Treadmill walking in water induces greater respiratory muscle fatigue than treadmill walking on land in healthy young men

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Self-Archiving by Author(s) Placed on: Osaka City University Treadmill walking in water induces greater respiratory muscle fatigue than treadmill walking on land in healthy young men

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Authors' contributions

YY, HY contributed to the design of the study. All authors conducted all experiments and collected the data. YY, HY conducted interpretation, statistical analysis, and drafting the manuscript. KO, DI, and TM contributed to revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Abstract

The purpose of the present study was to investigate the effect of walking in water on respiratory muscle fatigue compared with that of walking on land at the same exercise intensity. Ten healthy males participated in 40-min treadmill walking trials on land and in water at an intensity of 60% of peak oxygen consumption. Respiratory function and respiratory muscle strength were evaluated before and after walking trials. Inspiratory muscle strength and forced expiratory volume in one second were significantly decreased immediately after walking in water, and expiratory muscle strength was significantly decreased immediately and 5 min after walking in water compared with the baseline. The decreases of inspiratory and expiratory muscle strength were significantly greater compared with that after walking on land. In conclusion, greater inspiratory and expiratory muscle fatigue was induced by walking in water than by walking on land at the same exercise intensity in healthy young men.

Keywords

Walking in water; Walking on land; Respiratory function; Respiratory muscle strength

Introduction

The fatigue of muscles involved in inspiration, such as the diaphragm, external intercostal muscles, and parasternal intercostal muscles, induces shortness of breath resulting in impaired exercise tolerance [1, 2, 3]. Decreased respiratory muscle strength also reduces the ability to cough in order to eliminate respiratory secretions, thereby increasing risk of atelectasis and pneumonia following surgery or long-term recumbency [4, 5]. Thus, decreased respiratory muscle strength is a clinically important issue.

In recent years, aquatic exercise has generally been accepted as a component of health promotion activities in clinical rehabilitation and various sports facilities [6, 7]. As buoyancy helps to reduce weight-bearing in water, people with obesity [8], joint diseases, or lumbago [9] are likely to benefit from aquatic exercise as unnecessary exercise load on the joint can be avoided, allowing patients to perform exercise more safely. In addition, the muscles in the upper and lower extremities can be efficiently strengthened by aquatic exercise using water viscosity and pressure [10, 11].

With regard to the effect of submersion on the respiratory system, breathing underwater requires substantial effort, predominantly because of the following two aspects: first, blood volume shifts into the chest cavity because of the increased venous return from the lower extremities and second, inflexibility of the chest wall and a shift of diaphragm toward the cranial side caused by hydrostatic pressure lead to restricted pulmonary compliance [12, 13]. These conditions can add an aspect of resistance training on respiratory muscles to underwater aerobic exercise during chest expansion in the inspiratory phase. For this reason, exercising underwater may have utility in developing respiratory muscle strength and promoting a healthy lifestyle. However, few studies of respiratory dynamics or respiratory muscle strength during walking in water have been reported.

We hypothesized that walking in water can induce greater respiratory muscle fatigue than walking on land at the same exercise intensity. Therefore, the present study aimed to investigate the effects of walking in water on respiratory muscle strength in comparison with walking on land at the same exercise intensity measured by oxygen uptake rate.

Methods

Compliance with Ethical Standards

The present study was approved by the Institutional Review Board of Osaka City University Graduate School of Medicine (approval No. 2629) and registered with the University Hospital Medical Information Network-Clinical Trial Registry (study ID: UMIN000011736). The present study also conformed to the standard set by the Declaration of

Helsinki.

Subjects

We recruited volunteers from a mix of sedentary, healthy male college students aged between 20 and 29 years. Subjects with a phobia regarding water, a history of respiratory or cardiovascular disease, hypertension [resting systolic blood pressure (BP) \geq 140 mmHg and/or diastolic BP \geq 90 mmHg], diabetes, obesity [body mass index (BMI) \geq 30 kg/m²], or a habit of smoking were excluded. Eligible applicants who met the inclusion criteria participated in the study after familiarizing themselves with the experimental protocol, such as the spirometry measurement methods described below.

Informed consent

Written informed consent was obtained from all subjects prior to the initiation of the present study.

Experimental protocol

The study protocol is shown in Figure 1. Prior to the two experimental sessions, subjects performed a maximal graded exercise tests to evaluate peak oxygen consumption (peak VO₂). After an interval of at least 72 h following the maximal graded exercise test, subjects performed treadmill walking on land (land trial) and in water (water trial) in a randomized order with at least 48 h between trials. Before each trial, respiratory function and respiratory muscle strength were evaluated on land in the sitting position and recorded as baseline measurements. Respiratory function and respiratory muscle strength were re-evaluated immediately, 5 min, 10 min, and 15 min after completion of 40-min treadmill walking and were compared with baseline values.

Maximal graded exercise test

For determining peak VO₂, subjects performed a maximal graded treadmill exercise tests according to the modified Bruce protocol using a general treadmill [14, 15] (BM-2200, S&ME, Tokyo, Japan) after 5 min rest in the standing position on a treadmill. Expiratory gas was measured in order to evaluate VO₂ (ml/min) and carbon dioxide production (VCO₂, ml/min) using an electronic spirometry system integrated with a gas analyzer (AE-310S, Minato Medical Science, Osaka, Japan) in a breath-by-breath manner. BP and heart rate (HR) were continuously monitored throughout the test using an automated sphygmomanometer (STBP-780, Colin, Komaki, Tokyo, Japan) and an electrocardiograph (BSM7106, Life scope 8, Nihonkoden, Osaka, Japan), respectively. Rating of perceived exertion

(RPE) by the Borg scale was evaluated every minute. During the test, subjects were allowed to use the handrails for support. We considered peak load was attained when subjects met at least two of the following criteria [16]: 1) a HR of 85% of the age-predicted maximal HR, 2) RPE of 18 or greater, 3) respiratory exchange ratio (RER, VCO_2/VO_2) of 1.1 or greater, and 4) and no further increases in VO_2 regardless of increasing the load. The average VO_2 in the last 30 s of the graded test was defined as peak VO_2 .

Treadmill walking on land and in water

A general treadmill (BM-2200, S&ME, Tokyo, Japan) and an aquatic treadmill (AQUAEXMILL, Sanplatec, Osaka, Japan) allowing adjustment of water depth and belt speed and gradient were used for land and water trials, respectively. In both trials, room temperature of the laboratory was set approximately at 22°C with no significant difference between the trials. In land trials, the initial belt speed and gradient was set at the levels at which each subject attained 60% peak VO₂ in the each graded exercise test. In water trials, subjects wore swimming trunks and underwater walking shoes. Water depth was set at the height of the fourth rib and water temperature was maintained at 32.0° C ± 1.0°C [17]. Trials were started with treadmills set at the same gradient and two thirds of the speed at which subjects had attained 60% peak VO₂ in each graded exercise test. During the trials, subjects held the handrails on both sides. In both trials, subjects underwent 40-min treadmill walking after 5 min rest in the standing position on the treadmill (i.e., underwater in water trial) for baseline measurements. In addition to monitoring BP and HR, spirometry and expiratory gas analyses were continuously performed throughout the trial and respiratory rate (RR), minute ventilation (VE), VO₂, and RER were recorded. RPE was also evaluated every minute. We constantly attempted to adjust the belt speed to maintain exercise intensity at 60% peak VO₂ during the first 5 min of walking and thereafter.

Evaluation of respiratory function and respiratory muscle strength

Respiratory function was evaluated using a spirometer (AS-507, Minato, Osaka, Japan) and parameters, such as tidal volume (TV), inspiratory reserve volume (IRV), inspiratory capacity (IC), expiratory reserve volume (ERV), vital capacity (VC), forced expiratory volume in 1 s ($FEV_{1.0}$), and forced vital capacity (FVC) were extracted. The maximum expiratory (PEmax) and inspiratory (PImax) pressures in the oral cavity, considered surrogate indices of expiratory and inspiratory muscle strength, respectively [18], were evaluated using a sthenometer (AAM337, Minato, Osaka, Japan) attached to the spirometer.

The rate of change in each parameter of respiratory function and respiratory muscle strength from baseline was

calculated with the following formula:

rate of change (%) = [(measured value) – (baseline value)] / (baseline value) \times 100

Anthropometrical measurements

Weight and height were measured before maximal graded exercise tests. BMI (in kg/m²) was calculated as body weight (kg) divided by height (m) squared.

Statistical analyses

All statistical analyses were performed using statistical processing software (Stat View; SAS, Cary, NC, USA), and all values were presented as means \pm standard deviation (SD). Comparisons of baseline parameters and mean values during treadmill walking between trials were performed using the paired t-test. The effects of walking (on land or in water) and treadmill walking on the rate of change in respiratory function and respiratory muscle strength were examined by two-way (trial × time) analysis of variance (ANOVA) with repeated measurements. In cases where significant trial and time effects were detected, subsequent *post-hoc* multiple pairwise comparisons (Dunnett's method) were performed. P-values < 0.05 were considered statistically significant.

Results

Ten applicants who met the inclusion criteria were enrolled in the present study. The physical characteristics of the subjects are summarized in Table 1.

Respiratory functions, respiratory muscle strength, and clinical parameters from expiratory gas analysis at baseline on land and in water trials.

Values for respiratory functions, respiratory muscle strength, and parameters of expiratory gas analysis at baseline in both trials are shown in Table 2. At baseline, no significant differences in any parameters were observed between trials.

Exercise intensity and hemodynamics during land and water trials

Table 3 shows the mean values of respiratory and hemodynamic parameters and treadmill settings during the final 30 min of each trial. Mean RPE was significantly greater in water trials than in land trials (15.9% \pm 1.6% vs. 13.9% \pm 0.9%, respectively, P = 0.02). No significant differences in any other parameters were observed between trials.

The effects of treadmill walking on land and in water on respiratory functions and respiratory muscle strength

As shown in Table 4, FEV_{1.0} was significantly decreased immediately after water trials compared with the baseline, and the decrease was significantly greater compared with that after land trials ($-1.3\% \pm 5.8\%$ vs. $1.6\% \pm 4.4\%$, respectively, P = 0.01). No significant changes in any other parameters of respiratory function were observed both in water and land trials. Regarding respiratory muscle strength, as shown in Table 4 and Figure 2, PImax was significantly decreased from the baseline immediately after water trials, and the decrease was significantly greater than that immediately after land trials ($-12.7\% \pm 7.3\%$ vs. $-4.2\% \pm 2.3\%$, P = 0.02). PEmax was significantly decreased from the baseline immediately after water trials, and the decreases were significantly greater than that after land trials ($-8.2\% \pm 3.6\%$ vs. $-2.3\% \pm 5.3\%$, P = 0.01 and $-6.9\% \pm 3.4\%$ vs. $-0.7\% \pm 5.2\%$, P = 0.01 for immediately and 5 min after trials, respectively).

Discussion

In the present study, we investigated the effects of treadmill walking on land and in water on respiratory muscle strength. The primary finding of the present study was that greater decreases in inspiratory and expiratory muscle strength were induced by walking in water than by walking on land at the same exercise intensity according to VO_2 , indicating that respiratory muscle fatigue after walking in water was greater than after walking on land in healthy young men. Generally, training-associated factors which cause muscular fatigue, such as metabolic stress or local hypoxia, are necessary for muscular hypertrophy and strengthening [19]. Therefore, the results of the present study have important implications for exercise in water which not only help in avoiding unnecessary weight-bearing, particularly in people with obesity or joint diseases, but also produce favorable effects regarding the strengthening of respiratory muscles even in healthy persons.

We demonstrated that treadmill walking with an intensity corresponding to 60% of peak VO2 induced significant decreases in inspiratory and expiratory muscle strength only after walking in water. Respiratory functions, such as VC and FEV_{1.0}, are known to be reduced by water immersion [20-24]. When submerged, hydrostatic pressure against the chest and abdominal wall causes inflexibility of the thorax and elevation of the diaphragm toward the cranial side, this reduces lung compliance and alveolar size at the end-expiratory phase of the respiratory cycle. Therefore, we speculate that the 40-min walking in water trial at the depth of the fourth rib level required greater effort to dilate the thorax during the inspiratory phase due to hydrostatic pressure against the lower thorax and abdominal wall thereby inducing greater inspiratory muscle fatigue in comparison to trials on land. In general, TV and RR are predominant determinants of

inspiratory muscle workload [25], i.e., greater TV and RR causes greater inspiratory muscle fatigue. In the present study, these parameters throughout the treadmill walking were not observed to differ between trials. Therefore, it is unlikely that the difference in respiratory patterns observed between the trials explains the inspiratory muscle fatigue observed following walking in water.

At first, we predicted that walking in water would not cause expiratory muscle fatigue as hydrostatic pressure against chest wall would assist the expiratory muscle in contracting. However, contrary to expectation, we found that expiratory muscle strength was significantly decreased immediately and 5 min after the completion of walking in water compared with the baseline, whereas walking on land did not affect expiratory muscle strength. Fatigue of the abdominal muscles may be a possible reason for the expiratory muscle fatigue observed during walking in water. Abdominal muscular tone is crucial for stabilization of the body trunk in order to maintain standing posture during walking in water [26]. In addition, it has been reported that fatigue of the abdominal muscles causes decreased expiratory muscle strength [27]. In the water trial of the present study, we also observed a decrease in $FEV_{1.0}$, which represents the capacity to expire during the first second of forced expiration using abdominal muscular contraction. Therefore, it is possible that the decrease in expiratory muscle strength observed in the present study was due to fatigue of the abdominal muscles after 40-min of walking in water. However, abdominal muscular strain during walking in water was not evaluated in the present study.

Despite significant decreases in inspiratory and expiratory muscle strength in the water trial, vital capacity did not change following the water trial. It has been reported that at least 40 cmH₂O of inspiratory and expiratory pressure is sufficient to inflate the lungs [25]. No subjects were found to have a respiratory pressure below 40 cmH₂O, even after the treadmill walking, in the present study and this may explain the lack of decreased lung capacity observed following walking in water.

In the present study, we observed a decrease in inspiratory muscle strength immediately after walking in water that had recovered by 5 min after the cessation of walking. Suzuki *et al.* reported that inspiratory muscle strength was transiently decreased immediately after resistance load respiration training and this decrease was maintained for no longer than 5 min after the cessation of training [28]. The findings of the present study corroborate this report. Approximately 60% of muscle contained in the diaphragm consists of red muscle fibers [29], which are characterized by fatigue resistance and endurance strength. This may partly explain the fast recovery from inspiratory muscle fatigue after treadmill walking in the water trial of the present study.

The decrease in expiratory muscle strength immediately after walking in water recovered similarly within 10 min after the cessation of walking in the present study. Expiratory muscles mainly comprise the abdominal muscle group,

consisting of 55%–58% red muscle fiber [30]. Consequently, expiratory muscles are considered to be as fatigue-resistant as inspiratory muscles, and this may explain the rapid recovery of expiratory muscle fatigue following walking in water of the present study. In contrast, Suzuki *et al.* reported that expiratory muscle strength remained decreased for 60 min after the cessation of the 60-min load breathing at 66.0 cmH₂O \cdot 0.5l⁻¹ \cdot s [28]. Taken their result into consideration, submaximal expiration load may be a possible approach for further enhancement of expiration muscle strength. However, the present study demonstrated that walking in water alone was sufficient to result in fatigue of the expiratory muscles.

It is well known that cardiac stroke volume is increased during submergence due to increased venous return, which is accompanied by a decrease in HR. Therefore, it is generally difficult to adjust exercise intensity by targeting HR in underwater conditions. For this reason, 60% of peak VO₂ was adopted as the target exercise intensity during treadmill walking in both trials of the present study. Many previous reports have demonstrated that the belt speed of underwater treadmill walking corresponded to 1/2 times the speed by which the same VO₂ was attained by walking on land [31]. However, in the present study, treadmill speed was not found to differ between trials with the same exercise intensity according to VO₂. Our results were consistent with those of Conti A *et al.* who reported treadmill speed during walking in water with a water depth to the umbilicus was not different from that during walking on land at the same VO₂ [32]. The authors speculated that at certain water depths, i.e., comparatively shallow like the present study, the benefit from increased buoyancy exceeded the reduced propulsion force provided by hydrostatic pressure and water viscosity in underwater conditions. This may underlie the lack of difference observed in belt speed between the water trial and the land trial in the present study.

On the other hand, subjects perceived exertion during treadmill walking was greater in the water trial than in the land trial, and the result was also consistent with the findings of Conti A *et al.* [32]. Although, we did not separately evaluate RPE concerning dyspnea and leg fatigue, the results of the present study indicate that subjects felt greater fatigue of the lower extremities because of stride effort against hydrostatic pressure and water viscosity during walking in water, even in subjects able to maintain the same treadmill speed on land.

There are some limitations of the present study. The results of the present study in a small number of subjects suggest that walking in water is an effective method of increasing inspiratory and expiratory muscle fatigue. However, training producing greater respiratory muscle fatigue may not necessarily result in greater muscle strength. Interventional studies are required to clarify the training effect of walking in water on strengthening respiratory muscles. Further studies are required to validate the findings of the present study, performed in healthy young adults, in patients with chronic respiratory diseases whose respiratory muscles are already fatigued, even at rest. In addition, water resistance during aquatic exercise varies according to the kinetic rate, i.e., a lower kinetic rate is correlated with lower water resistance

during aquatic exercise. A lower respiration rate in water may reduce the load on the chest wall during aquatic respiration exercise. In contrast, the elevation of body temperature causes tachypnea. Although we unfortunately did not assess body temperature during the treadmill walking, the elevation of body temperature by exercise may have affected respiratory muscle fatigue. Therefore, the development of effective aquatic exercise protocols, including walking in water, is required to improve approaches for strengthening the respiratory muscles.

Conclusions

In conclusion, we demonstrated that greater inspiratory and expiratory muscle fatigue was induced by walking in water than by walking on land at the same exercise intensity in healthy young men. Further studies are required to evaluate the longitudinal effect of walking in water as a health promoting strategy for increasing respiratory muscle strength, particularly in patients with chronic pulmonary diseases.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. Protocol of land trial and water trial

Black arrows indicate the evaluation of respiratory function and respiratory muscle strength. Abbreviations: HR, heart rate; BP, blood pressure

Figure 2. Rate of change in respiratory muscle strength following treadmill walking

PImax was reduced immediately after walking in water compared to that at baseline, and the reduction was significantly greater than that after walking on land. PEmax was also significantly decreased immediately and 5 min after the walking in water from baseline, and the decrease was significantly greater than that in the walking on land. Values are means \pm SD.

Solid circle: land trial, solid triangle: water trial.

Other abbreviations are as Table 2.

[#]: p < 0.05 vs. Baseline. [§]: p < 0.05 vs. walking on land.

Tables and captions

Table 1. The physical characteristics of the subject
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Number	10		
Age (years)	23.1	±	3.6
Height (m)	1.64	±	0.1
Weight (kg)	63.9	±	7.3
BMI (kg/m ²)	23.5	±	2.4
Peak VO ₂ (ml/min)	2639	±	402

Data are means \pm SD.

Abbreviations: BMI, body mass index; Peak VO_2 , peak oxygen consumption.

	Land trial	Water trial
VO ₂ (ml/min)	242 ± 38	246 ± 25
VE (L/min)	9.2 ± 1.3	8.6 ± 1.0
RR (n/min)	15.7 ± 2.3	15.9 ± 3.1
RER	0.8 ± 0.0	0.8 \pm 0.0
HR (bpm)	66 ± 3	64 ± 3
SBP (mmHg)	117.7 ± 6.0	115.3 ± 4.7
DBP (mmHg)	78.8 ± 11.7	70.7 ± 7.3
TV (L)	0.7 ± 0.2	0.8 ± 0.3
IRV (L)	1.6 ± 0.3	1.4 \pm 0.4
IC (L)	2.4 ± 0.3	2.2 ± 0.4
ERV (L)	1.6 ± 0.3	1.5 ± 0.3
VC (L)	3.9 ± 0.4	3.8 ± 0.5
FVC (L)	3.7 ± 0.5	3.8 ± 0.3
FEV _{1.0} (L)	3.3 ± 0.3	3.5 ± 0.3
PImax (cmH ₂ O)	92.9 ± 24.9	98.6 ± 24.6
PEmax (cmH ₂ O)	99.9 ± 21.2	101.1 ± 20.1

Table 2. Respiratory functions, respiratory muscle strength and the parameters from expiratory gas analysis at baseline

Data are means \pm SD.

Abbreviations: VO₂, oxygen consumption; VE, minute ventilation; RR, respiratory rate; RER, respiratory exchange rate; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure. TV, tidal volume; IRV, inspiratory reserve volume; IC, inspiratory capacity; ERV, expiratory reserve volume; VC, vital capacity; FVC, forced vital capacity; FEV_{1.0}, Forced expiratory volume in one second; PImax, maximal inspiratory pressure; PEmax, maximal expiratory pressure.

	La	Water trial				
VO ₂ (ml/min)	1533	±	233	1524	±	237
% peak VO ₂ (%)	58.1	\pm	3.9	57.8	\pm	4.1
VE (L/min)	56.9	±	1.6	53.2	±	3.0
RR (n/min)	29.9	±	7.8	30.5	±	4.5
RER	0.9	±	0.0	1.0	±	0.0
HR (bpm)	149	\pm	4	141	±	8
SBP (mmHg)	167.0	±	15.7	161.3	±	7.3
DBP (mmHg)	66.3	\pm	11.1	71.9	\pm	4.7
Walking speed (m/min)	84.5	±	9.9	87.4	±	7.8
RPE	13.9	±	0.9	15.9	±	$0.6^{\$}$

Table 3. The mean values of the parameters for respiratory, hemodynamics and treadmill settings in each trial

Data are means \pm SD. Abbreviations: RPE, rating of perceived exertion.

Other abbreviations are as Table 2. $\S: p < 0.05$ vs. land trial.

Table 4. Changes in respiratory functions and respiratory muscle strength following the treadmill walking in both trials

_				Land t	rial	Water trial					
Rate of change (%)	0 min		5 min		10 min	15 min	0 min	5 min	10 min	15 min	
TV	32.1 ±	42.8	14.1 ±	39.9	-0.8 ± 14.1	12.1 ± 35.2	20.2 ± 46.5	40.3 ± 72.6	6.1 ± 35.8	3.4 ± 27.6	
IRV	-20.3 ±	16.2	-14.6 ±	19.4	4.2 ± 15.7	-8.8 ± 21.1	-0.3 ± 33.7	$6.4 \hspace{0.2cm} \pm \hspace{0.2cm} 25.5$	5.6 ± 31.1	8.9 ± 30.6	
IC	-5.8 \pm	8.6	-6.8 ±	7.6	2.1 ± 9.7	-1.8 ± 9.5	7.6 ± 15.3	6.8 ± 15.6	-0.4 ± 11.9	3.6 ± 17.7	
ERV	3.4 ±	15.2	4.1 ±	14.5	-6.9 ± 14.6	-3.3 ± 12.5	-5.6 ± 8.1	-9.2 ± 12.6	-3.8 ± 12.9	-8.9 ± 18.2	
VC	-2.1 ±	2.5	-2.8 ±	6.1	-1.1 ± 3.6	-2.9 ± 5.2	-0.2 ± 8.3	-1.5 ± 5.9	-3.1 ± 4.2	-1.5 ± 7.3	
FVC	-1.2 ±	10.6	-0.1 ±	8.9	0.8 ± 9.1	0.8 ± 9.7	-3.1 ± 5.2	-1.9 ± 5.1	-1.8 ± 5.1	-2.2 ± 5.5	
$\text{FEV}_{1.0}$	1.6 ±	4.4	2.9 ±	2.6	3.2 ± 3.8	2.8 ± 3.9	$-1.3 \pm 5.8^{*\$}$	-0.2 ± 3.9	0.2 ± 4.3	-3.6 ± 9.7	
PImax	-4.2 ±	2.3	-1.1 ±	3.9	1.0 ± 3.3	0.3 ± 2.6	$-12.7 \pm 7.3^{*\$}$	-5.3 ± 5.5	-2.9 ± 5.0	-2.2 ± 5.9	
PEmax	-2.3 ±	5.3	-0.7 ±	5.2	1.1 ± 4.7	0.9 ± 5.5	$-8.2 \pm 3.6^{*\$}$	$-6.9 \pm 3.4^{*\$}$	-3.6 ± 2.9	-0.1 ± 4.4	

Data are means \pm SD.

The abbreviations are as Table 2. *: p < 0.05 vs. baseline. [§]: p < 0.05 vs. land trial.

Figure 1.

Land trial	Sitting on land Room temperature: 22.1 ± 0.6 °C	Standing on land	Land treadmill walking Room temperature: 22.3 ± 0.7 °C	Room te	Sitting on land		
Water trial	Sitting on land Room temperature: 22.7 ± 0.8 °C	Standing in water with the depth of the fourth rib level	Underwater treadmill walking Water temperature: 32.0 ± 1.0 °C Room temperature: 22.8 ± 0.5 °C Depth of water bath: the fourth rib level	Room te	Sitting on land		
Start	5 min	5 min	40 min	5 min	5 min	5 min	Finish
	1		Î		1		



