscles on motor function and autonomic nervous system in each individual. In this study, we examined the effects of SS or DS of lower limb muscles on oxygen uptake, heart rate variability, skeletal muscle oxygenation and frequency of the electromyogram during incremental

Table 4. Comparison of work rate between the three types of WU.

		ergo	SS	DS
Work rate (W)	AT	85.0 ± 19.6	82.5 ± 16.2	87.6 ± 22.8
	peak	237.3 ± 42.4	232.7 ± 43.6	241.1 ± 45.9

Mean ± standard deviation.

Table 5. Comparison of frequency of the surface electromyogram between the three types of WU.

		ergo	SS	DS
MPF (Hz)	AT	72.2 ± 13.8	76.1 ± 13.9	79.4 ± 15.3
Vastus lateralis muscle	peak	70.2 ± 7.5	73.4 ± 13.5	77.9 ± 14.5
MPF (Hz)	AT	117.9 ± 12.6	111.9 ± 17.8	108.8 ± 19.6
Lateral head of gastrocnemius muscle	peak	112.1 ± 32.5	109.7 ± 21.0	109.9 ± 33.1

Mean ± standard deviation.
MPF, mean power frequency.

exercise.

It has been reported that the load intensities of ergo and DS were higher than that of SS, since $\dot{V}O_2$, which is an index that reflects the external load on the whole body [20], after ergo and DS were higher compared that after SS. After ergo and DS, the HR, LF/HF, $\dot{V}E$ and CO values were higher and HF nu values were lower compared with those after SS. Since it is known that HR, LF/HF, $\dot{V}E$ and CO increase and HF nu decreases as exercise load increases, the load intensity of ergo and DS were considered to be higher than that of SS. In addition, Oxy-Hb after ergo and DS showed significantly lower values than that after SS, indicating that peripheral oxygen consumption was increased by ergo and DS. SS does not induce active muscle contraction because signals from the stretched tendon suppress the excitability of the alpha motor neurons, thereby relaxing muscle tone [3]. Contrarily, the target muscle is stretched by repeatedly contracting antagonistic muscles and reciprocal inhibition in DS, and the temperature of the muscle has been reported to increase [21−24]. Cycle ergometer riding (ergo) as well as DS induce active muscle contraction, thus the load intensities of ergo and DS seemed to be higher than that of SS. These results suggested that ergo and DS significantly cause respiratory and circulatory activities, and skeletal muscle metabolism increases. Furthermore, ergo and DS were suggested to have preparatory effects for adaptation to exercise.

There were no significant differences in all parameters after the three types of WU at the exercise level of AT and peak. It has been reported that the exercise of ankle plantar dorsiflexion increased HR and LF/HF, decreased HF, and recovered 2 min after exercise [25]. It has been reported that HR recovered within 5 min after cycle ergometer riding at 75 watts [26]. These reports suggest that recovery after low-intensity and moderate-intensity exercise occurs within 2 min and 5 min, respectively. In this study, the effect of WU seemed to disappear at the AT level exercise, because it took over 3 min to reach AT. Furthermore, the incremental exercise might serve to reduce differences among all parameters after the three types of WU. These results suggest that ergo and DS might have preparatory effects to enhance fitness to exercise, however, a WU suitable for each individual's physical condition should be selected when performing incremental exercise. This conclusion is based on the result that no difference was found in the parameters in this study when the exercise intensity reached the AT level. However, further study on risk management is required in the case of steady or hard load exercises.

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